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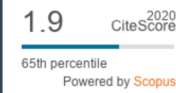
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## Vol. 7 (2017) No. 2

### Articles

#### Panoramic Image Communication for Mobile Application using Content-Aware Image Resizing Method

Jaejoon Kim

pages: 338-344 [Full text](#) DOI:10.18517/ijaseit.7.2.1751

#### Pseudo-Elliptic Bandpass Filters Using Closed-Loop Resonator

Norfishah Ab. Wahab, I. Pasya, M. F. Abdul Khalid, I. M. Yassin, S. H. Herman, Z. Awang

pages: 345-351 [Full text](#) DOI:10.18517/ijaseit.7.2.1362

#### Development of Electronic Nose with High Stable Sample Heater to Classify Quality Levels of Local Black Tea

Danang Lelono, Kuwat Triyana, Sri Hartati, Jazi Eko Istiyanto

pages: 352-358 [Full text](#) DOI:10.18517/ijaseit.7.2.1659

#### Modelling Effect of Aggregate Gradation and Bitumen Content on Marshall Properties of Asphalt Concrete

Arief Setiawan, Latif Budi Suparma, Agus Taufik Mulyono

pages: 359-365 [Full text](#) DOI:10.18517/ijaseit.7.2.2084

#### IoT and Public Weather Data Based Monitoring & Control Software Development for Variable Color Temperature LED Street Lights

Gandeva Bayu Satrya, Hafu Tasew Reda, Kim Jin Woo, Philip Tobianto Daely, Soo Young Shin, Seog Chae

pages: 366-372 [Full text](#) DOI:10.18517/ijaseit.7.2.1578

#### The Performance of Binary Artificial Bee Colony (BABC) in Structure Selection of Polynomial NARX and NARMAX Models

Azlee Zabidi, Noorilawati Md Tahir, Ihsan Mohd Yassin, Zairi Ismael Rizman

pages: 373-379 [Full text](#) DOI:10.18517/ijaseit.7.2.1387

#### Evaluation of the Pre-Cracked RC Beams Repaired with Sealant Injection Method

- Zaidir, Rendy Thamrin, Erick Dalmantias

pages: 380-386 [Full text](#) DOI:10.18517/ijaseit.7.2.2085

#### A Survey on Building Safety after Completing the Construction Process in Malaysia Using Statistical Approach

Nurnadiah Zamri, Fadhilah Ahmad, Amira Husni Talib, Mohamad Shafiq Mohd Ibrahim

pages: 387-398 [Full text](#) DOI:10.18517/ijaseit.7.2.1450

#### Role of Corridor in Territorial Meaning Formation in "Owned Low-cost Apartments" ("Rusunami") Bidara Cina, Jakarta, Indonesia

Fermanto Lianto, Lilanny Sigit Arffin, Y. Basuki Dwisusanto

pages: 399-405 [Full text](#) DOI:10.18517/ijaseit.7.2.2095

#### The Function of Religious Language in the Media: A Comparative Analysis of the Japanese, German and American Newspaper Coverage about the 2011 Great East Japan Earthquake and Tsunami

Yukiko Sato, Ikumi Waragai

pages: 406-417 [Full text](#) DOI:10.18517/ijaseit.7.2.2172

#### The Investigation of 1997 and 2015 El Nino Events in West Sumatera, Indonesia

Revalin Herdianto, Elvi Roza Syofyan, Suhendrik Hanwar, Bambang Istijono, - Dalrino

pages: 418-423 [Full text](#) DOI:10.18517/ijaseit.7.2.1594

#### Splitting Tensile Strength of Lightweight Foamed Concrete with Polypropylene Fiber

Frecy Raupit, Anis Saggaff, Cher Siang Tan, Yee Ling Lee, Mahmood Md Tahir

pages: 424-430 [Full text](#) DOI:10.18517/ijaseit.7.2.2096

#### Sample Biochemical Methane Potential from the Digestion of Domestic Mixed Sewage Sludge in Batch Tests

Roslinda Seswoya, Ahmad Tarmizi Abdul Karim

pages: 431-437 [Full text](#) DOI:10.18517/ijaseit.7.2.2094

#### Improving the Performance Of Single Cells In The Design Of Proton Exchange Membrane Fuel Cell (PEMFC) When Using Hydrogen

- Mulyazmi, Maria Ulfah, Silvi Octavia

pages: 438-445 [Full text](#) DOI:10.18517/ijaseit.7.2.1256

#### Risk Management Framework in Oil Field Development Project by Enclosing Fishbone Analysis

Abdul Hamid, Ishak Baba, Winardi Sani

pages: 446-452 [Full text](#) DOI:10.18517/ijaseit.7.2.1499

#### The Evaluation of Heavy Metals Concentrations in Cempaka Lake, Bangi, Selangor, Malaysia

Muhd. Barzani Gasim, Mohd. Ekhwani Toriman, Amal Barggig, Soaad Muttah, Norsyuhada Hairoma, Azizah Endut

pages: 453-459 [Full text](#) DOI:10.18517/ijaseit.7.2.1004

#### White-box Implementation to Advantage DRM

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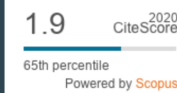
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Antonius Cahya Prihandoko, Hossein Ghodosi, Bruce Litow  
pages: 460-467 [Full text](#) DOI:10.18517/ijaseit.7.2.1445

#### Speed Effect to a Quarter Car ARX Model Based on System Identification

Dirman Hanafi, Mohd Syafiq Suid, Mohamed Najib Ribuan, Rosli Omar Omar, M Nor M. Than, M. Fua'ad Rahmat  
pages: 468-474 [Full text](#) DOI:10.18517/ijaseit.7.2.1500

#### Speaker Independent Speech Recognition of Isolated Words in Room Environment

M. Tabassum, M. A. Aziz Jahan, M. M. Rahman, S. B. Mohamed, M. A. Rashid  
pages: 475-481 [Full text](#) DOI:10.18517/ijaseit.7.2.1465

#### Validation on an Enhanced Dendrite Cell Algorithm using Statistical Analysis

Mohamad Farhan Mohamad Mohsin, Abdul Razak Hamdan, Azuraliza Abu Bakar, Mohd Helmy Abd Wahab  
pages: 482-488 [Full text](#) DOI:10.18517/ijaseit.7.2.1743

#### Rapid Simulation Model Building in Cellular Manufacturing using Cladistics Technique

Zainal Rasyid Mahayuddin, Nur Afiqah Khairuddin  
pages: 489-495 [Full text](#) DOI:10.18517/ijaseit.7.2.2136

#### An Efficient Cloud based Image Target Recognition SDK for Mobile Applications

Haslina Arshad, Meng Chun Lam, Waqas Khalid Obeidy, Siok Yee Tan  
pages: 496-502 [Full text](#) DOI:10.18517/ijaseit.7.2.1744

#### Assessing the Determinants of Cloud Computing Services for Utilizing Health Information Systems: A Case Study

Ahmed Meri Kadhun, Mohamad Khatim Hasan  
pages: 503-510 [Full text](#) DOI:10.18517/ijaseit.7.2.1814

#### A Comparative Review of Machine Learning for Arabic Named Entity Recognition

Ramzi Esmail Salah, Lailatul Qadri binti Zakaria  
pages: 511-518 [Full text](#) DOI:10.18517/ijaseit.7.2.1810

#### Interaction in Online System is A Favor Key for Learners' Success

Imane Kamsa, Rachid Elouahbi, Fatima El Khoukhi  
pages: 519-525 [Full text](#) DOI:10.18517/ijaseit.7.2.1475

#### Key Factors for Selecting an Agile Method: A Systematic Literature Review

Mashal Kasem Alqudah, Rozilewati Razali  
pages: 526-537 [Full text](#) DOI:10.18517/ijaseit.7.2.1830

#### Factors Affecting The Success of Incubators and The Moderating Role of Information and Communication Technologies

Qadri Kamal Alzaghal, Muriati Mukhtar  
pages: 538-545 [Full text](#) DOI:10.18517/ijaseit.7.2.1678

#### An Automatic Updating Process to Control The E-learning Courseware

Fatiha Elghibari, Rachid Elouahbi, Fatima El Khoukhi  
pages: 546-551 [Full text](#) DOI:10.18517/ijaseit.7.2.1871

#### Finding an Optimum Period of Oxidative Heat Treatment on SS 316 Catalyst for Nanocarbon Production from LDPE Plastic Waste

Praswasti P.D.K. Wulan, Satrio Bimo Wijardono  
pages: 552-558 [Full text](#) DOI:10.18517/ijaseit.7.2.2097

#### Identification of Nutrient Contents in Six Potential Green Biomasses for Developing Liquid Organic Fertilizer in Closed Agricultural Production System

- Fahrurrozi, Yenny Sariasih, Zainal Muktamar, Nanik Setyowati, Mohammad Chozin, Sigit Sudjatmiko  
pages: 559-565 [Full text](#) DOI:10.18517/ijaseit.7.2.1889

#### Treatment of Wastewater Containing Hexavalent Chromium Using Zeolite Ceramic Adsorbent in Adsorption Column

Tuty Emilia Agustina, Tine Aprianti, Siti Miskah  
pages: 566-572 [Full text](#) DOI:10.18517/ijaseit.7.2.2035

#### Identification of Plant Morphology of Taro as a Potential Source of Carbohydrates

Zulfadly Syarif, Nasrez Akhir, Benni Satria  
pages: 573-579 [Full text](#) DOI:10.18517/ijaseit.7.2.1323

#### Mixed Cooked Rice with Purple Sweet Potato is Potentially to be The Low Glycemic Index Food

Evawany Aritonang, Albiner Siagian, Fannisa Izzati  
pages: 580-586 [Full text](#) DOI:10.18517/ijaseit.7.2.1033

#### A Review of Minimum Quantity Lubrication Technique with Nanofluids Application in Metal Cutting Operations

Safian Sharif, Ibrahim Ogu Sadiq, Noordin Mohd Yusof, Amrifa Saladin Mohruni  
pages: 587-593 [Full text](#) DOI:10.18517/ijaseit.7.2.2141

#### Susceptible Phase of Chili Pepper Due to Yellow Leaf Curl Begomovirus Infection

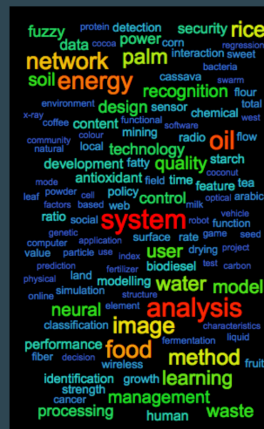
Dwi Wahyuni Ganefianti, Sri Hendrastuti Hidayat, Muhamad Syukur  
pages: 594-601 [Full text](#) DOI:10.18517/ijaseit.7.2.1872

#### Sweet Corn Performance and its Major Nutrient Uptake Following Application of Vermicompost Supplemented with Liquid Organic Fertilizer

Zainal Muktamar, Sigit Sudjatmiko, Mohammad Chozin, Nanik Setyowati, - Fahrurrozi  
pages: 602-608 [Full text](#) DOI:10.18517/ijaseit.7.2.1112

#### Secure Data Sensor In Environmental Monitoring System Using Attribute-Based Encryption With Revocation

- Munsyi, Amang Sudarsono, M. Udin Harun Al Rasyid



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pages: 609-624 [Full text](#) DOI:10.18517/ijaseit.7.2.2175

#### **A Novel Method to Detect Segmentation points of Arabic Words using Peaks and Neural Network**

*Jabril Ramdan, Khairuldin Omar, Mohammad Faidzul*  
pages: 625-631 [Full text](#) DOI:10.18517/ijaseit.7.2.1824

#### **Investigation of Home Agent Load Balancing, Failure Detection and Recovery in IPv6 Network-based Mobility**

*Anshu Khatri, Mathi Senthikumar*  
pages: 632-641 [Full text](#) DOI:10.18517/ijaseit.7.2.1787

#### **Feature Selection for Multi-label Document Based on Wrapper Approach through Class Association Rules**

*Roiss Alhutaish, Nazlia Omar*  
pages: 642-649 [Full text](#) DOI:10.18517/ijaseit.7.2.1040

#### **Local Color Voxel and Spatial Pattern for 3D Textured Recognition**

*Hero Yudo Martono*  
pages: 650-661 [Full text](#) DOI:10.18517/ijaseit.7.2.2173

#### **Molecularly Imprinted Polymers (MIP) Based Electrochemical Sensor for Detection of Endosulfan Pesticide**

*Yohandri Bow, Edy Sutriyono, Subriyer Nasir, Iskhaq Iskandar*  
pages: 662-668 [Full text](#) DOI:10.18517/ijaseit.7.2.1064

#### **Water Flow-Like Algorithm with Simulated Annealing for Travelling Salesman Problems**

*Zulaiha Ali Othman, Nasser Hamed Al-Dhwal, Ayman Srour, Wudi Yi*  
pages: 669-675 [Full text](#) DOI:10.18517/ijaseit.7.2.1837

#### **An Automatic Feature Extraction Method of Satellite Multispectral Images for Interpreting Deforestation Effects in Soil Degradation**

*Irene Erlyn Wina Rachmawan, Yasushi Kiyoki, Shiori Sasaki*  
pages: 676-687 [Full text](#) DOI:10.18517/ijaseit.7.2.2174

#### **Influence of Partial Solar Eclipse on the Radio Signal during 9 March 2016**

*Nur Zulaikha Mohd Afandi, Roslan Umar, Zainol Abidin Ibrahim, Nor Hazmin Sabri, Marhamah Mohd Shafie*  
pages: 688-694 [Full text](#) DOI:10.18517/ijaseit.7.2.1154

#### **Dual-Band Bandpass Filter with Dumbbell Shaped Defective Ground Structure**

*M. F. Abdul Khalid, Z. Ismail Khan, Z. Awang, I. Pasya, N. Ab Wahab, I. M. Yassin*  
pages: 695-701 [Full text](#) DOI:10.18517/ijaseit.7.2.1342

#### **Acrylic Acid Neutralization for Enhancing the Production of Grafted Chitosan Superabsorbent Hydrogel**

*Dhena Ria Barleany, Retno Sulisty Dhamar Lestari, Meri Yulvianti, Taufik Rachman Susanto, - Shalina, - Erizal*  
pages: 702-708 [Full text](#) DOI:10.18517/ijaseit.7.2.2340

#### **Physicochemical and Fatty Acid Profile of Fish Oil from Head of Tuna (*Thunnus albacares*) Extracted from Various Extraction Method**

*Novizar Nazir, Ayu Diana, Kesuma Sayuti*  
pages: 709-715 [Full text](#) DOI:10.18517/ijaseit.7.2.2339

#### **Structural Behaviour of Steel Building with Diagonal and Chevron Braced CBF (Concentrically Braced Frames) by Pushover Analysis**

*- Saloma, Yakni Idris, - Hanafiah, Nico Octavianus*  
pages: 716-722 [Full text](#) DOI:10.18517/ijaseit.7.2.2341

#### **Cost and Performance-Based Resource Selection Scheme for Asynchronous Replicated System in Utility-Based Computing Environment**

*Wan Nor Shuhadah Wan Nik, Bing Bing Zhou, Jemal H. Abawajy, Albert Y. Zomaya*  
pages: 723-735 [Full text](#) DOI:10.18517/ijaseit.7.2.1460



## Molecularly Imprinted Polymers (MIP) Based Electrochemical Sensors for Detection of Endosulfan Pesticide

Yohandri Bow<sup>#</sup>, Edy Sutriyono<sup>\*</sup>, Subriyer Nasir<sup>§</sup>, Iskhaq Iskandar<sup>&</sup>

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**Abstract**— The use of endosulfan pesticides in agriculture can cause environmental problems, such as pollution in aquatic environments that can lead to the destruction of fishery resources and drinking water. So, it has become imperative to detect and separate the hazardous pesticide endosulfan from contaminated water. In this work, molecularly imprinted membrane has been fabricated for the specific recognition by using methacrylic acid (MAA) as functional monomer and ethylene glycol dimethyl acrylate (EGDMA) as cross-linker. Scanning Electron Microscopy (SEM) confirmed the molecular imprinting of endosulfan on membrane matrix. Sensing of the endosulfan by voltammetry followed this. The electrochemical potential is additional information enhancing the selectivity of the sensor. It can be concluded that MIP-based voltammetric sensors are very promising analytical tool for the development of highly selective analytical sensor. The test results of electrode performance indicated that MIP endosulfan-based aluminum-carbon sensor had a detection limit of 0.02 mM, sensitive in the concentration range from 0.02 to 0.12 mM with Nernst factor > 0.059 V/decade and had good stability.

**Keywords**— endosulfan pesticide; molecularly imprinted polymer; electrochemical sensor

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### I. INTRODUCTION

Environmental contamination with pesticides is an undesired consequence of agricultural activities. Direct discharge of pesticide-containing wastewater into soil or natural water sources constitutes a major problem of point-source contamination. Inappropriate handling of pesticides during agriculture use results in a risk of contamination in tasks such as the spraying of pesticides on the field, their dilution and pouring in spraying tanks, the discharge of residues and cleaning of those tanks after application, and utilization in postharvest treatment [1], [2].

In recent years, farmers are using a higher percentage of chemical fertilizers and pesticides to enhance the yield of crops to fulfil growing demands of food crops. If pesticides are not used, crop yield has been reported to reduce by about 10%. It is well known that several pesticides used for agriculture purposes are associated with surface and groundwater contamination. Extravagant and enormous usage of endosulfan pesticide not only will leave a traceful

damage for the environment but also to the human living in it. This condition leads to the concern for people living in certain places such as children, pregnant women, farmers, farm workers and the elderly undergo negative health effects and can also cause acute poisoning, cancer, neurological damage, birth defects and reproductive as well as development harms. The excessive and uncontrolled uses of endosulfan pesticide in crops have raised and alarm for its toxicity not only in crops but also in drinking water. So, it has become imperative to detect and separate the hazardous pesticide endosulfan from contaminated water [3].

Persistent Organic Pollutants (POPs) are a set of chemicals that are toxic; persist in the environment for long periods of time, and biomagnify as they move up through the food chain. POPs have been linked to adverse effects on human health and animals. Because they circulate globally via the atmosphere, oceans, and other pathways, POPs released in one part of the world can travel to regions far from their source of origin [4].

Endosulfan is a man-made insecticide. It is used for control of a number of insects on such food crops as grains, tea, fruits, and vegetables and on such non-food crops like tobacco and cotton. It is also used as a wood preservative.

Endosulfan is sold as a mixture of two different forms of the same chemical (referred to as alpha- and beta-endosulfan). It is a cream-to-brown colour solid that may appear crystalline or be in flakes. It has a distinct odour similar to turpentine. Endosulfan does not burn. Its chemical name is 1,4,5,6,7,7-hexachloro-8,9,10-trinoborn-5-en-2,3-ylenebismethylene sulfite or 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepen 3-oxide. The empirical, structural formula and molecular mass are given as Fig. 1 below.

C<sub>9</sub>H<sub>6</sub>Cl<sub>6</sub>O<sub>3</sub>S  
406,9

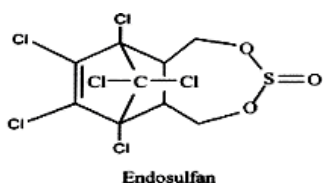


Fig. 1 Structure formula endosulfan

Endosulfan is an organochlorine insecticide and acaricide, and act as a contact poison in a wide variety of insects and mites [5]. It is easily absorbed by the stomach, lungs and through the skin, which meaning that all routes of exposure can pose a hazard. It enhances the effect of estrogens can pose a hazard. It enhances the effect of estrogens and acts as an endocrine disruptor, causing reproductive and development damage in animals and humans as well as cause cancer [6].

Endosulfan is a neurotoxic organochlorine insecticide of the cyclodiene family of pesticides. It is highly toxic and an endocrine disruptor and it is banned in the European Union, Philippine, and several countries. Due to extreme toxic of bioaccumulation and pollution in the environment, a worldwide ban for usage and production of endosulfan is applied as regulated by the Stockholm Convention [7].

Endosulfan is a broad-spectrum organochlorine insecticide and comes in  $\alpha$  and  $\beta$  isomers, both of which are found in commercial formulations. It is practically insoluble in water but is soluble in organic solvents. In the biological tissues and the environment, it is known to degrade into various metabolites, which include endosulfan sulphate, endosulfan alcohol, endosulfan ether and endosulfan lactone [8]. Consequently, it becomes the foremost important act to get the early detection and separation of this pesticide from the polluted water. Recent researches are reported developing methods in sensing and separating the pesticide from the environment. Biosensors and sensors are excellent tools for detecting the pesticides. Molecularly Imprinted Polymers (MIPs) are used for separating pesticides, which have created receptor structure artificially. There are many recent reports of molecularly imprinted polymer, which are used to develop detection systems for pharmaceuticals and environmental contaminants.

Molecular imprinting is a technology that is used to create specific cavities in the synthetic polymer with a memory for the molecules template. Until now molecularly imprinted

polymers (MIPs) have earned a strong position in material science and technology, expanding the list of functional materials significantly.

Molecular imprinting technology is a promising technique for creating recognition elements for selected compounds and has been successfully applied for the synthesis of environmental pollutants such as pesticides [9].

The molecular imprinting technique continues to be a fascinating field of analytical chemistry offering strategies for creating molecule-specific recognition matrices with recognition capabilities analogous to those of biological receptors [10]. The positions, shape, and size of the functional groups in the sites of recognition generated are complementary to its original analyte. Thus, molecularly imprinted polymers (MIPs) rebind their original analyte in preference to related molecules. MIPs have considerable potential for application in the areas of clinical analysis, medical diagnostic, environmental monitoring and drug delivery. Imprinted polymer materials possess several other virtues viz. physical and chemical stability, storage endurance and imprint memory, which is essential for the preparation of recognition membranes in a robust and reusable sensing device. Moreover, MIPs are usually cheaper and more accessible high-affinity recognition materials in contrast to many biological entities [11].

MIPs have been synthesized for large and small molecules, herbicides including atrazine [12] and 2,4,5 trichlorophenoxyacetic acid [13], environmental contaminants pentachlorophenol and organophosphorus such as paraoxon, dichlorovos [14]. Scheme for molecular imprinting is given as Fig. 2 [15].

MIPs are advantageous for sensors because they selectively bind the targeted compound while ignoring all others. Therefore, an MIP-based sensor should reduce the number of false positives that often plague other types of sensors (for example, pesticides often cause false alarms with nerve gas sensors) [16].

Potentiometric sensors, a subgroup of chemical sensors, are attractive for practical applications, as they are associated with small size, portability and low energy consumption and cost compared to another group of sensors [17], [18].

The development of MIP-based sensors with potentiometric transduction does not require the template or print molecule to be extracted from the membrane to create membrane potential and does not have to diffuse through the membrane so that there is no size restriction on the template molecule, the main Achilles heel of MIP's until recently. MIP-based potentiometric sensor for methylphosphonic acid, an ultimate degradation product of nerve agents by coupling surface imprinting technique with a nanoscale transduces indium tin oxide [19]. The literature reveals that the prepared imprinted polymer membranes can be effectively used for the detection of nerve agents by fabricating them into potentiometric sensors [20], [21].

The purpose of this study is to develop a molecularly imprinted polymer composite membrane to identify and recognize the contaminant (endosulfan) selectively in the water. The functions of selective matrix membrane are the identification and remove of selected analyte. In this research, MIPs composite membrane has been synthesized

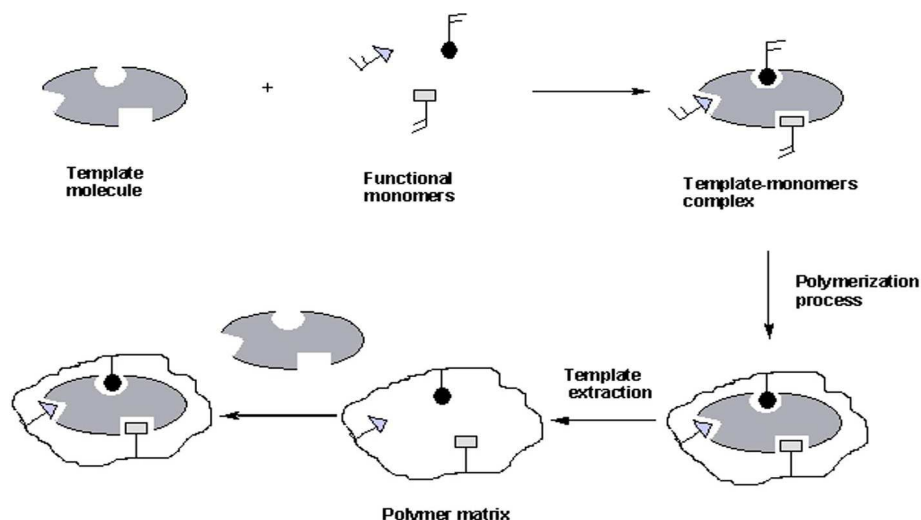


Fig. 2 Scheme of molecular imprinting

and used for sensing and separating the pesticide because of its exclusive stability and easy to adapt using an advanced instrumental technique like UV-Visible spectrophotometer and Scanning Electron Microscopy (SEM).

## II. MATERIAL AND METHOD

### A. Preparation of Endosulfan Template

All the reagents were of analytical grade. The procedure for the synthesis of MIP and NIP polymers as following procedure: Briefly, a template of endosulfan was taken in 0.025 gr endosulfan and 2.1 ml chloroform solvent, 0.525 ml EDMA cross-linker, 0.059 ml MAA functional monomer and 0.05 gr BPO initiator were added to it. The entire components were thoroughly mixed for 15 minutes. Blank polymer without endosulfan was also prepared. After that, the reaction vial was kept at  $-5^{\circ}\text{C}$  in the freezer for 1 hour and kept at  $70\text{-}120^{\circ}\text{C}$  for 150 minutes for complete polymerization.

The polymer was transferred from the vial to mortar and ground. The polymers were collected through acetonitrile solvent filtered and washed using methanol and water (aquabidest) for 1 hour. Remaining residues filtrate was also collected for drying and future use.

A control sensor was prepared in every case following the same procedure but in the absence of template molecule. The control (or non-imprinted polymer-modified electrode) had the same treatment as the imprinted sensor, to ensure that the effect is observed only due to the imprinting features and not to the subsequent treatments undergone by the sensor.

### B. Removal of Template and Confirmation by UV-Visible Spectrophotometer

Specific binding is confirmed by UV-Visible spectrophotometer. The MIPs were washed three times with methanol and also water to remove the print molecules. The imprinted template was eluted with methanol. The removal of the template was also confirmed by using a UV-Visible spectrophotometer.

### C. Characterization of MIP Composite Membrane by Scanning Electron Microscopy (SEM)

Prepared molecular imprinted composite membranes with and without template were categorized by using scanning electron microscopy (SEM, Model: Pro-X) and the micrograph was recorded of all the different samples of the membrane including non-imprinted and MIP composite membranes.

### D. Electrochemical Sensor Preparation

Electrochemical studies were performed using a potentiostat. The general procedure used to prepare the potentiometric sensor based MIP, using aluminium wire (1 mm diameter and 120 mm long) and carbon. The electrodes were polished with fine alumina slurries with a polishing cloth, sonicated in distilled water and dried in air. An unsaturated resin was glued to one end of the above aluminium wire. Endosulfan was dipped into the aluminium wire and added a solution of chloroform on the sensor surface that has been coated with endosulfan.

## III. RESULTS AND DISCUSSION

### A. MIP Endosulfan Characterization

Scanning Electron Microscopy (SEM) study usually used for recognizing the morphology structure and MIP surface because it has an excellent and very high resolution. The morphology surface SEM images of the IP endosulfan template under the magnificent 500X - 1.99KX and 30 resolution are shown in Fig. 3-6. SEM micrograph of MIP at various magnifications which generally shows rough MIP surface with the irregular pores. The globular and porous MIP has higher adsorption capacity toward the target (in this case endosulfan) compared to a NIP, possibly due to the porosity and higher surface area. The specific binding sites created also contribute to the higher adsorption of MIP towards endosulfan.

SEM analysis described both the morphological characteristics of the shape and surface structure of the MIP.



This was important information for the synthesis and applications of MIP.

Fig. 3, 4, 5 and 6 show the SEM micrographs of the MIP composite membrane imprinted by endosulfan template, which was recorded at 500X, 1 KX, 1.5 KX and 1.99 KX magnifications and at a resolution of 30  $\mu\text{m}$ . The surface texture clearly exhibits the cavities on the surfaces and within the pores, which is a clear indication of a change of porous behavior of MIP composite membrane after removal of the template. These things change in porous characteristic behavior in thermodynamical studies. Here were the results of SEM to the MIP obtained [22].

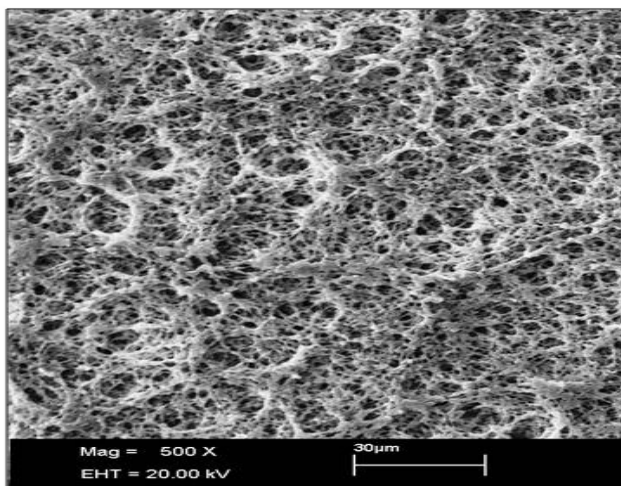


Fig. 3 SEM of the MIP endosulfan template at 500X magnification and 30  $\mu\text{m}$  resolution

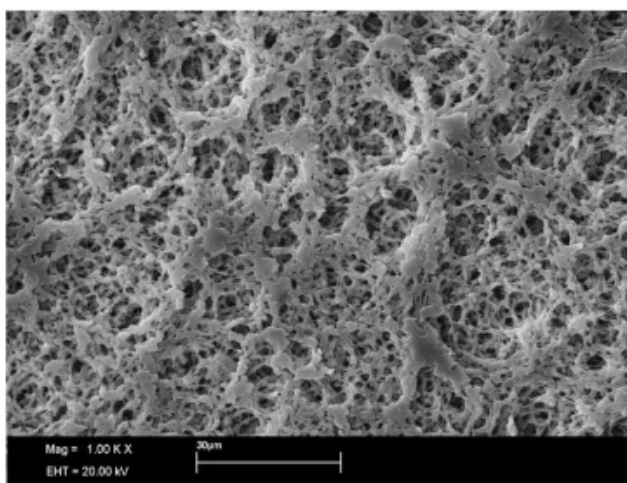


Fig. 4 SEM of the MIP endosulfan template at 1.0 KX magnification and 30  $\mu\text{m}$  resolution

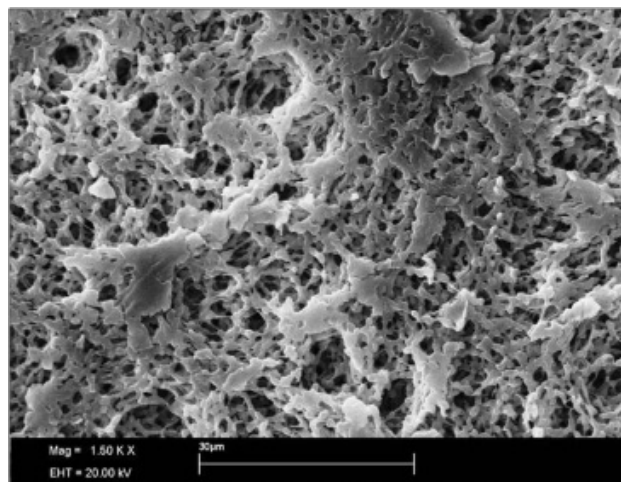


Fig. 5 SEM of the MIP endosulfan template at 1.5KX magnification and 30  $\mu\text{m}$  resolution

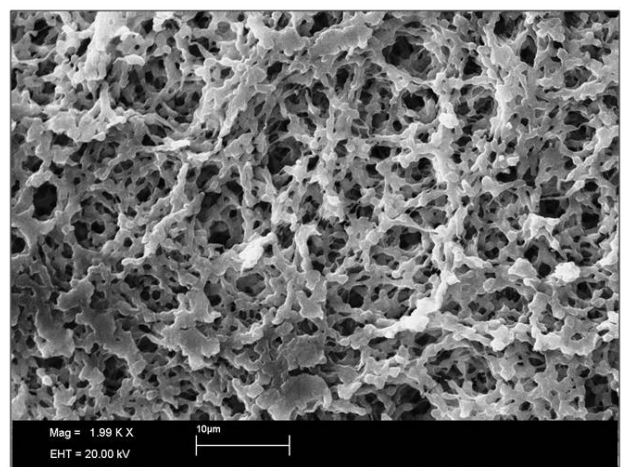


Fig. 6 SEM of the MIP endosulfan template at 1.99 KX magnification and 30  $\mu\text{m}$  resolution

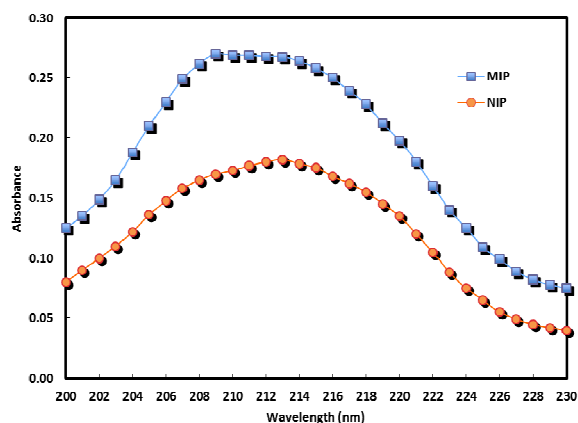


Fig. 7 Endosulfan concentration passed from MIP and NIP

### B. Specific Binding of Endosulfan

MIP has cavities that specific, which can be passed by template because every template has its sensitivity. Endosulfan MIP sensitivity can be measured using the UV-Visible Spectrophotometer and see the elution curve. From the curve, it can be known that MIP has a sensitivity to the template. Fig. 7 shows the graph of absorbance vs. wavelength of endosulfan concentration passed from MIP and non-imprinted polymer.

### C. The Concentration of Endosulfan Bound to the MIP

MIP endosulfan was fabricated with cooling - heating method for a variable heating time (90, 120, and 150 minutes), which includes the initial concentration (target), endosulfan concentrations of free and bound endosulfan concentrations of MIP. The relationship between the concentrations of endosulfan independent of the

concentration of endosulfan bound to the MIP can be seen in Fig. 8.

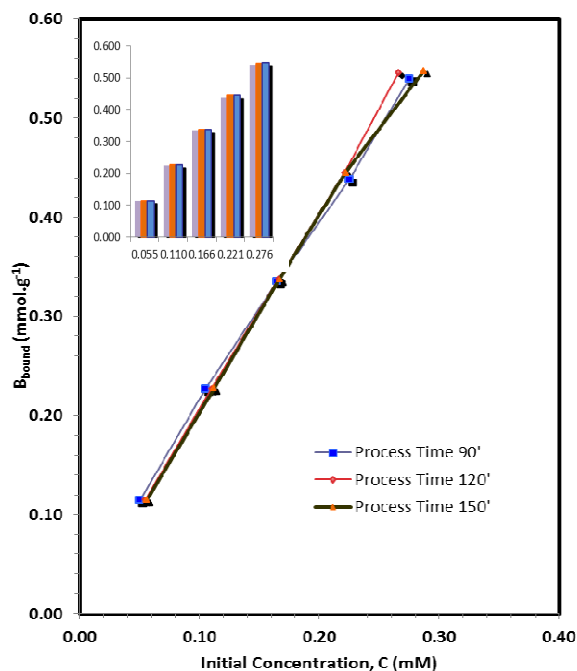


Fig. 8 The relationship between the concentrations of target bounds to the initial concentration of MIP

Furthermore, the concentration of endosulfan independent of the concentration of bound target can be seen in Fig. 8. It was explained that the polymer bound endosulfan concentrations increased with increasing concentrations of endosulfan free. Heating time of 150 minutes produces the least amount of free endosulfan than the heating time of 90 minutes and 120 minutes. This indicates that endosulfan binds to the MIP are most numerous in the old polymer prepared by heating 150 minutes. This result corresponds to the available cavities, where these polymers are more porous than the other polymers.

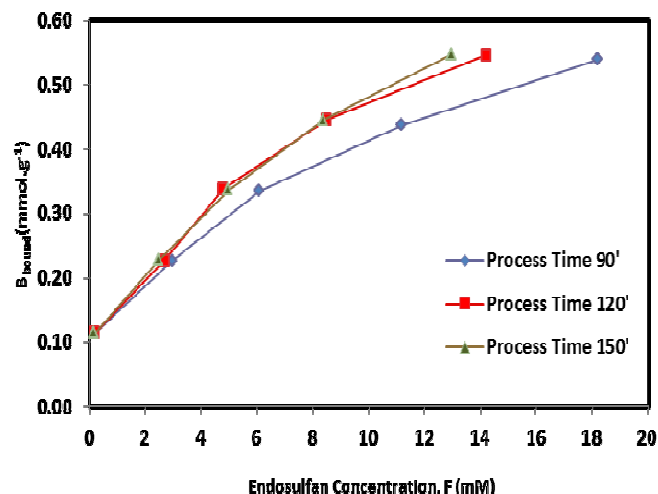


Fig. 9 The relationship between  $B_{bound}$  to the concentration of endosulfan free

From Scatchard Plots, it was found that the maximum amount of targets bounds to the MIPs was 10,03 mmol/gr for heating time 150 minutes, respectively. On the other hand,

the cavities of MIPs were 528 for heating time 150 minutes, as analyzed from the SEM images. These finding confirmed that the heating time of 150 minutes results in the endosulfan MIP with the best endosulfan sensing properties. The relationship between the concentrations of bounds to the initial concentration can be seen in Fig. 10.

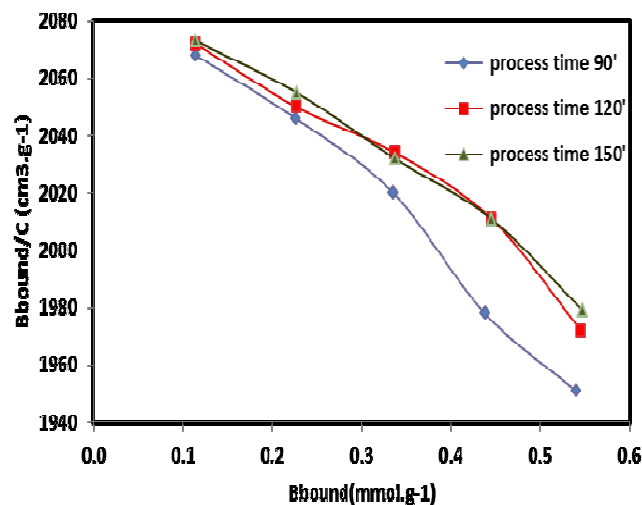


Fig. 10 Relationship  $B_{bound}/C$  to  $B_{bound}$

#### D. Sensor Performance Test

After testing the performance of the sensor by measuring the voltage (volts) on a standard solution with varying concentrations, linearity equation and Nernst factor were obtained.

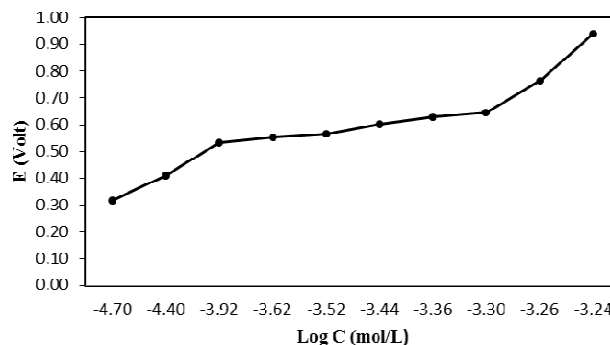


Fig. 11 Graph between MIP endosulfan potential to the target logarithm concentration on the aluminum-carbon sensor on the 1<sup>st</sup> day

Fig. 11 showed that the slope of the function  $E$  versus  $\log$  concentration increased with increasing concentrations of the target value. Based on the graph, linearity equation with three ranges of concentration was obtained. Based on the results of a linear regression on the graph of potential  $E$  (volts) to the logarithm concentration, the obtained parameter values were as in Table 1.

From the measurements performed in the concentration range from 0.02 to 0.58 mM obtained three N slopes with a value of 0.2682 in the concentration range from 0.02 to 0.12 mM, 0.3093 in the concentration range from 0.24 to 0.44 mM, and 4.2808 in the concentration range from 0.50 to 0.58 mM. These sensors had good sensitivity in the concentration range from 0.02 to 0.12 mM as indicated by the linearity value closed to 1,  $R^2 = 0.9977$ .

TABLE I  
THE OBTAINED PARAMETERS OF POTENTIAL PLOT E (VOLT) TO  
THE LOGARITHM CONCENTRATION AT 1ST DAY

Concentration Range	$E = K + N (\text{slope}) \log C$	Z	$R^2$
0.02 – 0.12	$1.5838 + 0,2682 \log C$	0.21626	0.9977
0.24 – 0.44	$1,6656 + 0,3093 \log C$	0.18752	0.9841
0.50 – 0.58	$14.763 + 4,2808 \log C$	0.01355	0.9120

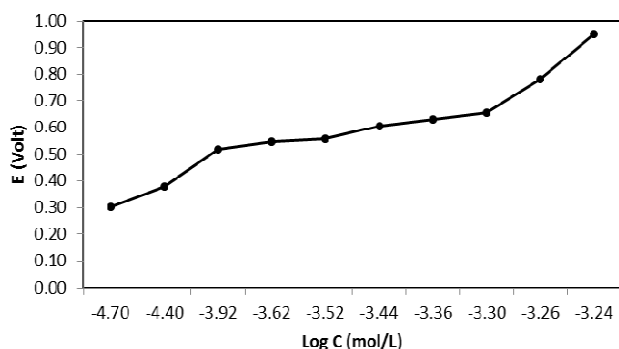


Fig. 12 Graph of MIP potential of endosulfan to the target of logarithm concentration on the aluminum-carbon electrodes on at 20<sup>th</sup> day

Based on the results of a linear regression on a graph of potential E (volt) to the logarithm of the concentration, the obtained parameters were as Table 2.

TABEL II  
OBTAINED PARAMETERS FROM E (VOLT) POTENTIAL PLOT TO  
THE LOGARITHM OF THE CONCENTRATION AT THE 20TH DAY

Concentration Range	$E = K + N (\text{slope}) \log C$	Z	$R^2$
0,02 - 0,12	$1,631 + 0,2837 \log C$	0,20444	0,9993
0,24 - 0,44	$1,7386 + 0,3302 \log C$	0,17565	0,9213
0,5 - 0,58	$14,87 + 4,3094 \log C$	0,01346	0,9330

From the measurements done on the 20<sup>th</sup> day in the concentration range from 0.02 to 0.58 mM N obtained three slopes with a value of 0.2837 in the concentration range from 0.02 to 0.12 mM, 0.3302 in the concentration range of 0.24 -0.44 mM, and 0.3094 in the concentration range 0.50 to 0.58 mM Measurement on the 20<sup>th</sup> day also showed sensors had good sensitivity in the concentration range from 0.02 to 0.12 mM with the linearity of 0.9993.

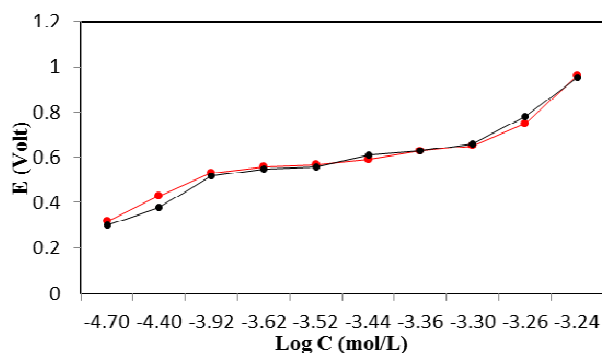


Fig. 13 Graph of stability of sensor the 1<sup>st</sup> and the 20<sup>th</sup> days

Based on Fig. 13, measurements of the 1<sup>st</sup> day and 4<sup>th</sup> were coincided, this indicated that this sensor had good stability.

The limit of detection indicated the lowest concentration of analyte ions addressed by the sensors the lowest measurement limit. Based on the experiment, the detection limit of the aluminum-carbon sensor was 0.02 mM Sensors responded well to a minimum level of 0.02 mM concentration of endosulfan.

#### IV. CONCLUSIONS

Molecularly imprinted polymers were successfully prepared for endosulfan using MAA as functional and EGDMA as cross-linking monomers. The method developed for the determination of residues of endosulfan. The molecularly imprinted polymer matrices specific to endosulfan pesticides were synthesized and used for the fabrication of MIP composite membranes. These MIP composite membranes were further characterized by using UV-Visible and SEM. In recent studies, for the specific recognition of endosulfan pesticide, the molecularly imprinted membrane is fabricated, widely used in controlling insects.

The test results of electrode performance indicated that MIP endosulfan-based aluminum-carbon sensor had a detection limit of 0.02 mM, sensitive in the concentration range from 0.02 to 0.12 mM with Nernst factor > 0.059 V/decade and had good stability.

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