Sensors & Transducers, Vol. 28, Special Issue, April-May 2018, pp. 7-12

Sensors & Transducers

Published by IFSA Publishing, S. L., 2018
http://www.sensorsportal.com

Molecularly Imprinted Polymer Sensing Layer for Tetracycline Chemical Sensor Based on Piezoelectric Quartz Crystal Transducer

*** Benilda S. EBARVIA and Isaiah E. UBANDO**
Department of Science and Technology-Industrial Technology Development Institute
DOST Compound, Gen. Santos Avenue, Bicutan, Taguig City, Metro Manila, 1632, Philippines
* Tel.: 632-837-0032 fax: 632-837-0032
* E-mail: bsebarvia@yahoo.com

Received: 28 March 2018 /Accepted: 28 May 2018 /Published: 31 May 2018

Abstract: Molecularly imprinted polymer (MIP) for tetracycline was prepared via precipitation polymerization at 60°C for 24 h using methacrylic acid as the monomer, trimethylolpropane methacrylate (TRIM) as the crosslinker, 2,2-azobis–isobutyronitrile as initiator, dichloromethane as porogen and tertracycline HCl as template. After template-removal by methanol-acetic acid solvent extraction, the MIP suspension was coated as sensing layer of a piezoelectric quartz crystal transducer. The molecular recognition of tetracycline in the MIP coating was indicated by a corresponding frequency shift of the sensor response. The sensor response was optimized by varying the amount of coating and pH of solution measured. Different parameters like linearity (r=0.9921), sensitivity (41 Hz/ln [conc]), response time (2.4 min), and dynamic range of solutions (1×10^{-6} to 1×10^{-3} µg/mL tetracycline) were obtained. A decrease in sensitivity and selectivity ratio was observed for oxy-tetracycline and chlortetracycline in the selectivity study done. Acceptable % recovery was observed for milk and honey matrices. The developed tetracycline sensor is a possible cheaper methodology for screening and quantitation of low levels of tetracycline in foods.

Keywords: Molecularly imprinted polymer, Tetracycline, Piezoelectric quartz crystal sensor.

1. Introduction

Antibiotics are extensively used to prevent diseases and as growth promoters in food producing animals. Antibiotic resistance is a natural phenomenon generally considered to be a consequence of the wide use – and misuse – of antibiotics [1]. Thus, the presence of these antibiotic residue in foods of animal origin poses health risks to human. A lot of countries worldwide including the UK, US, Canada, and Australia have set maximum residue limits (MRLs) to ensure food safety for consumers. The main concern regarding the ingestion of antibiotic residues is the development of strain resistance to the drug in human. It is an inevitable side-effect of using antibiotics resulting to reduced therapeutic options for patients infected by antibiotic-resistant pathogens as well as in the increased severity of symptoms of the infection [2].

Tetracycline which is one of the emerging contaminants from pharmaceutical industry [3], is present in high concentrations in soil fertilized with animal manure [4]. Due to its widespread use in farm animals, residues of tetracycline and other antibiotics can be found in milk, eggs, butter, cheese, animal tissues and other livestock products.

Some problems in the detection of antibiotic residues include very low level of detection and matrix

interference. Thus, the development of a reagent phase that is highly selective to tetracycline will help improve the selectivity, sensitivity and throughput of an analytical measurement for this antibiotic. One promising materials that can be used for the selective determination of residual antibiotics in food are the molecularly imprinted polymers (MIPs). These are synthetic polymers having a predetermined selectivity for a given analyte or a group of structurally related compounds that make them ideal materials to be used as selective sensing layers and as sorbents in separation processes. They have been used in several analytical methods, including liquid chromatography, capillary electrophoresis and capillary electro-chromatography, solid phase extraction, ligand binding assay, and sensor technology [5]. Several studies have demonstrated the application of MIPs in the determination of food additives, vitamins, nucleotides, pesticides, and drugs like anesthetics and antibiotics [6]. Molecularly imprinted polymer (MIP) offers greater stability, robustness, and enantioselectivity. It is less expensive and easy to prepare.

The selectivity of these MIPs could be integrated so some sensor transducers to meet the drawbacks in achieving very low detection limits at parts per billion ranges. The quartz crystal microbalance is one type of transducer that can detect very small mass change. That could be related to the change in resonant frequency of the quartz. This resulting frequency shift can then be used for the quantitation of the analyte measured.

Currently, there are a few methods capable of measuring residual concentrations of antibiotics at or close to the maximum residue limits in food [7-12]. These methods include high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), conventional spectrophotometry and microbial assay. They require sophisticated and expensive instrumentation as well as highly skilled personnel. In this study, characterization of MIP as sensing layer of a piezoelectric quartz crystal transducer was explored for tetracycline. The determination of tetracycline by this method integration of MIPs as recognition element could be an alternative way of determining very low levels of tetracycline in foods.

2. Experimental

2.1. Materials

All reagents were prepared from analytical reagent grade chemicals. Methacrylic acid (MAA), trimethylolpropane methacrylate (TRIM) and 2,2-azobis (isobutyronitrile) (AIBN), tetracycline HCL were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Polyvinyl Chloride (PVC) was obtained from Fluka Analytical. Tetrahydrofuran (THF) from J.T. Baker was used as received. For the preparation of universal buffer solutions, 0.040 M acetic acid, 0.040 M phosphoric acid, and 0.040 M boric acid were used. The piezoelectric quartz crystal (PQC) used with gold electrode (AT cut, 10 MHz, 5 mm dia.) was purchased from International Crystal Manufacturing, Oklahoma, USA.

2.2. MIP for Tetracycline Synthesis

The molecularly imprinted polymer (MIP) was synthesized by precipitation polymerization described elsewhere [13]. The tetracycline template molecule was dissolved in dichloromethane in a 500 mL screw-capped erlenmeyer flask. The mixture together with methacrylic acid, TRIM and AIBN was sonicated for about 5 minutes followed by purging with nitrogen. The said polymerization was carried out by placing the flasks in a 60 °C water bath with agitation at 120 cycles per minute for 24 hours. After polymerization, dichloromethane was removed by filtration. A non-imprinted polymer (NIP) was prepared in the same way but without the addition of template. The template was then removed from the resulting polymer by Soxhlet extraction with methanol containing 10 % acetic acid (v/v) for 24 h or until no template could be detected. Polymer particles was finally washed with acetone and dried in an air-oven at 60 °C.

2.3. Tetracycline Sensor Assembly and Measurement

The extracted MIP was mixed with the polyvinyl chloride and THF in a 6:2:1 ratio in a small vial. A small amount of this mixture was dropped onto the center of the gold electrode on one side of the quartz crystal, spin-coated for 5 seconds, and allowed to dry, and kept at 25°C. A reference sensor was also prepared by coating one electrode of a quartz crystal with the NIP, using similar method.

The instrumentation set-up consists of an ICM Lever Oscillator (35366-10, 10000 MHz), and a digital frequency counter (Agilent Technologies 53181A, 225 MHz) interfaced to a computer. The MIP-coated quartz was encased in a Teflon flow cell such that only the coated electrode was exposed to the measurand solution. A Masterflex C/L (Cole Parmer) pump, was used to deliver the solution to the flow cell.

A stopped-flow technique was adopted in the measurements. The blank solution was run through the flow cell at a constant flow rate of 1.2 mL per minute. The flow is stopped, and the oscillation frequency of the quartz crystal was monitored until a steady frequency reading (F_a) was achieved. Then, the tetracycline standard solution was allowed to flow through the cell for about 1 to 2 minutes. After the flow is stopped, the frequency was read until it remains constant as the (F_b) and this value was recorded. The frequency shift (Eq. 1) for each concentration was calculated as the sensor response:

$$\Delta F = F_b - F_a \quad (1)$$

3. Results and Discussion

3.1. Sensor Response

The sensor response was optimized by using different parameters such as thickness of coating as sensing layer of the sensor and pH of tetracycline solution. Curing time of about two 2 hours was used in all sensor preparation as this does not affect the sensor performance [13]. The amount of MIP as a function of volume of the coating deposited onto the transducer's surface affects the sensor performance. Two volumes (10 µL and 20 µL) of the MIP suspension in PVC-THF (1:6:2 v/v) were tried as sensing layer (Fig. 1). Sensor response for 10 µL MIP coating exhibited a sensitivity of about 15 Hz/ln [conc]. Very good linearity for the sensor (r= 0.9965) and a dynamic linear range of 1×10^{-6} to 1 µg/mL tetracycline were observed.

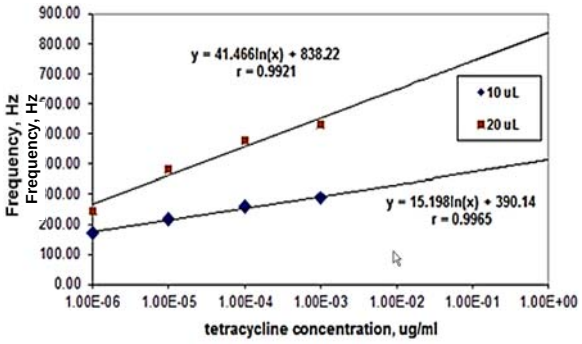


Fig. 1. Effect of amount of MIP coating on sensor response.

On the other hand, for the 20 µL MIP coating (Fig. 2) the dynamic range observed for the sensor was from 1×10^{-6} to 1×10^{-3} µg/mL tetracycline. When the range of the concentration was increased up to 1 µg/mL the response of the sensor appeared to be constant. This could be due to the saturation of the available binding sites in the sensing layer of the sensor when exposed to > 1×10^{-3} µg/mL tetracycline and thus additional analyte in the solution resulted to damping of sensor response and could no longer be recognized. More than twice sensitivity, (m = 41 Hz/ln [conc] was achieved. Very good linearity, (r = 0.9921) of the sensor response was observed (1×10^{-6} to 1×10^{-3} µg/mL tetracycline) using this quartz crystal microbalance transducer. The optimized coating with higher sensitivity and better linearity was using the 20 µL coating. The response time of the tetracycline sensor using 20 µL coating of MIP was t_{95}=2.4 min as presented in Fig. 3.

The regeneration of the sensing layer of the senor for about 2 - 3 times was executed. This was done by allowing the flow of diluted acetic acid (10 %) to release the bounded tetracycline on the surface of the MIP layer. An increase in the frequency reading of the sensor could be observed indicating the release of the tetracycline from the MIP layer. The process was followed with washing with water until a stable response was attained. Furthermore, the used quartz crystal could be cleaned using piranha solution for several times. The cleaned quartz crystal can then be coated again. The lifetime of the quartz crystal can be estimated to be good up to three times usage or until the frequency response for the said crystal is still stable. Checking of the frequency response of the bare quartz crystal is always done before deposition of the sensing layer by spin coating was performed.

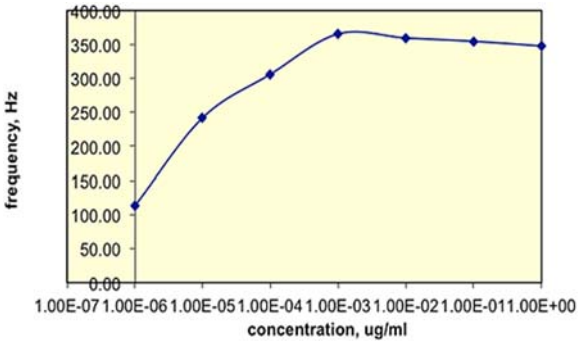


Fig. 2. Calibration graph for the sensor using 20 µL of MIP coating.

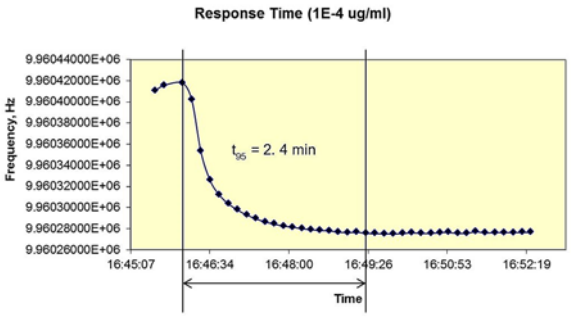


Fig. 3. Response time of the tetracycline sensor using 1×10^{-4} µg/mL tetracycline standard solution.

3.2. Effect of pH of Solution in Sensor Response

The effect of pH on the response of the sensor was also undertaken in this study. A universal buffer was prepared by dissolving about 2.30 mL acetic acid, 2.4732 g boric acid and 3.92 g phosphoric acid in one liter of water. This solution was adjusted to different pH (4-12) by adding diluted NaOH. Then different pH solutions of 1×10^{-3} µg/mL tetracycline were prepared and measured. A low response was observed from pH 4 - pH 5 (Fig. 4) and it gradually increase and seem to slightly flatten in alkaline solution up to pH 11. Result of measurements of the sensor response to these

different solutions revealed a high response from pH 7-9. Adjustment to this condition could be required in measurement of samples. Too basic solution (pH 12) could damage the quartz crystal and not recommended for sample measurement.

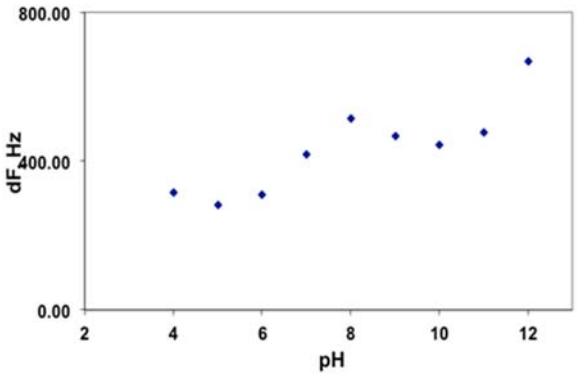
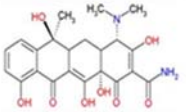
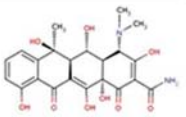
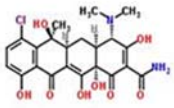


Fig. 4. Effect of pH on sensor response.

3.3. Selectivity Study

The selectivity of the sensor was investigated using test solutions of pure compounds of other antibiotics that are structurally related to tetracycline like oxy-tetracycline and chlortetracycline as shown in Table 1.

Table 1. Comparison of Structures of compounds tested for the selectivity study using the developed sensor.

Compound	Sensitivity index, m_i	Linearity, r
tetracycline 	1	0.9921
oxytetracycline 	0.32	0.8193
chlortetracycline 	0.46	0.8759

Different concentrations of these compounds were tested using the developed sensor. A decrease in sensitivity index or the selectivity ratio ($m_{tetracycline}/m_{intererence}$) was observed for these compounds as compared to the sensitivity using tetracycline standard solutions. Likewise, the linearity, r of the sensor also decreased when exposed to oxy-tetracycline and chlortetracycline standard solutions. A plot of the sensitivities of the sensor to these compounds is presented in Fig. 5. This observation also support the preference of the MIP synthesized with the same template to tetracycline and have better selectivity.

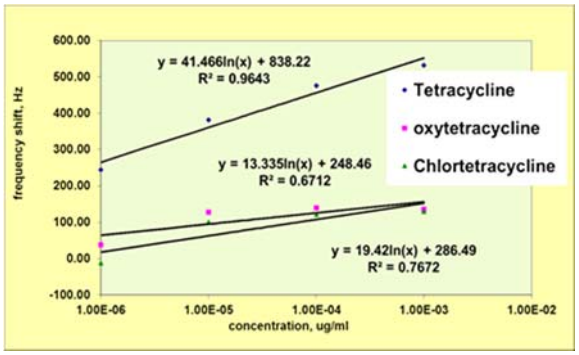


Fig. 5. Selectivity study for the tetracycline sensor.

The molecular recognition capability of the MIP sensing layer in combination with high sensitivity of the quartz crystal transducer contributes to the high selectivity of the sensor to tetracycline. The proposed rebinding of tetracycline to the binding sites of the MIP sensing layer is shown in Fig. 6. The rebinding of the tetracycline can be facilitated in a similar way the imprinting was carried out through non-covalent hydrogen-bonding interactions [14] between carboxyl (-COOH) group of MAA and oxygen (=O) and hydroxyl (-OH) group of tetracycline. Electrostatic interactions are also likely taking place between the amine groups (-NH_2 and -$N(CH_3)_2$) of tetracycline and carboxyl group of MAA.

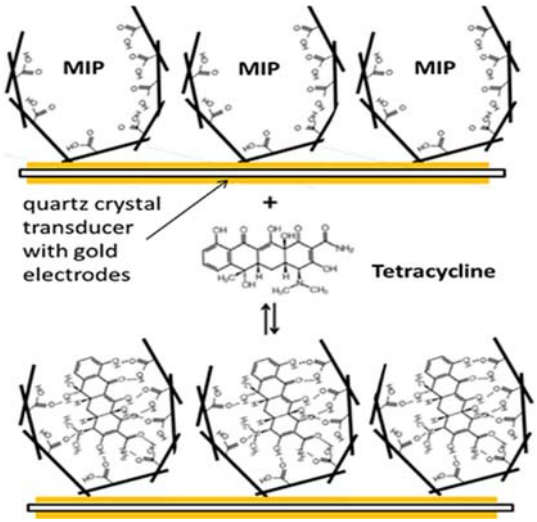


Fig. 6. Highly selective rebinding of tetracycline with MIP modified quartz crystal transducer.

3.4. Performance Characteristics of the Tetracycline Sensor

The average limit of detection for three replicates is calculated as 3×10^{-8} µg/mL based on 3x standard deviation of the blank. The limit of quantitation (10 × LOD) was 3×10^{-7} µg/mL. The repeatability

(rsd = 12, n=3) and dynamic range 1×10^{-6} to 1×10^{-3} µg/mL were achieved for the sensor.

3.5. Application of the Sensor to Real Sample

The performance of the sensor was validated using different sample matrix by standard addition technique. This technique was often recommended to correct the matrix effect on the measurements. The sensor was applied to quantitatively determine spiked tetracycline (1×10^{-4} µg/mL tetracycline) food matrices like milk and honey. A diluted 1 mL of milk sample was used. The recovery obtained for milk and honey spiked samples were 106.5 % and 83.5 % respectively. These recovery rates are acceptable according to the guidelines for recoveries as set by the Association of Official Analytical Chemists [15].

4. Conclusions

The study demonstrated the feasibility of integrating molecularly imprinted polymer for tetracycline prepared by precipitation polymerization and the quartz crystal microbalance transducer. The sensor response attributed to the molecular recognition capability to tetracycline of the prepared MIP was observed using the highly sensitive quartz crystal sensor. As sensing layer to the said transducer, the sensor response was affected by the amount of MIP coating and pH change. The observed performance characteristics of the sensor are very good linearity, high sensitivity and selectivity and wide dynamic range. Acceptable % recovery was achieved using this sensor for food matrices like milk and honey. The sensor for tetracycline with MIP coating is a promising alternative inexpensive approach possibly for screening and quantitation of low levels of tetracycline in foods.

Acknowledgements

The financial support by the National Research Council of the Philippines of the Department of Science and Technology (NRCP-DOST) is gratefully acknowledged for this work.

References

[1]. W. T. M. Jansen, J. T. van der Bruggen, J. Verhoef, A. C. Flui, Bacterial resistance: A sensitive issue Complexity of the challenge and containment strategy in Europe, *Drug Resistance Updates*, Vol. 9, Issue 3, 2006, pp. 123-133.

[2]. M. P. Doyle, M. C. Erickson, Emerging microbiological food safety issues related to meat, *Meat Science*, Vol. 74, Issue 1, 2006, pp. 98-112.

[3]. S. Babic, A. Horvat, M. Kastelan-Macan, D. Pavlovic, Sample Preparation in analysis of pharmaceuticals, *Trends in Analytical Chemistry*, Vol. 26, Issue 11, 2007, pp. 1062-1075.

[4]. G. Hamscher, H. Hoper, H. Nau, S. Sczesny, Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry, *Analytical Chemistry*, Vol. 74, Issue 7, 2002, pp. 1509-1518.

[5]. L. I. Andersson, Molecular imprinting: developments and applications in the analytical chemistry field *Journal of Chromatography B: Biomedical Sciences and Applications*, Vol. 745, Issue 1, 2000, pp. 3-13.

[6]. O. Bruggemann, K. Haupt, L. Ye, E. Yilmaz, K. Mosbach, New configurations and applications of molecularly imprinted polymers, *Journal of Chromatography A*, Vol. 889, Issue 1-2, 2000, pp. 15-24.

[7]. C. Blasco, Y. Picó, C. M. Torres, Progress in analysis of residual antibacterials in food, *TrAC Trends in Analytical Chemistry*, Vol. 26, Issue 9, 2007, pp. 895-913.

[8]. L. Nozal, L. Arce, A. Ríos, M. Valcárcel, Development of a screening method for analytical control of antibiotic residues by micellar electrokinetic capillary chromatography, *Analytica Chimica Acta*, Vol. 523, Issue 1, 2004, pp. 21-28.

[9]. F. I. Cetinkaya, A. Yibar, G. E. Soyutemiz, B. Okutan, A. Ozcan, M. Y. Karaca, Determination of tetracycline residues in chicken meat by liquid chromatography-tandem mass spectrometry, *Food Addit Contam Part B Surveill*, Vol. 5, Issue 1, 2012, pp. 45-49.

[10]. N. Alavi, A. A. Babaei, M. Shirmardi, A. Naimabadi, G. Goudarzi, Assessment of oxytetracycline and tetracycline antibiotics in manure samples in different cities of Khuzestan Province, Iran, *Environmental Science and Pollution Research*, Vol. 22, Issue 22, 2015, pp. 17948-17954.

[11]. A. Pena, C. M. Lino, R. Alonso, D Barcelo, Determination of Tetracycline Antibiotic Residues in Edible Swine Tissues by Liquid Chromatography with Spectrofluorometric Detection and Confirmation by Mass Spectrometry, *Journal of Agricultural and Food Chemistry*, Vol. 55, Issue 13, 2007, pp. 4973-4979.

[12]. M. E. Lindsey, M. Meyer, E. M. Thurman, Analysis of Trace Levels of Sulfonamide and Tetracycline Antimicrobials in Groundwater and Surface Water Using Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry, *Anal. Chem.*, Vol. 73, Issue 19, 2001, pp. 4640-4646.

[13]. B. S. Ebarvia, I. E. Ubando, F. B. Sevilla III, Biomimetic Piezoelectric Quartz Crystal Sensor with Chloramphenicol-Imprinted Polymer Sensing Layer, *Talanta*, Vol. 144, 2015, pp. 1260-1265.

[14]. B. S. Ebarvia, C. A. Binag, F. B. Sevilla III, Biomimetic Piezoelectric Quartz Sensor for Caffeine Based on a Molecularly Imprinted Polymer, *Analytical and Bioanalytical Chemistry*, Vol. 378, Issue 5, 2004, pp. 1331-1337.

[15]. AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, 2002, pp. 1-38. https://www.aoac.org/aoac_prod_imis/AOAC_Docs/StandardsDevelopment/SLV_Guidelines_Dietary_Supplements.pdf (accessed 05.05.18).

AMSTERDAM
SEIA'18

4th International Conference on Sensors and Electronic Instrumentation Advances

19-21 September 2018
Amsterdam, The Netherlands

Call for Papers

http://www.seia-conference.com/

Venue

SEIA' 2018 will take place in Amsterdam, The Netherlans, 19-21 September 2018

Conference Hotel

Mövenpick Hotel Amsterdam City Centre
Piet Heinkade 11, 1019 BR Amsterdam, The Netherlands

One event - three publications !

1. All registered abstracts will be published in the conference proceedings.

2. Selected extended papers will be published in Sensors & Transducers journal.

3. The best full-page papers will be recommended to extend to book chapters for 'Advances in Sensors: Reviews' Book Series.

Social Programme

20 September 2018 (18:00-22:00). The Gala Dinner will take place in the Amsterdam Dinner Train - only train restaurant in Western Europe. The Amsterdam Dinner Train takes our participants through the so called 'Green Heart' of the Netherlands, while savouring a gala dinner. Departing from Amsterdam Central station in front of the 'royal waiting area' on platform 2B, we will ride through Breukelen, Gouda, Rotterdam noord, Den Haag HS, Leiden and Haarlem. Back to Amsterdam central at the end (21:45). We welcome our guests 15 minutes prior to the tour's departure at 19:15.

Organizing Committee and Contacts

Chairmen:

Prof., Dr. Yurish, Sergey (IFSA, Spain),
e-mail: SYurish@sensorsportal.com
Dr. Daneshmand Malayeri, Amin (Asia-SAME, UAE)
e-mail: amin.daneshmand87@gmail.com

Advisory Chairmen

Prof. Gennaro Conte (University Roma Tre, Italy)
Dr. Pavel Shuk (Emerson Process Management, USA)
Dr. Marius Gheorghe (Ideal Aerosmith Inc., USA)
Dr. Paolo Dabove (Politecnico di Torino, Italy)

Conference and Publication Manager

Tetyana Zakharchenko (IFSA Publishing, S.L., Spain)

Sponsors:

IFSA Publishing · Axcelera · MY DEAR DRONE

Media Partners:

sensors · chemosensors · micromachines