Advanced Materials Research Vol. 896 (2014) pp 89-94
© (2014) Trans Tech Publications, Switzerland
doi:10.4028/www.scientific.net/AMR.896.89

Effect of Heating Time on Atrazine-based MIP Materials Synthesized via the Cooling-heating Method

Idha Royani[1,3,a], Widayani[2,b], Mikrajuddin Abdullah[1,c], Khairurrijal[1,d*]

[1]Physics of Electronic Materials Research Division,

[2]Nuclear Physics and Biophysics Research Division,

Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Indonesia

[3]Dept. of Physics, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indonesia

[a]royaniidha@yahoo.co.id, [b]widayani@fi.itb.ac.id, [c]din@fi.itb.ac.id, [d]* E-mail: krijal@fi.itb.ac.id

Keywords: molecular imprinting, atrazine, cavity, template, Scatchard plot.

Abstract. Molecular imprinting is a technique to produce a polymer called as molecularly imprinted polymer (MIP) that provides cavities to form a particular space generated by removing the template when the polymer has been formed. It will recognize a target that has the shape and physico-chemical properties similar or identical with those of template molecule. In this study, MIPs using atrazine as template have been made via the cooling-heating method. Initially the pre-polymer solution was cooled at a refrigerator for 1 h. Next, the polymerization was carried out at 70 °C for heating times of 90, 120, and 150 min. without nitrogen flow which is generally done for polymerization process. Characterizations were performed by employing a reversed-phase high performance liquid chromatograph (HPLC) and scanning electron microscope (SEM). From Scatchard plots, it was found that the equilibrium dissociation constant K_D and the apparent maximum number of binding sites B_{max}, which are written as (K_D, B_{max}), are (4.69 μM, 9.87 mmol/g), (4.54 μM, 9.56 mmol/g) and (3.52 μM, 7.44 mmol/g) for the heating times of 150, 120, and 90 min., respectively. This is verified by their SEM images showing that the broadest pore size distribution with the highest number of pores is in the MIP prepared under the heating time of 150 min. The MIPs therefore could be applied as an atrazine sensor and the MIP prepared under the heating time of 150 min. would give its best characteristics compared to the others.

Introduction

Molecular imprinting technique is a technique to produce polymer having cavities with a shape according to the template used. The polymer made by imprinting technique can be used as an active component of a sensor that will recognize other molecules that have same or similar physico-chemical properties to that of the template molecules [1,2]. Because of the sensing properties owned by these polymers, a molecularly imprinted polymer (MIP) is possible to be applied in many fields, such as synthesis protein and catalysts [3, 4], chromatography [5,6], solid-phase extraction [7,8], food analysis [9,10], as well as health [11]. For example, MIP can be used in a sensor to detect the presence of morphine.

Atrazine is included in the triazine group which is harmful toxins contained in herbicides. These herbicides are used to control weeds in crops such bullies corn, sorghum, sugarcane, and others. Although widely used, triazines are known to be harmful to animals and humans. The use of herbicides is tightly regulated in the EU, because it can cause health problems. Triazines are banned from use in most European countries. European legislation allows a maximum of 0.1 mg/L in drinking water [12]. More recently, a MIP sensor has been made to detect the presence of atrazine pesticides [13, 14]. This sensor showed good response. In this study, an atrazine-based MIP material has been prepared for application as sensors. But not as commonly done by previous researchers, in which they drain nitrogen in the polymerization process [15-18], in this study, pre-polymerization solution was not immersed in nitrogen flow, but it was cooled in a refrigerator [19] and heated in an oven to form a solid polymer. Characterization results showed that this method has

good opportunity to use in MIP synthesis procedure. In this paper, we report the study and discuss the obtained results thoroughly.

Materials and Methods

Chemicals. Atrazine pestanal (molar mass of 215.68 g/mol), methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), benzoyl peroxide (BPO), methanol, acetonitrile, chloroform, acetic acid, and aquabidest. All the chemicals were obtained from Sigma Aldrich.

Methods. The picture given in Fig. 1 shows the phases in synthesis of atrazine-based MIP, starting from dissolving atrazine as the template, MAA as the monomer, EDMA as cross-linker, and the last BPO as an initiator into the solvent ($CHCl_3$). The solution was stirred for 15 min., before it was cooled in a refrigerator for 1 h. and heated in an oven at 70 °C for 90, 120, and 150 min. After the polymer was formed, then by using consecutive solvents (acetonitrile, acetic acid, and aquabidest), the template molecules were removed so that the remaining polymer (a MIP) contains cavities which have similar shape with the template. When target/guest molecules, which have similar structure and properties with those of the template, are exposed to the MIP, the MIP will recognize and be able to coordinate/bind the target/guest effectively.

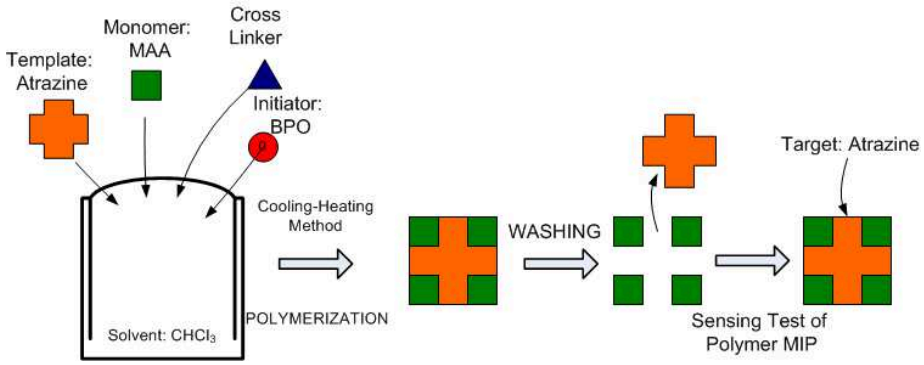


Fig. 1. The phases in synthesis of atrazine-based MIP and testing its sensing characteristic.

From the description, the steps to make an atrazine-based MIP and to test the sensing characteristic of the MIP are as follows.

Polymerization Process. Atrazine as template (0.025 g), MAA as the functional monomer (0.059 mL), EDMA as cross-linker (0.525 mL), benzoyl peroxide (BPO) as initiator (0.5 g) were put into 2.1 mL of chloroform ($CHCl_3$) under constant stirring for 15 min. Different with other researchers, we did not flow nitrogen to the pre-polymerization solution. Then, the pre-polymerization solution was poured into 3 vials and then we closed the vials. These vials were put in a refrigerator for 1 h., and finally they were put in an oven at temperature of 70 °C for 90, 120, and 150 min. After heating, the translucent solid polymer is produced.

Template Removal Process. The obtained solid polymer was crushed into fine powder. The powder obtained via synthesis using three different heating times were put into three different vials. The polymer powder was then soaked in acetonitrile for 24 h. to release the bound between the template and MIP. Other procedures can also be carried out. The polymer can be washed in methanol /acetic acid (0.625 mL/12.5 mL), methanol / aquabidest (6375 mL/12.5 mL), each for 1 h., and next the powder was soaked in methanol (3 mL) for 48 h. Then, free template atrazine polymer particles were collected and dried.

Sensing Test. Firstly, atrazine standard solution of 5000 ppm as stock solution was prepared by weighing atrazine (50 mg) and dissolving it in 10 mL of methanol. A series of diluted stock solution was made by diluting 0.25, 0.5, 0.75, 1.0, and 1.25 mL of the stock solution with 5 mL of methanol/acetic acid with the ratio of 1:1. The series are 250, 500, 750, 1000, and 1250 ppm.

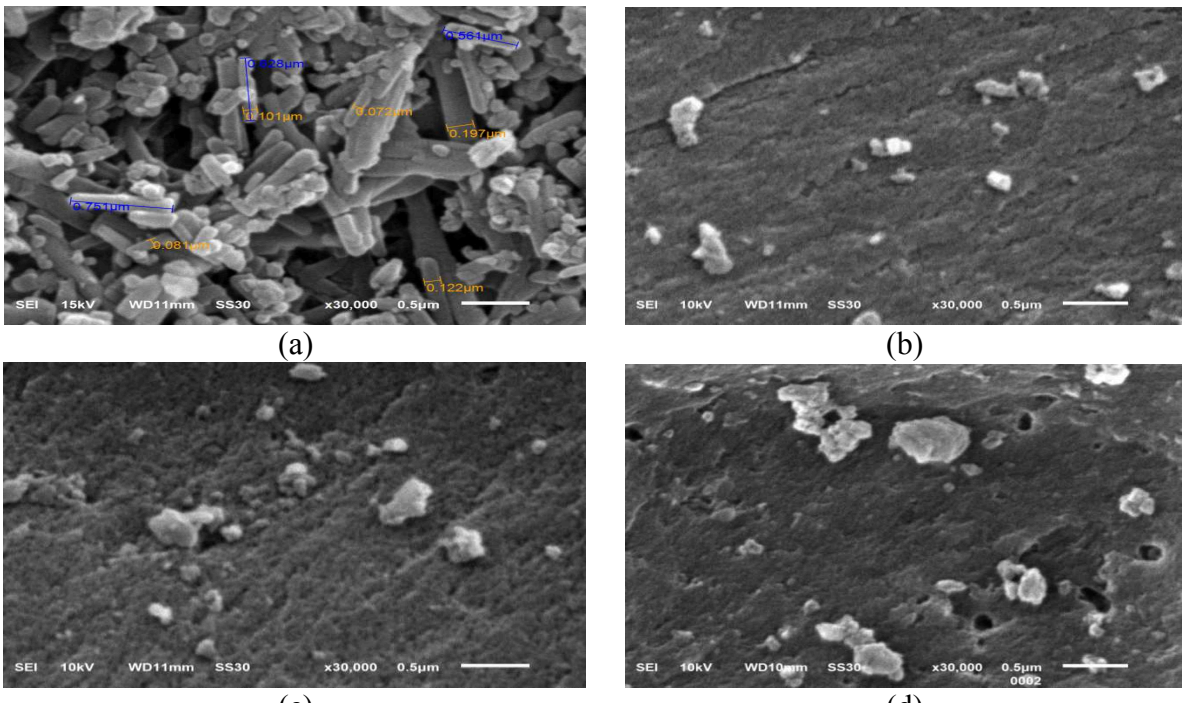
Next, the MIP particles with certain mass (m) were put in 6 sample tubes and 1 mL of the diluted stock solution was added into the sample tubes. To obtain the initial concentration (C) of atrazine, 5 mL of chloroform, 5 mL of acetic acid, and 10 mL of methanol were added into these mixtures. Thus, a series of 0.055, 0.110, 0.116, 0.221, and 0.276 mM initial concentrations were obtained. The sample tubes were then stirred in for 12 h., put in a centrifuge for 15 min. to precipitate the polymer, and filtered with 45-micron filter papers to collect the polymer. This polymer may contain bound atrazine.

Subsequently, the concentration of free atrazine in the remaining solution, which is called as R_{remain}, was determined by a reversed-phase high performance liquid chromatograph (HPLC). The difference in values of C and R_{remain} is therefore the number of guests bound to the MIP particles, which is called as B_{bound}. For all measurements, the injection volume was 50 µL, the mobile phase was 0.01 M of acetic acid in acetonitrile (50:50), the flow rate was 1 mL/min and the detection employed UV light at 260 nm.

Results and Discussion

Characterization of Polymer Surfaces. The vials containing pre-polymerization solution were put in a refrigerator for 1 h. This procedure is to slow down the reaction between compounds existing in the solution with oxygen. Next, the vials were heated in the oven at temperature 70 °C for 90, 120, and 150 min. This heating process is done to help speed up the evaporation process that accelerates the formation of solid polymer.

After the template is removed, the MIP containing cavities is formed. It is expected that the MIP can recognize target molecules that have the same or similar properties with those of the template molecules. The obtained MIP was characterized using a scanning electron microscope (SEM). The SEM images show the difference between atrazine standard and atrazine-based MIPs, as shown in Fig. 2.



(a) (b)

(c) (d)

Fig. 2. SEM images of (a) atrazine standard and atrazine-based MIPs produced by heating times of (b) 90, (c) 120, and (d) 150 min.

It can be seen that the cavities left by the template in the MIPs dispersed almost evenly. Each cavity generally has a size in the range of 80-230 nm such as owned by atrazine particles (see Fig. 2. (a)). Pore size distribution of the MIPs can be seen in Fig. 3. By increasing heating time, the pore size distribution becomes broader and the number of pores that have a size in the same range with

atrazine particles also increases. The MIP obtained using the heating time of 150 min. shows the broadest pore size distribution with the highest number of pores. So it is expected that the capability of this MIP to capture the target molecules is the highest compared to those prepared under the heating times of 90 and 120 min.

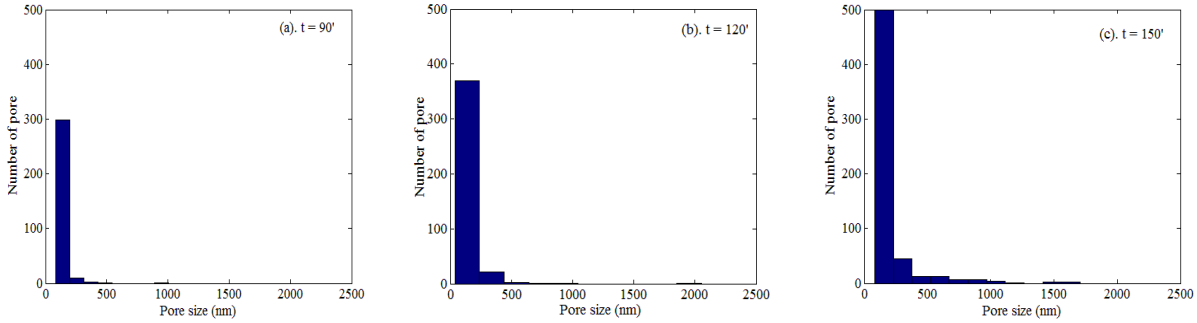


Fig. 3. Histograms of pore size distribution of atrazine-based MIPs produced by the heating times of (a) 90, (b) 120, and (c) 150 min.

The number of pores of MIPs was obtained from SEM images with the same size (253 x 337 mm^2). The total number of pores of the MIPs produced by the heating times of 90, 120, and 150 min. are 299, 370, and 499, respectively. Thus the highest number of pores is in the MIP obtained under the heating time of 150 min. These results indicate that heating time significantly influences properties of the obtained MIPs. Furthermore, these results will be correlated with the characterization results using a HPLC.

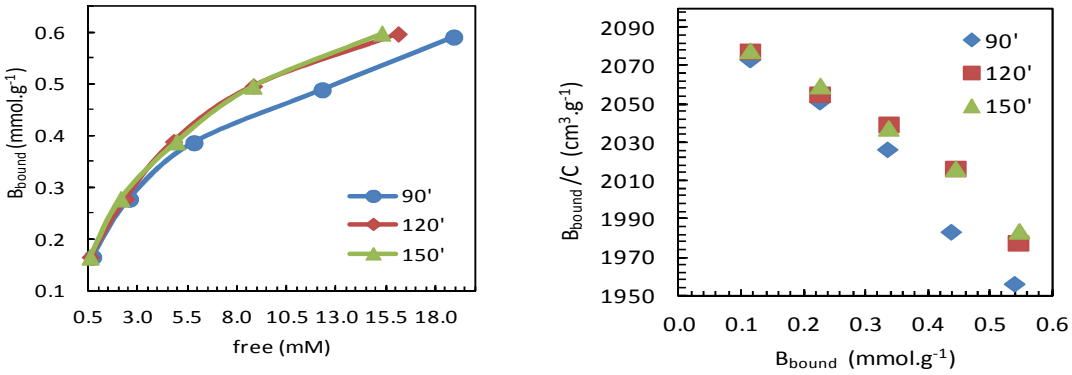
Concentration of atrazine bound to MIP. Table 1 shows the measurement variables including free atrazine concentration and the obtained concentration of atrazine bound to the MIP for different heating times and initial concentration of atrazine.

Table 1. Initial concentrations of atrazine along with their measured free atrazine concentrations and atrazine bound to the MIPs for different heating times.

Heating time (minute)	Initial concentration of atrazine, C(mM)	Atrazine free (mM)	B_{bound} (mmol/g)	B_{bound}/C (cm^3/g)
90	0.055	0.0007	0.1144	2073
	0.110	0.0026	0.2264	2051
	0.166	0.0058	0.3355	2026
	0.221	0.0123	0.4379	1983
	0.276	0.0189	0.5399	1956
120	0.055	0.0006	0.1147	2077
	0.110	0.0024	0.2269	2055
	0.166	0.0048	0.3377	2039
	0.221	0.0088	0.4452	2016
	0.276	0.0161	0.5457	1977
150	0.055	0.0006	0.1147	2078
	0.110	0.0021	0.2274	2060
	0.166	0.0049	0.3374	2037
	0.221	0.0088	0.4452	2016
	0.276	0.0153	0.5475	1984

The curves in Fig. 4.(a), which were obtained from the data in Table 1, show that the amount of atrazine bound to the cavities of the MIPs increases with increasing the concentration of free atrazine for all heating times. The curves for the heating times of 120 and 150 min. are almost similar, while that for the heating time of 90 min. is slightly lower. It means that the average number of free atrazine (not bound to the MIP) in the MIP prepared under the heating time of 150

min. is less than those in the MIP did under the heating times of 90 and 120 min for the same concentration of atrazine bound to the MIP. Thus it is expected that the MIP prepared under the heating time of 150 min. has higher number of cavities. By having more cavities, this MIP will be able to catch or bind targets better.



(a) (b)

Fig. 4. Binding isotherm or saturation binding curves. (a) B_{bound} of atrazine against free concentration and (b) Scatchard plots between bound concentration/ initial concentration against bound concentration.

To determine the apparent maximum number of binding sites and the equilibrium binding constant, the data in Table 1 is re-plotted as a Scatchard plot according to Eq. (1).

$$\frac{B_{bound}}{C} = -\frac{1}{K_D} B_{bound} + \frac{B_{\max}}{K_D} \quad (1)$$

where B_{bound} is the concentration of atrazine bound to the MIP, C is the guest initial concentration of atrazine, B_{max} is the apparent maximum number of binding sites, and K_D is the equilibrium dissociation constant. The K_d value is a parameter that indicates the dimensions of concentration. If the value is smaller than the initial concentration of atrazine, shows a strong and selective guest binding. By using the Scatchard plot, the obtained values of (K_D, B_{max}) are (4.69 µM, 9.87 mmol/g), (4.54 µM, 9.56 mmol/g) and (3.52 µM, 7.44 mmol/g) for the heating times of 150, 120, and 90 min., respectively (Fig. 4.(b). Since the obtained values of K_D are lower than the highest guest initial concentration, the guests are strongly and selectively bound to the MIPs. For the MIP prepared under the heating time of 150 minutes has the highest B_{max}. This indicates that the number of guests bound to its cavities is also the largest.

Conclusion

A series of atrazine-based MIPs has been synthesized via the cooling-heating method, i.e. cooling the pre-polymer solution for 1 h. followed by heating at 70 ^{o}C with the heating times of 90, 120, and 150 min. The MIPs have been characterized using an SEM and HPLC. SEM images showed that the MIP prepared under the heating time of 150 min. has the broadest pore size distribution with the highest number of pores. From the HPLC test, it was shown that the MIP prepared under the heating time of 150 min. has the greatest value of apparent maximum number of binding sites namely 9.87 mmol/g. It means that the MIP has the most number of targets bound to the MIP compared to those prepared under the heating times of 90 and 120 min. Therefore, the MIP prepared using the heating time of 150 min. produces the best characteristics for sensor applications compared to the others.

Acknowledgment

This work was financially supported by Competitive Research Grant from Directorate General of Higher Education of the Ministry of Education and Culture through Sriwijaya University.

References

[1] K. D. Shimizu, Binding Isotherms, in: M. Yan and O. Ramstrom, Molecularly Imprinted Materials: Science and Technology, Marcel Dekker, New York, USA, 2005, pp. 419–434.
[2] S. Scorrano, L. Mergola, R. D. Sole, and G. Vasapollo, Synthesis of Molecularly Imprinted Polymers for Amino Acid Derivates by Using Different Functional Monomers, Int. J. Mol. Sci. 12, 3 (2011) 1735–1743.
[3] A. Bossi, F. Bonini, A. P. F. Turner, and S. A. Piletsky, Molecularly Imprinted Polymers for the Recognition of Proteins: the State of the Art, Biosens. Bioelectron. 22, 6 (2007) 1131 – 1137.
[4] G. Wulff, Enzyme-like Catalysis by Molecularly Imprinted Polymers, Chem. Rev. 102, 1 (2002) 1–28.
[5] L. I. Andersson, A. Paprica, and T. A. Arvidsson, A Highly Selective Solid Phase Extraction Sorbent for Pre-concentration of Sameridine Made by Molecular Imprinting, Chromatographia. 46, 1-2 (1997) 57 – 62.
[6] S. Wei and B. Mizaikoff, Molecularly Imprinted Polymers: Present and Future Prospective, J. Sep. Sci. 30, 11 (2007) 1794-1805.
[7] O. Ramström and K. Mosbach, Synthesis and Catalysis by Molecularly Imprinted Materials, Curr. Opin. Chem. Biol. 3, 6 (1999) 759-764.
[8] C. Baggiani, L. Anfossi, and C. Giovannoli, Solid Phase Extraction of Food Contaminants Using Molecular Imprinted Polymers, Anal. Chim. 59, 1 (2007) 29 – 39.
[9] I. Kubo, R. Shoji, Y. Fuchiwaki, and H. Suzuki, Atrazine Sensing Chip Based on Molecularly Imprinted Polymer Layer, Electrochemistry. 76, 8 (2008) 541-544.
[10] T. Kitade, K. Kitamura, T. Konishi, S. Takegami, T. Okuno, M. Ishikawa, M. Wakabavashi, K. Nishikawa, and Y. Muramatsu, Potentiometric Immunosensor Using Artificial Antibody Based on Molecularly Imprinted Polymers, Anal. Chem. 76, 22 (2004) 6802–6807.
[11] G. Vasapollo, R. D. Sole, L. Mergola, M. R. Lazzoi, A. Scardino, S. Scorrano, and G. Mele, Molecularly Imprinted Polymers: Present and Future Prospective, Int. J. Mol. Sci. 12, 9 (2011) 5908-5945.
[12] L. Chimuka, M. V. Pinxteren, J. Billing, E. Yilmaz, and J. Å. Jönsson, Selective Extraction of Triazine Herbicides Based on a Combination of Membrane Assisted Solvent Extraction and Molecularly Imprinted Solid Phase Extraction, J. Chromatogr. A, 1218, 5 (2011) 647- 653.
[13] C. Malitesta, M. R. Guascito, E. Mazzotta, and R. A. Picca, X-Ray Photoelectron Spectroscopy Characterization of Electrosynthesized Poly(3-thiophene acetic acid) and Its Application in Molecularly Imprinted Polymers for Atrazine, Thin Solid Films. 518, 14 (2010) 3705.
[14] S. G. Derouich, M. N. Nguyen, A. Madani, N. Maouche, P. Lang, C. Perruchot, and M. M. Chehimi, Aryl Diazonium Salt Surface Chemistry and ATRP for the Preparation of Molecularly Imprinted Polymer Grafts on Gold Substrates, Surf. Interface Anal. 42, 6-7 (2010) 1050-1056.
[15] M. S. Tehrani, M. T. Vardini, P. A. Azar, and S. W. Husain, Molecularly Imprinted Polymer Based PVC-Membrane-Coated Graphite Electrode for the Determination of Metroprolol, Int. J. Electrochem. Sci. 5, 1 (2010) 88 - 104.
[16] H. Zeng, Y. Wang, C. Nie, J. Kong, and X. Liu, Preparation of Magnetic Molecularly Imprinted Polymers for Separating Rutin from Chinese Medicinal Plants, Analyst. 137, 10 (2012) 2503- 2512.
[17] S. Scorrano, L. Mergola, R. D. Sole, and G. Vasapollo, Synthesis of Molecularly Imprinted Polymers for Amino Acid Derivates by Using Different Functional Monomers, Int. J. Mol. Sci. 12, 3 (2011) 1735 - 1743.
[18] K. Balamurugana, K. Gokulakrishnan, and T. Prakasama, Preparation and Evaluation of Molecularly Imprinted Polymer Liquid Chromatography Column for the Separation of Chathine Enantiomers, Saudi Pharm. J. 20, 1 (2012) 53-61.
[19] I. Royani, Widayani, M. Abdullah, and Khairurrijal: Characterization of an Atrazine Molecularly Imprinted Polymer Prepared by a Cooling Method. Int. Conf. Math. Nat. Sci. (Bandung, Indonesia, 8-9 Nov. 2012).