

# **Synthesis and Evaluation of Molecularly Imprinted Polymer As a Selective Material for Vanillin**

**Thitiporn Pengkamta<sup>1</sup>, Molthicha Mala<sup>1</sup>, Chanapa Klakasikit<sup>1</sup>, Patcharawan  
Kanawuttikorn<sup>1</sup>, Pornanan Boonkorn<sup>2</sup>, Angkana Chuajedton<sup>2</sup>, Weeranuch  
Karuehanon<sup>1,\*</sup>**

<sup>1</sup>Chemistry Program and Center of Excellence for Innovation in Chemistry, Faculty of Science, Lampang Rajabhat University,  
Lampang 52100, Thailand

<sup>2</sup>Biology Program, Faculty of Science, Lampang Rajabhat University, Lampang 52100, Thailand

Corresponding author e-mail: \* [w.karuehanon@lpru.ac.th](mailto:w.karuehanon@lpru.ac.th)

Received: 18 December 2019 / Revised: 31 December 2019 / Accepted: 7 January 2020

## **Abstract**

Molecularly imprinted polymers (MIPs) using vanillin (Val) as a template molecule were synthesized and evaluated. MIPs were prepared by precipitation polymerization using various types of functional monomers with using ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM) as cross linkers and polymerization was done at 80°C for 6 h. The binding efficiency of polymers was evaluated including types of monomers, binding time and reusability. Results showed that methacrylic acid was the selective monomer for vanillin and the highest performance of MIPs, which including the binding efficiency and selectivity, was obtained when bound polymer with vanillin for only 2 h and could be reused as least five times.

**Keywords:** Vanillin, Molecularly imprinted polymer, Selective material

## **1. Introduction**

Molecularly imprinted polymers (MIPs) are a very attractive materials in various fields, including separation (Hong & Chen, 2013), purification (Mokgadi, Batlokwa, Mosepele, Obuseng, & Torto, 2013), sensors (Lee, Oh, Kim, & Jang, 2015), pharmaceutical (Diouf, Motia, El Alami El Hassani, El Bari, & Bouchikhi, 2017) and catalysts (Wulff, 2002). The composition of this materials are template molecule (or target molecule), functional monomer, cross-linker and initiator. Generally, the preparation of MIPs include 3 steps which are self-assembly, polymerization and template removal (Mayes & Whitcombe, 2005). Firstly, self-assembly was done by interacted template molecule with functional monomer which can be covalent or non-covalent interaction then cross-linker and initiator are added for polymerization reaction. After polymerization, polymer which contains template molecule can be obtained. Finally, to create imprinted polymer, the template is removed from polymer using suitable solvent extraction method to leave the cavity of template inside the polymer which exhibited high template recognition affinity.

Vanillin (Val) is the valuable compound in food industry which provide as an ingredient in wide range such as food flavor, beverage, pharmaceutical and perfume (Esposito et al., 1997). This compound can be originally obtained from a seed of vanilla which is a very unique and highly prize compound due to its various applications (Mohamad Ibrahim, Sipaut, & Mohamad Yusof, 2009). Other natural source of vanillin is extracted from softwood lignin by catalytic decomposition (Borges da Silva et al., 2009) and recrystallization method which are very expensive process and high amount of impurities will be occurred (Wu et al., 2015). Solid-phase extraction (SPE) with solid-sorbent such as C18 or ion-exchange resin has generally applied for purification of interested compounds. However, the high cost sorbent materials with cannot be reused and non-selective extraction and have been observed (Xu, Yang, & Liu, 2014), therefore, high selective materials with low cost production and reusable are still promising.

In present study, we have synthesized and evaluated molecularly imprinted polymer selective to vanillin (MIP-Val) with various types of functional monomers and cross-linkers. The binding

study with template molecule was applied to indicate the efficiency of MIP-Val and the effect of types of polymers, binding time and reusability were investigated.

## 2. Materials and Methods

### 2.1 Synthesis of molecularly imprinted polymer for vanillin (MIP-Val)

All polymers were synthesized according to the literature report (Karuehanon, Wongthep, & Wangnorn, 2018). For MIPs-Val synthesis, in 150 ml round-bottom flask, vanillin 0.25 mmol and functional monomer 1 mmol were added and dissolved in 25 ml of acetonitrile. After incubated the solution for 15 min, 5 mmol of cross-linker was added following by 0.125 mmol of benzoyl peroxide and 25 ml of acetonitrile, solution was then stirred at room temperature for 5 min before bubbled with N<sub>2</sub> gas for 15 min. The precipitation polymerization was carried out at 80°C for 6 h and washed the obtained polymer with acetonitrile. Template extracting step was done by stirring synthesized polymers in 100 ml off methanol:acetic acid solution (8:2 v/v) for 2 h x 5 times until no trace of vanillin was observed by UV-Visible spectrophotometer ( $\lambda_{\max}=274$  nm) then washed with distilled water until pH of washing solution was neutral following by acetonitrile and acetone before dried in hot air oven at 80°C, overnight. Non-Imprinted polymers (NIPs) were synthesized using the same procedure with MIPs in the absence of vanillin. The yield of synthesized MIPs was calculated according to equation (1).

$$\%Yield_{MIP} = \frac{W_{MIP}}{W_m + W_c} \times 100 \quad (1)$$

When

$W_{MIP}$  is weight of synthesized MIP after template removal (mg)

$W_m$  is weight of functional monomer used in synthesis (mg)

$W_c$  is weight of cross-linker used in synthesis (mg)

### 2.2 Binding study of MIP-Val

The binding study of synthesized MIP-Val and its corresponding NIPs was done by re-binding polymers with vanillin solution (template molecule). In 1.5 ml of micro centrifuge tube, 5 mg of polymer and 1 ml of vanillin in concentration of 10 ppm were added then carefully close the cap and wrap with parafilm. All prepared tubes were then incubated

with the shaking incubator at room temperature with investigated binding time then centrifuged at 6,000 rpm for 5 min. The supernatant was then separated to another micro centrifuge tube and the absorbance of vanillin was determined using UV-Visible spectrophotometer at  $\lambda_{\max}=274$  nm. The concentration of vanillin in supernatant was then calculated from calibration curve of vanillin ( $y=0.0385x+0.0325$ ,  $R^2=1$ ). %Bound and imprinting factor ( $\alpha$ ) of polymer was calculated following equation (2) and (3) which indicated efficiency of re-binding and selectivity of polymer (Karuehanon, Lee, Nimmanpipug, Tayapiwatana, & Pattarawarapan, 2009), respectively.

$$\%Bound = \frac{Q_0 - Q}{Q_0} \times 100 \quad (2)$$

When

$Q_0$  is initial concentration of vanillin before binding with polymer (ppm)

$Q$  is concentration of vanillin in supernatant after binding with polymer (ppm)

$$\alpha = \frac{\%Bound_{MIP}}{\%Bound_{NIP}} \quad (3)$$

When

$\%Bound_{MIP}$  is %Bound of MIP

$\%Bound_{NIP}$  is %Bound of corresponding NIP

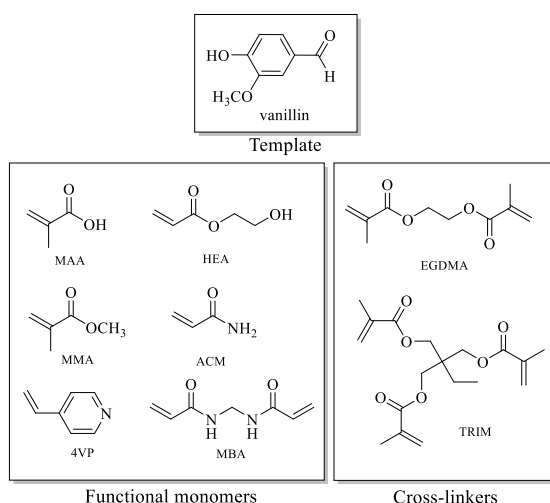
### 2.3 Reusability of MIP-Val

The reusability of MIP was observed with the selected polymer which showed the highest performance of binding study. Vanillin solution (10 ppm) was added to selected MIP in ratio of MIP:vanillin as 5 mg : 1 ml. Firstly, the binding study was done as described method with 50 mg of MIP in 10 ml of vanillin solution for the first cycle. After analyzed the supernatant by UV-Visible spectrophotometer, the left MIP was then washed with 100 ml of methanol:acetic acid solution (8:2 v/v) for 2 h x 5 times to remove vanillin from MIP and washed with water, acetonitrile and acetone, respectively, before dried by hot air oven. The prepared MIP was then weighted as 40 mg for second cycle of binding study with 8 ml of vanillin solution (as previously ratio of MIP:vanillin as 5 mg : 1 ml) due to prevent the loss of MIP during template removal and washing step which recovered less than 50 mg. And the reusability was continued to fifth cycle with using 30, 20 and 10 mg of MIP with 6, 4 and 2 ml of vanillin solution, respectively

### 3. Results and Discussions

#### 3.1 Synthesis of MIPs

Six types of functional monomer, including methacrylic acid (MAA), 2-hydroxyethyl acrylate (HEA), methyl methacrylate (MMA), acrylamide (ACM), 4-vinylpyridine (4VP) and N,N-methylene bis acrylamide (MBA), with two types of cross-linkers; ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM), were used for synthesis (Figure 1). All MIPs using vanillin as a template molecule and their corresponding NIPs were synthesized by precipitation polymerization method. All polymers were obtained as white powder and the %yield was showed in Table 1.



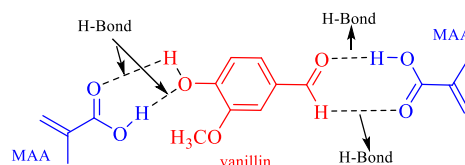
**Figure 1.** Structure of vanillin, functional monomers and cross-linkers used in this study.

From results showed in Table 1., %Yield of synthesized polymer was in the range of 17.63 – 90.50% for using EGDMA as cross-linkers (entry 1 – 6) and 57.67 – 98.88% which less than when using TRIM as a cross-linkers (entry 7 – 12). Since TRIM has three branch chain containing three vinyl group ( $-\text{CH}=\text{CH}_2$ ) in molecule, this can be favorable structure for the polymerization more than EGDMA which contains only two vinyl groups in structure. For considering the effect of types of functional monomers to the yield of polymer, three types of functional monomers including acidic (MAA), neutral (HEA and MMA) and basic (ACM, 4VP and MBA) were applied in this study. The lowest yield of MIPs prepared by using 4VP, strong organic base monomer, were observed in both of cross-linkers (entry 5, 11). This could be due to the weak interaction between vanillin and nitrogen atom in structure of vinylpyridine. On the other hand, acidic monomer as MAA (entry 1, 7) exhibited the highest

%yield of MIPs in both of cross-linkers, since MAA which contains carboxylic group ( $-\text{COOH}$ ) has demonstrated H-bonding interaction with template molecule among others monomers (Figure 2) (Mohamad Ibrahim et al., 2009). Moreover, it was found that most of prepared NIPs were achieved in higher yield than MIPs. This could be caused by the synthetic process which NIPs could be prepared by only polymerization step while the synthesis of MIPs initiated by self-assembly step before polymerization, which binding interaction between monomer and template was the major effect to the completion of polymerization reaction.

**Table 1.** Synthesis of MIPs.

Entry	Functional monomer	Cross-linker	%Yield	
			MIP	NIP
1	MAA	EGDMA	65.17	67.68
2	HEA	EGDMA	38.29	90.50
3	MMA	EGDMA	38.30	69.43
4	ACM	EGDMA	46.41	83.13
5	4VP	EGDMA	17.63	85.08
6	MBA	EGDMA	53.61	88.45
7	MAA	TRIM	98.88	94.10
8	HEA	TRIM	73.96	93.42
9	MMA	TRIM	97.40	80.13
10	ACM	TRIM	87.34	57.67
11	4VP	TRIM	64.86	78.25
12	MBA	TRIM	80.05	88.16



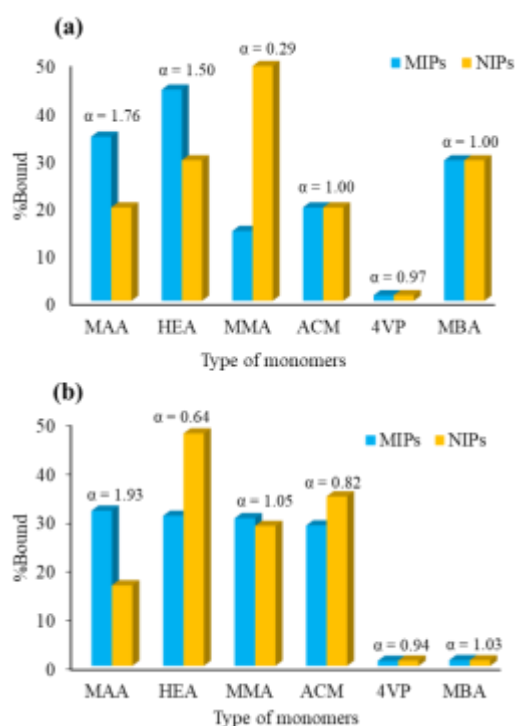
**Figure 2.** Binding interaction between vanillin and MAA.

#### 3.2 Binding study of MIPs

The efficiency of MIPs was investigated by binding study which re-bound vanillin to MIPs and their corresponding NIPs. %Bound and imprinting factor ( $\alpha$ ) indicated the binding affinity and selectivity of MIPs which calculated as previously describe. Firstly, 12 types of synthesized polymer according to Table 1 were investigated with using 5 mg polymers bound with 1 ml of vanillin solution (10 ppm), for 24 h at room temperature. Results of binding study were shown in Figure 3 (a) and Figure 3 (b) for using EGDMA and TRIM as cross-linkers, respectively. Surprisingly, non-selective binding was observed in the most of polymers that could be

shown by the low value of imprinting factor ( $\alpha$  nearly or less than 1) which indicated that NIPs showed higher binding affinity than MIPs, since

NIPs were synthesized by the absence of vanillin and had no cavity inside polymer. However, MIPs synthesized by MAA in both of cross-linkers showed good binding efficiency to template molecule with imprinting factor value as 1.76 (%Bound = 34.45) and 1.93 (%Bound = 31.78) for EGDMA and TRIM, respectively. This would be caused by the strong binding interaction between the cavities inside MIPs with functionalized by carboxylic acid of MAA as shown in Figure 2, therefore, these two types of MIPs and their corresponding NIPs were selected for further study.



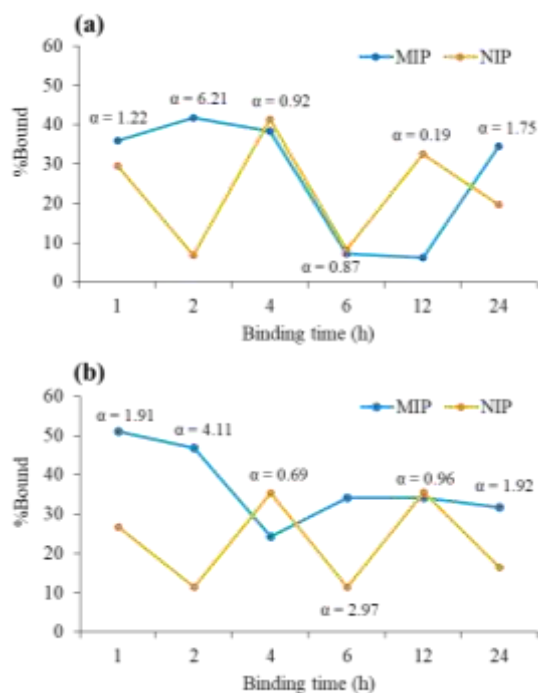
**Figure 3.** Binding affinity of polymers synthesized by using (a) EGDMA and (b) TRIM as a cross-linker.

### 3.3 Effect of binding time

Two types of polymer using MAA as a functional monomer with EGDMA and TRIM as a cross-linkers were then selected to study the effect of binding time. The binding experiments were also done by previously procedure with varied incubation time as 1, 2, 4, 6, 12 and 24 h at room temperature. Results in Figure 4 showed the uncertainly %Bound of NIPs when increasing of time which could be explained by non-specific binding, the binding interaction was only occurred at the surface of NIPs

(Kantarovich, Belmont, Haupt, Bar, & Gheber, 2009). For MIPs, high %Bound was observed from both of polymers from 1 h of binding time then slightly decreased when increasing of time before increased and stabled. This binding behavior attributed by the rebinding of template at the cavities created from self-assembly process and binding equilibrium (Ansell, 2015). Nevertheless, very high imprinting factor value as 6.21 (41.87%Bound) and 4.11 (46.88%Bound) were showed in both MIPs with binding time as 2 h. Although for MAA-TRIM at 1h (Figure 4 (b)), the highest %Bound of MIP (51.06%) was obtained but imprinting factor was two times lower than at 2 h. Therefore, as the moderate affinity in which of both %Bound and imprinting factor value, MIPs using MAA-EDMA and MAA-TRIM synthesis system showed the highest performance when bound with template for 2 h and this protocol was selected to study the reusability of MIPs.

**Figure 4.** The effect of binding time of MIP-Val



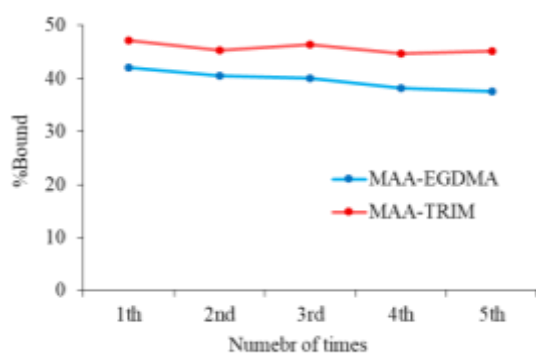
synthesized by (a) MAA-EGDMA and (b) MAA-TRIM.

### 3.4 Reusability of MIPs

The reusability of MAA-EGDMA and MAA-TRIM were studied in five cycles for binding with

10 ppm of vanillin for 2 h and results showed in Figure 5. The ratio between MIP:vanillin solution was 5 mg:1 ml and all MIPs were scaled-up to 50 mg in the first cycle to prevent the losing of MIPs during study in five cycles. It could be seen that MAA-TRIM showed higher %Bound than MAA-EGDMA and %Bound of MIPs were slightly decreased when continuously reused from first cycle to fifth cycles which was from 42.10% to 37.56% for MAA-EGDMA and 47.22% to 45.12% for MAA-TRIM, respectively. However, these MIPs could be reused at least five times with less than 5% of %Bound decreasing.

**Figure 5.** Reusability of MAA-EGDMA and MAA-TRIM



#### 4. Conclusion

Molecularly imprinted polymers (MIPs) selective to vanillin were synthesized using various types of functional monomer and cross-linkers by precipitation polymerization. The binding efficiency of synthesized MIPs were evaluated with various effects including types of polymers, binding time and reusability. Methacrylic acid (MMA) was the suitable functional monomer for synthesis of MIPs using vanillin as a template molecule. Both of ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM) could be used as cross-linkers to achieve the highest performance of MIPs with high binding affinity and high selectivity at 2 h of binding time. Moreover, the studied MIPs could be reused at least five times with moderate affinity compare with first time usage.

#### 5. Acknowledgement

The authors would like to thank Center of Excellence for Innovation in Chemistry (PERCH-CIC) for providing facilities. The financial and

facilities support from Faculty of Science, Lampang Rajabhat University is also gratefully appreciated.

#### 6. Publication Ethic

Submitted manuscripts must not have been previously published by or be under review by another print or online journal or source

#### 7. References

- Ansell, R. J. (2015). Characterization of the binding properties of molecularly imprinted polymers. *Advances in Biochemical Engineering/Biotechnology*, 150, 51-93.
- Borges da Silva, E. A., Zabkova, M., Araujo, J. D., Cateto, C. A., Barreiro, M. F., Belgacem, M. N., & Rodrigues, A. E. (2009). An integrated process to produce vanillin and lignin-based polyurethanes from Kraft lignin. *Chemical Engineering Research and Design*, 87(9), 1276-1292.
- Diouf, A., Motia, S., El Alami El Hassani, N., El Bari, N., & Bouchikhi, B. (2017). Development and characterization of an electrochemical biosensor for creatinine detection in human urine based on functional molecularly imprinted polymer. *Journal of Electroanalytical Chemistry*, 788, 44-53.
- Esposito, L. J., Formanek, K., Kientz, G., Mauger, F., Maureaux, V., Robert, G., & Truchet, F. (1997). Vanillin. In *Kirk-Othmer Encyclopedia of chemical technology*. New Jersey: John Wiley & Sons.
- Hong, Y., & Chen, L. (2013). Extraction of quercetin from *Herba Lysimachiae* by molecularly imprinted-matrix solid phase dispersion. *Journal of Chromatography B*, 941, 38-44.
- Kantarovich, K., Belmont, A. S., Haupt, K., Bar, I., & Gheber, L. A. (2009). Detection of template binding to molecularly imprinted polymers by Raman microspectroscopy. *Applied Physics Letters*, 94, 194103.
- Karuehanon, W., Lee, V. S., Nimmanpipug, P., Tayapiwatana, C., & Pattarawarapan, M. (2009). Synthesis of molecularly imprinted polymers for nevirapine by dummy template imprinting approach. *Chromatographia*, 70, 1531-1537.
- Karuehanon, W., Wongthep, T., & Wanggnorn, C. (2018). Synthesis of molecularly imprinted polymers for extraction of quercetin from mulberry leaves. *Prawarun Agricultural Journal*, 15, 96-103.
- Lee, J. S., Oh, J., Kim, S. G., & Jang, J. (2015). Highly sensitive and selective field-effect-transistor nonenzyme dopamine sensors

- based on Pt/conducting polymer hybrid nanoparticles. *Small*, *11*, 2399-2406.
- Mayes, A. G., & Whitcombe, M. J. (2005). Synthetic strategies for the generation of molecularly imprinted organic polymers. *Advanced Drug Delivery Reviews*, *57*(12), 1742-1778.
- Mohamad Ibrahim, M. N., Sipaut, C. S., & Mohamad Yusof, N. N. (2009). Purification of vanillin by a molecular imprinting polymer technique. *Separation and Purification Technology*, *66*, 450-456.
- Mokgadi, J., Batlokwa, S., Mosepele, K., Obuseng, V., & Torto, N. (2013). Pressurized hot water extraction coupled to molecularly imprinted polymers for simultaneous extraction and clean-up of pesticides residues in edible and medicinal plants of the Okavango Delta, Botswana. *Molecular Imprinting*, *1*, 55-64.
- Wu, J., Yang, Z., Chen, N., Zhu, W., Hong, J., Huang, C., & Zhou, X. (2015). Vanillin-molecularly targeted extraction of stir bar based on magnetic field induced self-assembly of multifunctional Fe<sub>3</sub>O<sub>4</sub>@ Polyaniline nanoparticles for detection of vanilla-flavor enhancers in infant milk powders. *Journal of Colloid and Interface Science*, *442*, 22-29.
- Wulff, G. (2002). Enzyme-like catalysis by molecularly imprinted polymers. *Chemical Reviews*, *102*, 1-27.
- Xu, Z., Yang, Z., & Liu, Z. (2014). Development of dual-templates molecularly imprinted stir bar sorptive extraction and its application for the analysis of environmental estrogens in water and plastic samples. *Journal of Chromatography A*, *1358*, 52-59.