

Recent studies of synthetic antibody-based 3-MCPD determination technology

Penelitian terkini dari teknologi deteksi 3-MCPD berbasis antibodi sintesis

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Abstrak

3-kloro-1,2-propanadiol (3-MCPD) diklasifikasikan oleh International Agency for Research on Cancer sebagai bahan bersifat karsinogen dan menjadi salah satu permintaan dari Uni Eropa yang mensyaratkan tingkat maksimum konsentrasi dari 3-MCPD dalam minyak sawit hingga 2,5 ppm. Meskipun metode GCMS dan HPLC-FLD yang dilaporkan menunjukkan sensitifitas dan selektifitas yang tinggi pada pengukuran 3-MCPD, semua metode tersebut membutuhkan bahan kimia yang banyak dan proses pengerjaan dengan waktu yang lama untuk preparasi dan analisis sampel. Molecularly Imprinted Polymer (MIP) atau antibodi sintetik bisa digunakan untuk mengenali 3-MCPD. MIP lebih stabil dalam kondisi suhu dan pH yang ekstrim. Artikel ini akan membahas tentang pemanfaatan MIP pada ekstraksi sampel dan analisis sampel untuk mendeteksi 3-MCPD. MIP disintesis melalui polimerisasi monomer-monomer yang bergugus fungsi di sekitar 3-MCPD sebagai targetnya. Kemudian, 3-MCPD diekstrak dari MIP dengan meninggalkan sisi aktif. Oleh karena itu, sisi-sisi aktif inilah yang dapat mengikat kembali 3-MCPD baik dengan ikatan kovalen maupun non kovalen. Simulasi komputer dan eksperimen dapat menginvestigasi komposisi dari MIP. MIP dapat diproduksi menjadi kolom ekstraksi berbasis MIP (MIPSPE) dan sensor berbasis MIP. Kedua produk tersebut menunjukkan parameter analitik yang signifikan, yaitu nilai recovery lebih dari 90% dan limit deteksi kurang dari 2,5 ppm. Berdasarkan hasil kajian ini, penggunaan MIP dapat fleksibel, digunakan baik untuk ekstraksi maupun analisis sampel dalam penentuan 3-MCPD. Teknologi berbasis MIP ini akan menjadi instrument yang prospektif untuk mendeteksi 3-MCPD. Produksi MIP dalam skala industri akan menjadi sebuah tantangan dalam memonitor tingkat konsentrasi 3-MCPD dalam produk minyak sawit.

[Kata kunci: 3-MCPD, molecularly imprinted polymer (MIP), kolom ekstraksi berbasis MIP, sensor berbasis MIP]

Abstract

3-Chloro-1,2-propanediol (3-MCPD) is classified by the International Agency for Research on Cancer as carcinogenic material. 3-MCPD will also become one of the European Union's requirements, proposing the maximum level of the 3-MCPD in palm oil until 2.5 ppm. Although the reported technologies GCMS and HPLC-FLD demonstrated high sensitivity and selectivity on 3-MCPD determination, those technologies invest in chemical and time-consuming sample preparation and analysis. Molecularly imprinted polymer (MIP), or a synthetic antibody, can be used to recognize 3-MCPD. MIP is more robust under extreme environments such as temperature and pH. This paper, therefore, aims to discuss the application of MIP on sample extraction and analysis to detect 3-MCPD. MIP is synthesized by polymerization of functional monomers surrounding 3-MCPD as a template. 3-MCPD is then removed from the MIP, leaving active cavities. Thus, these sites can either covalently or non-covalently rebind to 3-MCPD. Computational or empirical studies could investigate the composition of MIP. MIP can be manufactured as MIP-based solid phase extraction (MIPSPE) and MIP-based sensor. Both applications showed significant analytical parameters, such as recovery higher than 90% and detection limit lower than 2.5 ppm. Therefore, the application of MIP can be flexible for sample preparation and analysis on the 3-MCPD determination. MIP-based technology would be a prospective instrument to detect 3-MCPD. In the future, producing MIP on an industrial scale will be a challenge to monitor the 3-MCPD level in palm oil.

[Keywords: 3-MCPD, molecularly imprinted polymer (MIP), MIP-based SPE, MIP-based sensor]

Introduction

3-chloro-1,2-propanediol (3-MCPD) is one of the critical substances for palm oil exporters, particularly in Indonesia. This material will be one of the palm oil requirements for export trade proposed by the European Commission (Suwastoyo, 2020). At this moment, the valid regulation for 3-MCPD is only focussing on hydrolyzed vegetable protein and soy sauce with a maximum level of 0.02 ppm (EU, 2018). Surprisingly, the new regulation for 3-MCPD conferred in palm oil up to 2.5 ppm has recently been proposed by the European Commission. Although this regulation is still being discussed, it is necessary to develop innovative technology for 3-MCPD control.

The incidence of 3-MCPD was first reported in 1984 by Cerbulis and friends (Cerbulis *et al.*, 1984). Fatty acid ester and bound 3-MCPD were found in goat's milk. Free and bound 3-MCPDs have also been identified and reported by Zelinková *et al.* (2006) in several edible oils such as virgin seed oils, virgin olive oils, virgin germ oils, refined seed oils, and refined olive oils from several European countries such as France, Italy, Germany, Spain, Greece, the Czech Republic, and Hungary. Some researchers in Indonesia, Lanovia and colleagues (Lanovia *et al.*, 2014) reported that 3-MCPD was present in palm oil with a 14-35 ppm concentration range. Concerning the above, it is said that the presence of 3-MCPD or derivatized 3-MCPD in our foodstuffs should be more attentive.

The International Agency for Research on Cancer (IARC) reported the carcinogenicity of 3-MCPD in their monograph book (IARC, 2013), although no data were found in human cases. Several methods, such as oral and subcutaneous administration, and dermal application, have investigated the incidence of tumors in mice and rats (Van Duuren *et al.*, 1974; Weisburger *et al.*, 1981; Cho *et al.*, 2008; Jeong *et al.*, 2010), showing damage to multiple organs, such as liver and kidney. From these data, it is believed that to investigate the 3-MCPD residue in our food, the technique for controlling 3-MCPD is essential;

therefore, organ damage would be immediately anticipated.

This paper aims to discuss the relevant information on the latest technology based on molecularly imprinted polymer, particularly its performance and application in actual samples. General information on 3-MCPD has been provided, including physical and chemical properties. The authors also described earlier technologies from the past, such as Gas Chromatography-Mass Spectroscopy (GC-MS) and High-Performance Liquid Chromatography Fluorescence Detector (HPLC-FLD). Molecularly Imprinted Polymer (MIP) was mentioned as a synthetic antibody, including composition, production, and for solid-phase extraction (SPE) and sensor application. In the end, the future development of MIP-based technology has been identified from previously reported developments.

3-MCPD: physical and chemical characteristics

The 3-MCPD has more than ten names and 3-monochloro-1,2-propanediol is the popular one (IARC, 2013). 3-MCPD has a molecular weight of 110.54 gmol⁻¹ and is soluble in water, alcohol, diethyl ether, and acetone. From the chemical formula, 3-MCPD contains one chloride and two hydroxyl groups (Figure 1). It is called the unbound or free 3-MCPD. Other forms of 3-MCPD are bound 3-MCPD containing the ester chain and could be hydrolyzed to be a free form (Barocelli *et al.*, 2011; Abraham *et al.*, 2013).

The formation of 3-MCPD can be triggered in palm oil during fat frying by sodium chloride (NaCl), water content, heating temperature, and heating time (Zhou *et al.*, 2014). From these results, careful processing of palm oil could be recommended. There are many cases in which 3-MCPD may be formed during degumming (Zulkurnain *et al.*, 2012; Matthäus & Pudel, 2013), neutralisation (Matthäus & Pudel, 2013), bleaching (Razak *et al.*, 2012; Zulkurnain *et al.*, 2012) and deodorisation (Franke *et al.*, 2009; Hrnčirik & van Duijn, 2011). Due to these issues, the 3-MCPD is easily left in food.

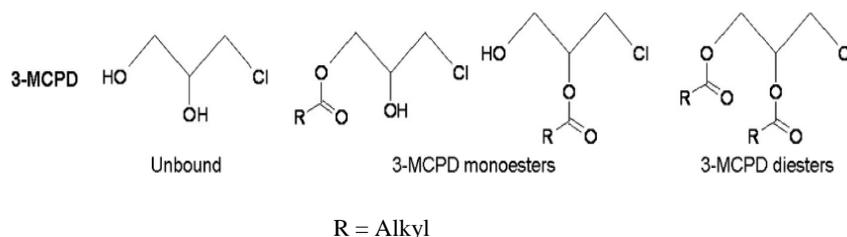


Figure 1. Chemical structures of free 3-MCPD and bound 3-MCPD

Gambar 1. Struktur kimia 3-MCPD bebas dan 3-MCPD terikat

Source: modified from Andres and colleagues (2013)

Sumber: dimodifikasi dari Andres *et al.* (2013)

The current technologies for 3-MCPD detection

Most of the techniques used to detect 3-MCPD are based on chromatography methods. One of them is GC-MS. This method is probably not easily accessible due to lengthy procedures for sample extraction and analysis, even though the European Commission recommended the GC-MS method (EU, 2014). Miyazaki and colleagues (Miyazaki *et al.*, 2012) tried to analyze 3-MCPD of spiked palm oil. The oil sample was hydrolyzed and derived before going to GC-MS. It is claimed that 3-MCPD was read as 3-MCPD derivatization, i.e., dipalmitic 3-MCPD dioleic, 3-MCPD, 3-MCPD dilinoleic, 3-MCPD 1-palmitate, and 3-MCPD 1-oleate. Results from a recovery test of 93-106% were quite good. They also applied this method to other samples with a different matrix, such as rapeseed oil, safflower oil, olive oil, soya bean oil, corn oil, rice bran oil, sesame oil, and sunflower oil, with a recovery test of between 89 and 108%.

The following method is HPLC-FLD, reported by Hu *et al.* (2013), testing 3-MCPD in vegetable oils, including corn oil, rice bran oil, and soya oil. The procedure is different from the one before. The 3-MCPD was cleaved by chloroacetaldehyde and

derivatized by adenine, finally producing ϵ Ade (1-N⁶-ethenoadenine). FLD then analyzed this final product through Fluorescence Derivation. The results of this experiment were terrific since the recovery test was approximately 93 – 97%, and the detection limit was 0.36 ppb. The last example is from Lanovia *et al.* (2014), measured free 3-MCPD and total 3-MCPD in palm oil measured by GC-MS. The instrument was modified by the Weißhaar method (Weißhaar, 2008). The experiments claimed that the results were outstanding for several reasons, such as concentration range: 0.008-0.377 ppm, detection limit: 0.06 ppm and recovery test: 96-113%.

Thus far, the current technologies (GCMS and HPLC FLD) for 3-MCPD detection show powerful results even though they are still laborious and time-consuming. The methods use many chemicals and are not easy for people to operate the instrument. Besides, there is no guarantee that 3-MCPD will be fully converted to its derivatization product event though the methods enable to measure the derivatisation of 3-MCPD. As a result, MIP-based technology could minimize the problem of those technologies to identify better 3-MCPDs than previously reported (Table 1).

Table 1. Comparison of the techniques for 3-MCPD analysis between MIP-based technologies and conventional methods (GC-MS and HPLC-FLD)

Tabel 1. Perbandingan metode analisis 3-MCPD antara teknologi berbasis MIP dan Metode konvensional (GC-MS and HPLC-FLD)

Methods for 3-MCPD analysis <i>Metode analisis 3-MCPD</i>	Advantages <i>Kelebihan</i>	Disadvantages <i>Kekurangan</i>
GC-MS	<ul style="list-style-type: none"> - High sensitivity and selectivity - Enable to detect multianalyte with different analogs 	<ul style="list-style-type: none"> - Long protocol for sample preparation consuming the plenty of chemicals - Long procedure for sample analysis; therefore, a trained person is needed - The maintenance fee is expensive - The price for sample analysis is expensive
HPLC-FLD	<ul style="list-style-type: none"> - High sensitivity and selectivity - Enable to detect multianalyte with different analogs 	<ul style="list-style-type: none"> - Long protocol for sample preparation consuming the plenty of chemicals - Long procedure for sample analysis; therefore, a trained person is needed - The maintenance fee is expensive - The price for sample analysis is expensive
MIP-based technologies	<ul style="list-style-type: none"> - Short protocol for sample preparation consuming less chemicals - Short procedure for sample analysis and no need to hire a trained person - Low cost for maintenance fee - The price for sample analysis is cheap - High sensitivity and selectivity 	<ul style="list-style-type: none"> - Cannot detect multianalyte with different analogs because the MIP is produced for only one single target.

*Molecularly Imprinted Polymer (MIP)-based technologies**MIP composition for 3-MCPD*

In general, several chemicals such as functional monomers (FMs), crosslinkers (CL), initiators (I), and solvents (S) are needed to produce MIP. FMs are critical materials because these chemicals should have a proper interaction directly with 3-MCPD; therefore, the MIP has an active cavity recognizing 3-MCPD correctly. Published articles (Leung *et al.*, 2003; Li *et al.*, 2014; Sun *et al.*, 2014; Fang *et al.*, 2019; Yaman *et al.*, 2020) show that FMs for 3-MCPD had different materials among experiments (Table 2). It is indicated that FM materials can be flexible as long as these chemicals can react with 3-MCPD. Similarly, the solvents used in each MIP production are different depending on the solubility of FMs and 3-MCPDs. These reagents are also crucial to produce MIP on the polymerization step. CL is probably a similar reagent for different MIP production methods and different MIP applications, unlike FMs and solvents. Similarly, the use of an initiator occurred, as shown in Table 2. For instance, ethylene glycol dimethacrylate and Azodi isobutyro nitrile are popular materials as CL and Initiator, respectively. They can be commonly used for bulk polymerization and both sample preparation and analysis purposes. Besides, not all of the MIP production needs CL and Initiator. For example, Sun *et al.* (2014) did not use CL, while Yaman *et al.* (2020) did not use I to generate MIP. The main reason is that they used the method of electropolymerization for the construction of MIP. Therefore, the composition of the MIP is very versatile, and it would be an opportunity for the researcher to explore the new composition of the MIP occasionally.

The role of each component of the MIP is accurate because every element supports the quality of the MIP. For example, the FMs form active MIP sites. In this way, FMs should have an appropriate functional group that can interact either covalently or non-covalently with 3-MCPD. Generally, hydrogen bonding is a type of interaction between FMs and 3-MCPD because this interaction is the strongest than other bonding. Attention should be paid to the chemical structures of 3-MCPD to select the best FMs. Here, 3-MCPD contains two hydroxyls and one chloride, forming a fast hydrogen bonding. It appears that the FMs for 3-MCPD is abundant. For example, methacrylic acid (Table 2) can be used appropriately and tested experimentally to react with 3-MCPD (Li *et al.*, 2014; Fang *et al.*, 2019).

Other compositions, such as CL, also have specific responsibility for maintaining interaction between FMs and 3-MCPD; therefore, the active cavity on the MIP is more stable and rigid. The best CL should have double or triple bonds because they will help forming the MIP body. The existence of CL is designed to control polymer morphology, such as swelling and mesh size, to stabilize polymer binding sites and to convey the mechanical stability of matrix polymers (Mayes & Mosbach, 1996; Yu & Mosbach, 2000; Wong *et al.*, 2015; Mane *et al.*, 2015). Furthermore, the initiator is undoubtedly a vital material forming a free radical substance, and this part will initiate polymerization for generating MIP. However, in some cases, the initiator operates optimally under light, thermal or chemical conditions (Mijangos *et al.*, 2006). Finally, the solvent holds the essential component as a medium for the production of MIP and provides the opportunity for other components to interact and form the morphology of MIP.

Table 2. The composition, method, and application for 3-MCPD imprinted polymer

Tabel 2. Komposisi, metode, dan aplikasi dari 3-MCPD imprinted polymer

MIP composition/ <i>Komposisi MIP</i>				Method <i>Metode</i>	Application <i>Aplikasi</i>	Reference <i>Referensi</i>
FM	CL	I/ES	S			
p-amino thiophenol	-	Tetrabutyl ammonium perchlorate	Methanol	electropolymerisation	sample analysis	(Sun <i>et al.</i> , 2014)
4-vinyl phenyl boronic acid	Ethylene glycol dimethacrylate	Azodi isobutyro nitrile	chloroform	bulk polymerization	sample analysis	(Leung <i>et al.</i> , 2003)
Methacrylic acid			Methanol: water		sample extraction	(Li <i>et al.</i> , 2014)
			ethanol		sample analysis	(Fang <i>et al.</i> , 2019)
Pyrrrole	Graphene oxide	-	Methanol	Electropolymerization	sample analysis	(Yaman <i>et al.</i> , 2020)

Notes:

FM= Functional Monomer, CL= Cross Linker, I/ES=Initiator/Electrolyte Solvent, S= solvent

Keterangan:

FM=monomer fungsional, CL=bahan pengikat, I/ES= inisiator/pelarut elektrolit, S=pelarut

There are many reported MIP production methods, such as conventional polymerization (e.g., bulk polymerization, suspension polymerization) (De Smet *et al.*, 2009), supercritical fluid technology (Scholsky, 1993), solid-phase synthesis (MIP in nanoscale, called nanoMIP) (Munawar *et al.*, 2020a) and electropolymerisation (Munawar *et al.*, 2020b). Unfortunately, according to the published article on the development of MIP for 3-MCPD, only two methods are described here, bulk polymerization (Li *et al.*, 2014) and electropolymerization (Sun *et al.*, 2014; Yaman *et al.*, 2020).

MIP based SPE (MIPSPE) for 3-MCPD

The preparation of MIPSPE is more straightforward than reported SPE, and there is no need to spend many chemicals. Figure 2 showed how to form the MIP for 3-MCPD modified from Li *et al.* (2014). The silica gel was activated by sodium hydroxide and silanized by APTES, obtaining the modified silica gel. This silica gel was then used for copolymerization of MAA (FM) and EGDMA (CL) in the presence of 3-MCPD. Copolymerization occurred due to the presence of AIBN, resulting in a non-covalent MAA and 3-MCPD complex. In this case, 3-MCPD was predicted to form hydrogen bonding with MAA (Figure 2(a)). MCPD was removed by methanol: water (9:1, v / v) to obtain pure MIP. Li also

provides computational simulation data to prove the interaction between 3-MCPD and MAA (Figure 2b). They claim that the best ratio is 1:2 for 3-MCPD and MAA, respectively, for MIP production. In addition, the morphology of the produced MIP for 3-MCPD was characterized using SEM. FTIR also observed the identification of the structure of MIP. The MIP profile could, therefore be more comprehensive and reliable.

The quality of the MIP-based SPE can be seen objectively compared to the previous SPE (Table 3) concerning analytical parameters such as the recovery and detection limit or the type of reagent used. Thus, it can be seen which SPEs are more efficient, effective, and safe. Unfortunately, not much data has been found on the development of MIP-based SPE for 3-MCPD. There are probably few commercialized MIPSPEs; therefore, MIP may be unknown to some stack holders. This case shows that it is necessary to disseminate the benefit of MIP and its application to stack holders or users.

In general, MIPSPE has outstanding accuracy in binding 3-MCD compared to other SPEs. The recovery test of MIPSPE is higher than 90%, and the limit detection is around 0.002 ppm, indicating that the MIP was a powerful application for sample extraction. In addition, an elution solvent is not much more organic than the others, only acetonitrile and water. Therefore, MIPSPE is safer than the other SPEs using more organic solvents.

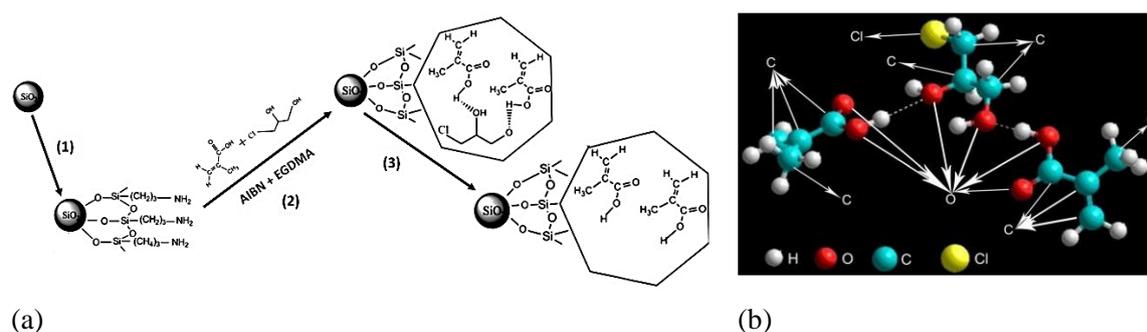


Figure 2 (a) Illustration of MIP production: (1) activation and silanization of silica gels (2) copolymerization of MIP on the surface of modified silica gels (3) removal of 3-MCPD for getting active cavities of MIP. (b) Associated complexes formed between 3-MCPD and MAA; the dotted lines indicate the hydrogen bonds using HyperChemV8.0.1.

Gambar 2 (a) Ilustrasi dari produksi MIP: (1) aktivasi dan silanisasi dari gel silika (2) kopolimerisasi MIP pada permukaan gel silika yang sudah dimodifikasi (3) pelepasan 3-MCPD untuk mendapatkan sisi aktif dari MIP. (b) Kompleks senyawa antara 3-MCPD dan MAA; garis titik titik mengindikasikan ikatan hydrogen menggunakan HyperChemV8.0.1.

Source: modified from Li *et al.* (2014)
Sumber: dimodifikasi dari Li *et al.* (2014)

MIP-based sensor for 3-MCPD

The next application, MIP, may also be used for sample analysis. Yaman *et al.* (2020) indicated that the MIP might be manufactured as a 3-MCPD sensor. Based on its outcome, this sensor is assumed to be quite good. The sensor performance was worked on the concentration range at 5-500 nM and the detection limit was 1.82 nM. The interesting point is how they manufactured the sensor using MIP (Figure 3), even though they still used old-fashioned electrode systems, including pencil electrode graphite (working electrode), Platinum wire (counter electrode), and Ag / AgCl/3

M KCl (reference electrode), instead of the screen-printed electrode used recently by so many sensor developers.

Manufacturing MIP-based sensor uses electropolymerization that is simpler than bulk polymerization. Here, Yaman and colleagues (Yaman *et al.*, 2020) used a pencil graphite electrode (PGE) as a working electrode where the MIP attachment is located. The protocol is a one-time step, but they instantly get both the MIP and the MIP-based sensor. For this reason, electropolymerization is not complicated and is also more economical because few chemicals are used. Figure 3 shows the production of MIP and

Table 3. Comparison of sample extraction using MIPSPE and other SPEs

Tabel 3. Perbandingan ekstraksi sampel dengan MIPSPE dan SPE lain

Type of sample <i>Tipe sample</i>	Type of SPE <i>Tipe SPE</i>	Reagent <i>Pelarut</i>	Recovery <i>Recovery</i> (%)	LoD <i>Limit deteksi</i> (ppm)	Reference <i>Referensi</i>
Soya sauce	MIP SPE	acetonitrile: water	93	0.002	(Li <i>et al.</i> , 2014)
Cookies and Margarine	diatomaceous earth extraction	ethyl ether: hexane	99 - 108	NA	(Becalski <i>et al.</i> , 2015)
Soy sauce, dehydrated soup, toasted bread, vegetable oils, salami, sausage, and cheese	Extrelut [®] NT20 cartridges	ethyl acetate	92 – 100	NA	(Retho & Blanchard, 2005)
Vegetable oil	C18 powder- packed syringe	acetonitrile: 2- propanol	94 – 108	0.0001–0.02	(C. Li, Nie, Zhou, & Xie, 2015)
Infant Formula Milk Powder	alkaline diatomite SPE column	acetic ether: diethyl ether	98 – 111	30	(Wang <i>et al.</i> , 2016)
Corn oil	DVB/CAR/PDMS packed column	NA	93	0.004 – 0.005	(Xu, Jin, Yang, Rao, & Chen, 2020)
Extra virgin olive	(Si-SAX and PSA)-packed SPE tube and (Z-Sep+ and PSA)-packed SPE	diethyl ether: hexane and ethyl acetate: acetonitrile	71 – 123	10 – 20	(Custodio- Mendoza <i>et al.</i> , 2018)

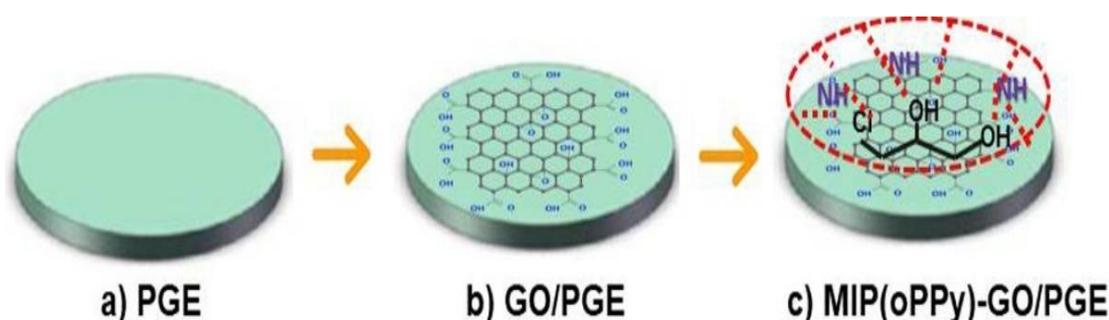


Figure 3. The illustration of MIP-based sensor for 3-MCPD using pencil graphite electrode (PGE)

Gambar 3. Ilustrasi dari sensor berbasis MIP untuk 3-MCPD menggunakan elektroda grafit pensil

Source: modified from Yaman *et al.* (2020)Sumber: dimodifikasi dari Yaman *et al.* (2020)

sensor. Step one, the graphene oxide (GO) was immobilized on the surface of the PGE. The pyrrole and 3-MCPD were then electropolymerized by Cyclic Voltammetry in optimum condition (Britton Robinson buffer, pH 4), potential range 0.0-+1.2 V (vs. Ag/AgCl), scan rate 50 mVs⁻¹, five cycles) on the GO-modified PGE (GO / PGE) surface. The last step was to wash the PGE with methanol: acetic acid (9:1, v / v) to remove 3-MCPD from the system and leave the active MIP-based sensor (MIP(oPPy)/GO / PGE) cavities. The sensor is finally used for 3-MCPD detection on actual samples, soya sauces from the local market, using electrochemical impedance spectroscopy (EIS) with the optimum setting (AC potential +0.20 V, frequency range 0.1 Hz-100 kHz, amplitude ten mV, ferri/ferrocyanide redox system). However, the data is likely to be validated to determine the recovery percentage in further study; therefore, the sensor is more reliable.

Sun and Colleagues (Sun *et al.*, 2014) also applied MIP on sensor technology. However, the manufacturing process of the sensor is more complicated than the previous explanation, even though both used the same conventional electrode system. Herein, they used a modified carbon glass electrode (GCE) as a working electrode, a platinum wire as a counter electrode, and a saturated carbon electrode (SCE) as a reference electrode. Therefore, it is believed that there is an opportunity

to develop a 3-MCPD sensor with a screen-printed electrode because it would be more portable and moveable. The sensor was manufactured in several stages (Figure 4) because the GCE should be modified by tetrachloroaurate (III) acid (HAuCl₄) to form Au nanoparticles (AuNPs) deposit. The surface of the AuNP modified GCE (AuNPs / GCE) was then self-assembled by *p*-amino thiophenol (*p*-ATP) through interaction between the gold and thiol groups (-SH) of the *p*-ATP to form an Au-S bond. The 3-MCPD was continuously immobilized on that electrode, employing hydrogen bonding between the amino groups of *p*-ATP and the hydroxyls of 3-MCPD. Finally, the MIP was electropolymerized by Cyclic Voltammetry with optimum condition (10 cycles, potential range -0.3 – +1.2 V, scan rate 50 mVs⁻¹).

The outstanding results have been reported, although the protocol for manufacturing the MIP-based sensor has been complicated. The produced sensor was tested for its 3-MCPD solution with a concentration range of 1.0 × 10⁻¹⁷ to 1.0 × 10⁻¹³ M, and the sensor is quite sensitive because the detection limit is lower than 1 nM. Soya sauce was also used for the recovery test, resulting in a recovery percentage of more than 90%. There is, however, no data on the consistency of the sensor during the time of storage and no explanation of the sensor's reproducibility.

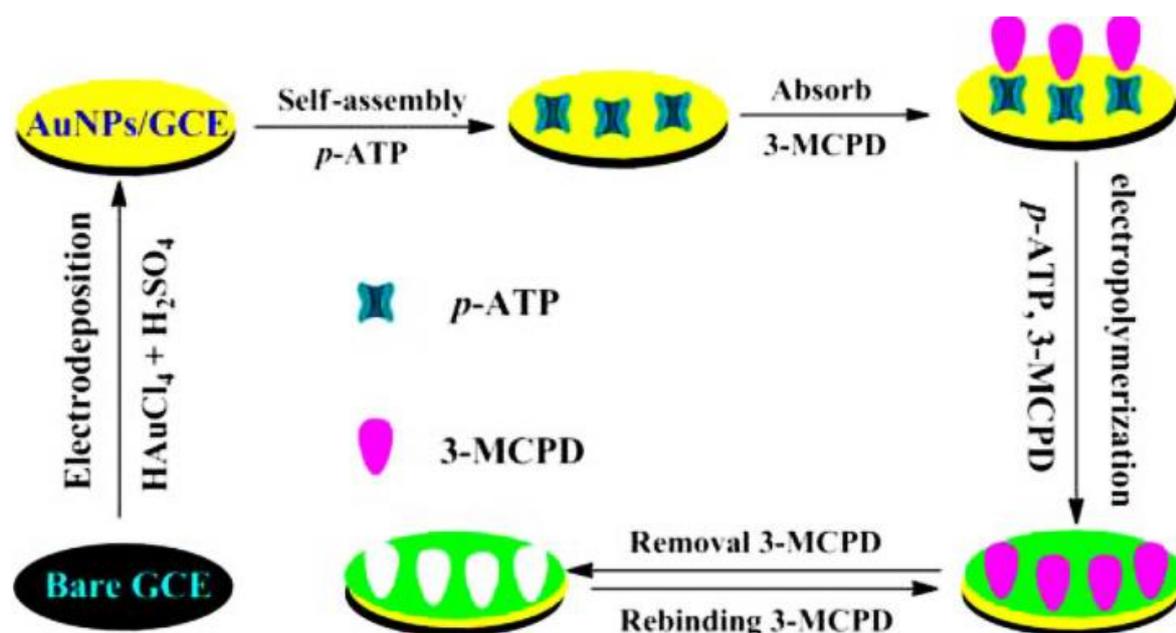


Figure 4. The illustration of MIP-based sensor for 3-MCPD using glassy carbon electrode (CGE)

Gambar 4. Ilustrasi dari sensor berbasis MIP untuk 3-MCPD dengan menggunakan elektroda karbon gelas

Source: adapted from Sun *et al.* (2014)

Sumber: diadaptasi dari Sun *et al.* (2014)

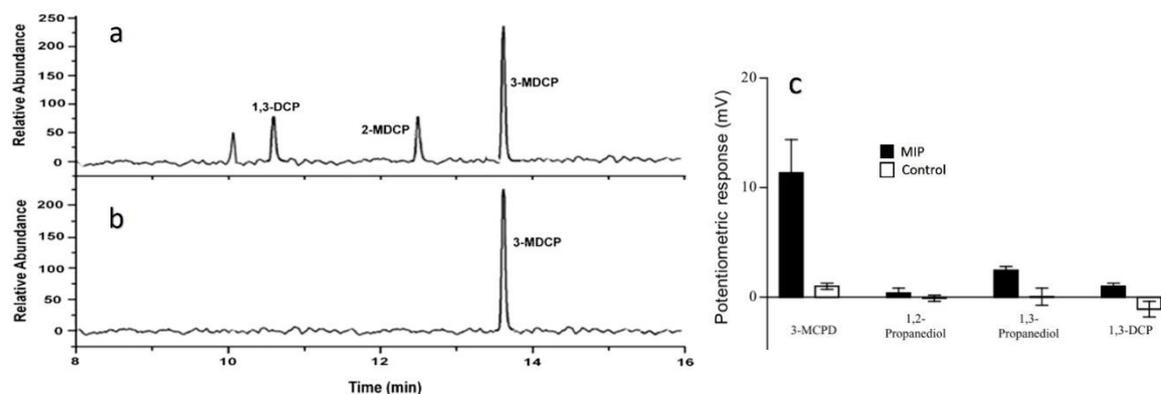


Figure 5 Chromatograms of the mixture solution (3-MCPD, 2-MPCD, and 1,3-DCP) without (a) and with (b) MIP-SPE column. (c) The selectivity testing of the MIP-based sensor on 3-MCPD and other analogs diols (1,2-propanediol; 1,3-propanediol; and 1,3-DCP)

Gambar 5. Kromatogram dari larutan campuran (3-MCPD, 2-MPCD, and 1,3-DCP) tanpa (a) dan dengan (b) MIP-SPE column. (c) Uji selektifitas dari sensor berbasis MIP pada 3-MCPD dan senyawa diols lainnya (1,2-propanediol; 1,3-propanediol; and 1,3-DCP)

Source: adapted from Leung *et al.*, (2003) and Li *et al.*, (2014)

Sumber: diadaptasi dari Leung *et al.*, (2003) dan Li *et al.*, (2014)

The selectivity of MIP-based technologies

MIP selectivity was reported from Li and Colleagues (2014), showing results from MIP-based SPE. Figures 5a and 5b illustrated the chromatograms of mixture solution including 3-MCPD, 2-MPCD, and 1,3-DCP, without and with MIP-based SPE pre-treatment. The performance of MIP was very outstanding in distinguishing 3-MCPD from Li's work with other targets. In addition, the selectivity of MIP was observed by Leung *et al.* (2003), producing an electrochemical sensor based on MIP. The 3-MCPD sensor may differ from other analog diols such as 1,2-propanediol and 1,3-propanediol, and 1,3-DCP (Figure 5c). It appears that the sensor was unable to recognize the other targets as the same as those responses from the control material. Similar results have been shown in the following reports (Sun *et al.*, 2014; Fang *et al.*, 2019; Yaman *et al.*, 2020). More analog diols (e.g., glycerol, 2,3-DCP, ethylene glycol, 2-MCPD, L-glutamic acid, L-tryptophan and L-phenylalanine) were used to demonstrate the selectivity of MIP in the platform sensor, and the performance of MIP did not significantly recognize those targets. Unfortunately, no observation has been reported about the selectivity of MIP on free and bound 3-MCPDs. It would be an excellent opportunity for future researchers to investigate this challenge.

Conclusion

Molecular Imprinting Polymer (MIP) technology is an alternative method for controlling 3-MCPD with many benefits. The few chemicals used are advantageous to MIP. For this reason, the use of MIP has so far been safer than other methods. The application of MIP is also applicable

to the extraction and analysis of samples. For some analysts/users, the extraction step is laborious, not only chemicals used but also the time and effort needed. The MIPSPE would be more effective. Therefore, the improvement of this innovative SPE should be made to achieve not only acceptable but also reliable results. The following future, probably the validation step, is an improvement to achieve better MIPSPE quality than previously reported. Furthermore, the MIP-based sensor technology showed the robust performance of the soya sauce even though the old-fashionable electrode systems are still used. Further research, therefore, requires the use of a screen-printed electrode to make the sensor more portable. Testing on actual samples is also required in a variety of foods containing 3-MCPD or bound 3-MCPD.

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