



# Methyl methacrylate magnetic molecularly imprinted polymer for gluten determination

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## Abstract

The gluten protein is found in some rice and flour. The allergy of gluten, a little bit of gluten in diet cause long-term damage and dangerous to the body. Even tiny amounts of gluten in diet may bring enormous symptoms. The rapid and simple method for gluten detection is molecularly imprinted polymers (MIP) combined with electrochemical analysis. In addition, magnetic molecularly imprinted polymers (MMIP) as known as Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles have used in combination with electrochemical measurement as well to improve the sensitivity of detection. In this work, the MMIP was combined with electrochemical technique. The Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles were synthesized by chemical reaction and then encapsulated with methyl methacrylate (MMA) as a functional group for gluten detection. Dynamic light scattering measurement clearly illustrates the average size of as-synthesized Fe<sub>3</sub>O<sub>4</sub> nanoparticles as low as 150 nm. Chemical bonding, morphology, crystal structure and magnetic properties were characterized by fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), X-ray diffractometer (XRD), and vibrating sample magnetometer (VSM), respectively.

## 1. Introduction

The U.S Food and Drug Administration (FDA) defines that the level in gluten-free products must contain less than 20 ppm gluten [1,4]. Gluten detection in foods is necessary for celiac patient to ensure product. The low level of gluten in diet can be detected by enzyme-linked immunosorbent assay (ELISA) [5], mass spectrometry (MS) [6,7] and high-performance liquid chromatography (HPLC) [8,9], but these methods are expensive, time-consuming and require trained operators. However, the electrochemical analysis has gained more interest recently as measurement for organic molecule detection due to the various advantages such as simple, rapid, accurate, fast response and low cost. Moreover, electrochemical cells have been developed as a convenient and portable device. Although electrochemical analysis takes many advantages, the selectivity of this method is limited by non-specific binding [10-13]. Thus, molecularly imprinted polymers (MIPs), is a surface modification electrode, has been gained attention to

improve the selectivity, owing to functional group, shape and size [14,15]. Moreover, MIP has been developed by superparamagnetic iron oxide nanoparticles (SPIONs), a magnetite (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles, as known as magnetic molecularly imprinted polymers (MMIP). That can improve the electrochemical signal for analytic detection by external magnetic field [16,17]. Many researches on the application of MMIP in electrochemical sensors have been reported. Molecularly imprinted sensor combined with magnetic molecularly imprinted for determination of dibutyl phthalate. Determination system was successfully applied to the determination of DBP in soybean milk and milk samples with DVP mode [18]. Molecularly imprinted star polymer-modified SPIONs for trace level sensing of mancozeb [19]. That was detected with square wave stripping voltammetry (SWASV) techniques, which shows a good sensitivity, selectivity and reproducibility.

The aim of this study, Fe<sub>3</sub>O<sub>4</sub> nanoparticles were synthesized by the chemical synthesis. MMIP for gluten detection was prepared by polymerization of

methyl methacrylate (MMA) as functional group and gluten as template. The ethylene glycol dimethacrylate (EGDMA) and 2,2-azobisisobutyronitrile (AIBN) was used as cross-linker, and initiator, respectively. Then, carbon paste electrode was modified with gluten-MMIP. Finally, the electrochemical measurement was used to confirm the gluten detection. The modified carbon paste electrode with gluten-MMIP, MIP and NIP were tested gluten with amperometric method. Finally, the modified carbon paste electrode was tested in 7 samples such as rice flour, glutinous rice flour, tapioca starch, wheat flour, corn starch and gluten-free corn starch for gluten determination.

## 2. Experimental

### 2.1 Chemicals and apparatus

Methyl methacrylate (MMA), ethylene glycol dimethacrylate (EGDMA), 2,2-azobisisobutyronitrile (AIBN), Iron (III) chloride ( $\text{FeCl}_3$ ), polyvinyl alcohol (PVA) and gluten were purchased from sigma-aldrich. Iron (II) chloride ( $\text{FeCl}_2$ ) and sodium hydroxide (NaOH) were purchased from Carlos.

Electrochemical measurements were performed with a  $\mu\text{STAT}$  400 potentiostat (Dropsens, Spain) controlled by dropview 8400 software. Fourier transform infrared spectroscopy (FTIR-Spectrum two, PerkinElmer) was recorded wave numbers ranging from 400-4,000  $\text{cm}^{-1}$ . The crystalline structure was observed by X-ray diffraction (XRD-smartlab, rigaku) with Cu  $K\alpha$  radiation ( $\lambda=1.5406 \text{ \AA}$ ). The morphology of SPIONs was observed by transmission electron microscope (TEM, JEOL-JEM-2100). The sample was prepared on copper grid covered with carbon film. The modified carbon electrode morphologies were observed by field-emission scanning electron microscope (FE-SEM, JEOL-JSM-7600F). Magnetic properties were measured at room temperature using vibrating sample magnetometer (VSM, Lakeshore-model 7404)

### 2.2 Synthesis of $\text{Fe}_3\text{O}_4$ magnetic nanoparticle

$\text{Fe}_3\text{O}_4$  magnetic nanoparticles were synthesized via chemical solution process. 2.76 g of  $\text{FeCl}_3$  was mixed with 1.59 g of  $\text{FeCl}_2$  by controlling the molar ratio as 2:1. The mixture was dissolved in 50 ml of deionized water and stirred at 80°C under  $\text{N}_2$  atmosphere for 30 min. Next, 100 ml of 1 M NaOH

was added in solution and carried out for 30 min. The color of solution changes from yellow to black. Then, 0.2% PVA was added to prevent the agglomeration. The mixture was cooled to room temperature. Finally,  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles were separated with external magnetic and washed with deionized water several times.

### 2.3 Preparation of magnetic molecularly imprinted polymer (MMIP) for gluten detection

Gluten-magnetic molecularly imprinted polymer was prepared by the following procedure. Firstly, 0.05 g of gluten was dissolved in 10 ml of PBS, followed by 10 ml of SPIONs, 1.0 ml of MMA and 5 ml of chloroform, respectively. The solution was stirred at 60°C for 5 min. Then, 9.4 ml of EDGMA was added into the mixture and carried out for 5 min. Next, 0.96 ml of AIBN was added into the mixture and dropped onto the carbon paste electrode. Finally, the modified electrode was washed using deionized water and ethanol several times for eluting the template. In comparison, non-imprinted polymer (NIP) and molecularly imprinted polymer (MIP) was prepared by the same procedure of MMIP except the addition of template and SPIONs, respectively.

### 2.4 Electrochemical measurement

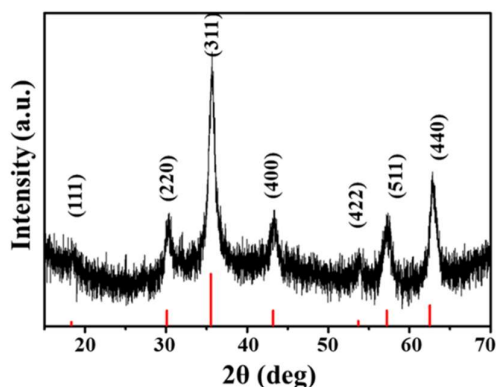
Modified carbon paste electrodes were investigated for gluten detection by electrochemical measurement. Cyclic voltammetry (CV) measurement was performed with the scan range from 0.3 V to -0.9 V at the scan rate 50  $\text{mV}\cdot\text{s}^{-1}$ . An amperometry mode was tested at fixed voltage as -0.47 V for 300 s. All sample solutions were dissolved in 0.1 mM phosphate buffer solution. In this work, the modified carbon paste electrode was tested in flour samples such as rice flour, glutinous rice flour, tapioca starch, wheat flour, corn starch and gluten-free corn starch for gluten determination.

## 3. Results and discussion

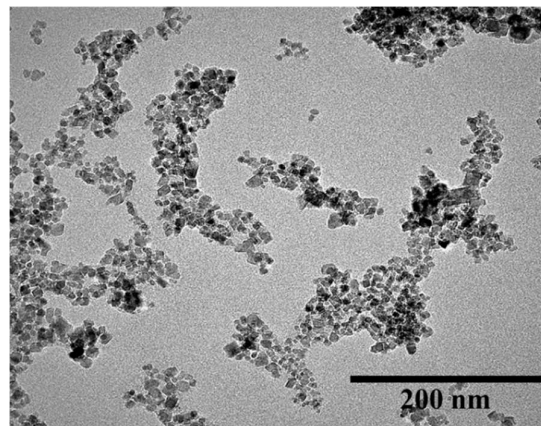
### 3.1 Synthesis and characterization of $\text{Fe}_3\text{O}_4$ magnetic nanoparticle

$\text{Fe}_3\text{O}_4$  magnetic nanoparticles were synthesized via chemical solution process. PVA as stabilizer prevented the agglomeration of  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles. X-ray diffraction diagram indicated

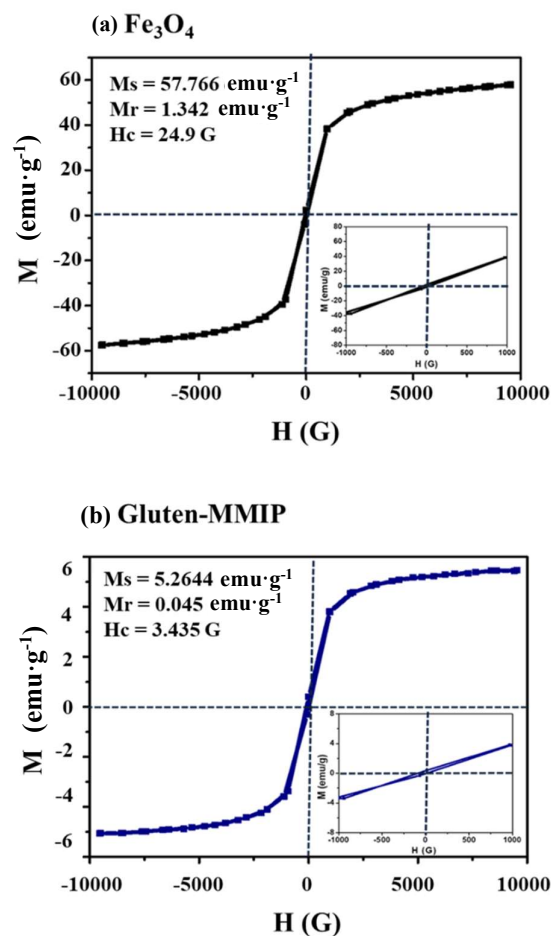
the molecular form at seven characteristic peaks,  $18.3^\circ$ ,  $30.2^\circ$ ,  $35.6^\circ$ ,  $43.2^\circ$ ,  $53.5^\circ$ ,  $57.2^\circ$  and  $62.8^\circ$ . The peak position can be indexed to (111), (220), (311), (400), (422), (511) and (440) as shown in Figure 1. Diffractogram indicated cubic inverse spinel structure and magnetite ( $\text{Fe}_3\text{O}_4$ ) phase. The characteristic peaks of  $\text{Fe}_3\text{O}_4$  were confirmed by JCPDS file (PDF No. 00-019-0629). Moreover, the crystalline size was calculated by using the Debye-Scherrer equation ( $d = K\lambda/(\beta\cos\theta)$ ) where  $K = 0.9$ ,  $\lambda = 0.15406$  nm,  $\beta$  is full width at half maximum (FWHM). By using this equation, it showed the crystalline size of 9.75 nm. TEM image presented morphology and size of  $\text{Fe}_3\text{O}_4$  nanoparticles as shown in Figure 2.  $\text{Fe}_3\text{O}_4$  with diameter about 10 nm were found in TEM image which related to the diameter size from Debye-Scherrer equation.  $\text{Fe}_3\text{O}_4$  is ferromagnetic, but  $\text{Fe}_3\text{O}_4$  is superparamagnetic with single domain. Superparamagnetic property provides magnetic field when apply external magnetic field [20,21]. In this work, the magnetic properties were measured with vibrating sample magnetometer.  $\text{Fe}_3\text{O}_4$  nanoparticles exhibited superparamagnetic properties with magnetization ( $M_s$ ) =  $57.766 \text{ emu}\cdot\text{g}^{-1}$  as shown in Figure 3. From literature,  $\text{Fe}_3\text{O}_4$  nanoparticles (size in range 2-20 nm) have superparamagnetic properties [22] which according to this work. In addition, FTIR spectra were used to provide information for functional groups of  $\text{Fe}_3\text{O}_4$ . The FTIR spectra presented bands at  $573 \text{ cm}^{-1}$  of the Fe-O vibration as shown in Figure 4 [20,22]. Therefore, superparamagnetic  $\text{Fe}_3\text{O}_4$  nanoparticles were produced by synthesis via chemical solution process.



**Figure 1.** X-ray diffraction (XRD) pattern of  $\text{Fe}_3\text{O}_4$ .



**Figure 2.** TEM morphology of  $\text{Fe}_3\text{O}_4$  with an average diameter about 6-10 nm.



**Figure 3.** Hysteresis loop for  $\text{Fe}_3\text{O}_4$  nanoparticles (a) and MMIP (b).

### 3.2 Synthesis and characterization of magnetic molecularly imprinted polymer

Carbon paste electrode modified MMIP was prepared using methyl methacrylate (MMA) as a functional group to interact with gluten. The ethylene glycol dimethacrylate (EGDMA) and 2,2-azobisisobutyronitrile (AIBN) were used as cross-linker, and initiator, respectively. The scheme of gluten detection was illustrated in Figure 5. The carbon paste electrode was modified with MMIP using gluten as template. Then, the gluten was eluted with ethanol and DI to prepare for gluten detection. The MMIP on carbon paste electrode which was eluted the gluten templates could have the cavities and functional groups for gluten detection. Figure 4 showed FTIR spectra analysis of MMIP and non-imprinted polymer (NIP). The FTIR spectra were showed in the same signal of PMMA, gluten-MMIP and NIP at the wavenumber at 2950  $\text{cm}^{-1}$  and 1720  $\text{cm}^{-1}$  (stretching vibration from C-H stretching and C=O double bond stretching, respectively). In addition, vibration of C-O-C single bond stretching showed at wavenumber 1144  $\text{cm}^{-1}$  were found and presented the functional group of PMMA. Gluten-MMIP and NIP were eluted the gluten template. Thus, gluten functional group presented 3000  $\text{cm}^{-1}$  of -OH stretching, 1635  $\text{cm}^{-1}$  of C=O double bond stretching, 1531  $\text{cm}^{-1}$  of -NH bending and 1234  $\text{cm}^{-1}$  of -CN stretching whereas gluten-MMIP and NIP was not presented their spectra. SEM image were confirmed modification of gluten-MMIP on carbon paste electrode that showed morphology between NIP and MMIP. MMIP was observed cavity of eluted gluten template whereas NIP was smooth of carbon paste

electrode as shown in Figure 6. Magnetic properties of gluten-MMIP were magnetization ( $M_s$ ) = 5.264  $\text{emu} \cdot \text{g}^{-1}$  as shown in Figure 3. Compared with pure SPIONs, the saturation magnetization of gluten-MMIP decreased because of the coating molecularly imprinted polymer on the SPIONs surface. However, the gluten-MMIP also presented superparamagnetic of iron oxide nanoparticles confirmed by VSM, additionally, has no hysteresis loop. Thus, gluten-MMIP could be controlled with external magnetic.

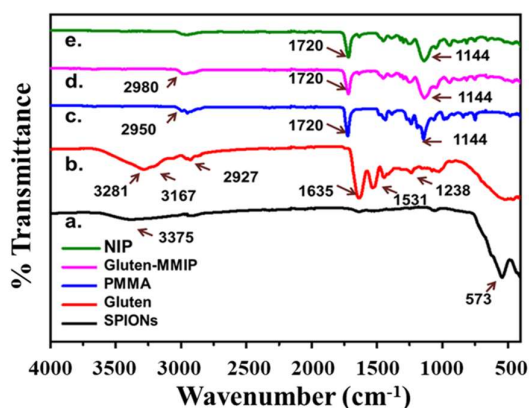


Figure 4. FTIR spectrum of  $\text{Fe}_3\text{O}_4$  (a), gluten (b), PMMA (c), gluten-MMIP (d) and NIP (e).

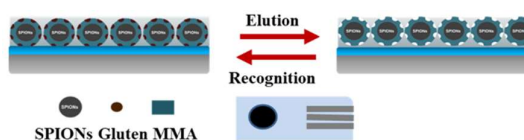


Figure 5. Schematic illustration of magnetic molecularly imprinted polymer for gluten detection.

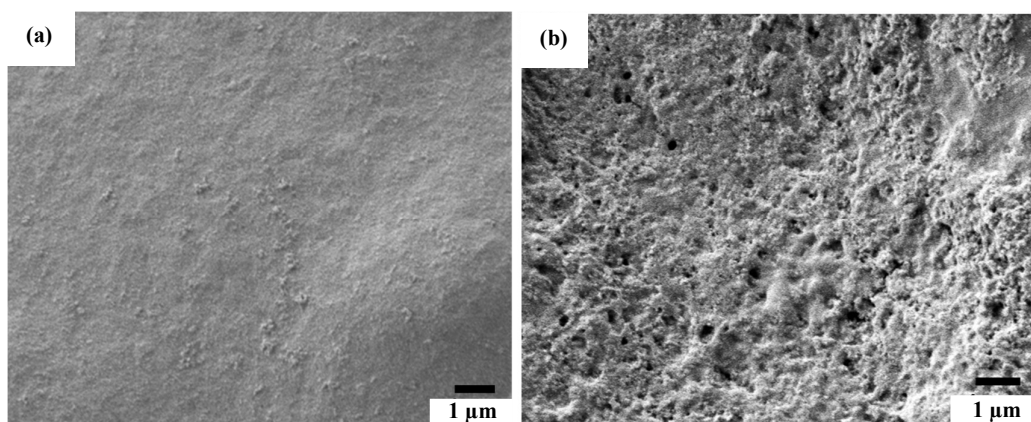


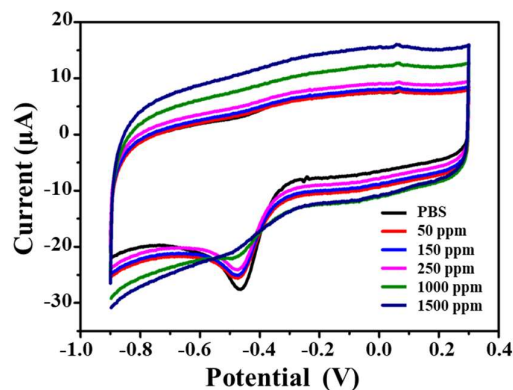
Figure 6. SEM images of NIP (a) and MMIP (b)



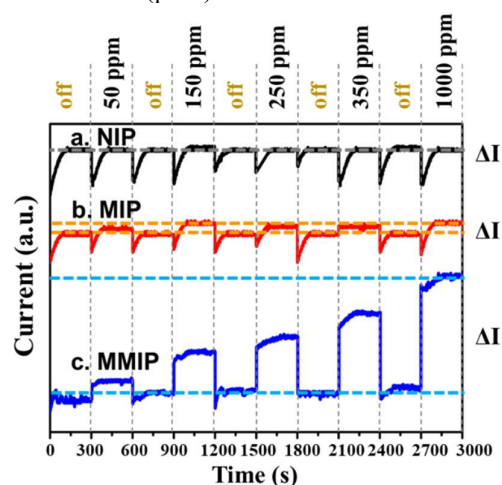


### 3.3 Electrochemical of gluten-MMIP detection

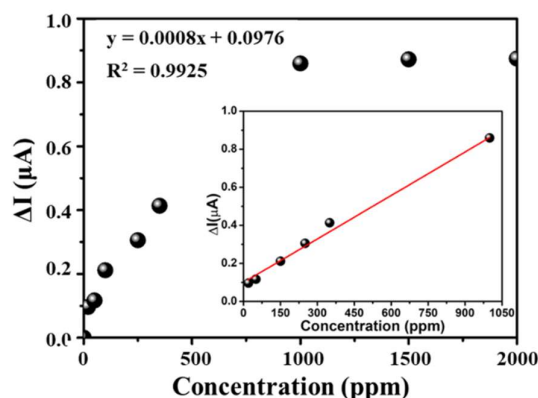
Gluten-MMIP was applied on carbon paste electrode for gluten detection using electrochemical technique. Cyclic voltammetric measurement (CV) was performed to evaluate the recognition of the gluten-MMIP with various concentration of gluten template (50, 150, 250, 1000 and 1500 ppm) in PBS buffer. CV curves were showed changeable peak at -0.47 V. in the potential window from +0.3V. to -0.9 V as shown in Figure 7. The current signal vs. time was measured by amperometric method. The current signal was measured for 300 s/sample. The initial potential was defined at -0.47 V. Figure 8 showed the recorded current signal to time. The current signal was changed and saturated at 100 s when the gluten detections were performed. Thus, the relative current signal from the base line which is PBS buffer was brought to determine the gluten detection. Firstly, NIP showed insignificant change of current signal while the gluten concentration increase. MIP was found that slight change of current signal with increasing gluten concentration due to its potentiality to detect gluten from molecularly imprint. But the relative current signal cannot be remarkably observed. Finally, MMIP was performed to detect the gluten concentration. The results were indicated that the MMIP has the highest relative current signal. Moreover, the increase of gluten concentration related to increase of MMIP current signal. That can be implied MMIP could be used to enhance the relative current signal for gluten detection. After that the calibration curve of MMIP for gluten detection was performed. The calibration curve of gluten-MMIP was plotted between concentration and relative current signal. A linearity in the range of 20-1000 ppm was obtained. The linear regression equation was  $y = 0.0008x + 0.0976$  with a correlation coefficient 0.9925 as showed in Figure 9. Limit of detection (LOD) was calculated from equation  $LOD = 3.3 (S.D.)/M$  where S.D. is the standard deviation of the response and M is the slope of the calibration curve [24]. LOD of gluten-MMIP can be calculated following the equation as mentioned above is 12.38 ppm with linear calibration curve in the range of 20-1000 ppm as shown in Figure 9. LOD of gluten-MMIP is less than permission level in products with gluten-free tag from FDA organization (less than 20 ppm) [3,4].



**Figure 7.** Cyclic voltammetric of gluten-MMIP with various concentration of gluten in 0.1 mM phosphate buffer solution (pH 7).



**Figure 8.** Amperometric responses of gluten determination as NIP (a), MIP (b) and gluten-MMIP (c) in various concentration of gluten at 50, 150, 250, 350 and 1000 ppm in 0.1 mM phosphate buffer.



**Figure 9.** Calibration curves of gluten-MMIP determination.

### 3.4 Determination of gluten in flour sample.

Determination of gluten in flour sample was tested using carbon paste electrode. It was modified surface with gluten-MMIP by MMA. Amperometric measurement was tested with fixed initial potential at -0.5 V, 300 s. Six flour samples (rice flour, glutinous rice flour, tapioca starch, wheat flour, corn starch and gluten-free corn starch) were tested for determination of gluten. 0.1%w/v of flour samples were dissolved in 0.1 mM phosphate buffer solution. Rice flour, glutinous rice flour, and tapioca starch are contained less than 5% of total protein in products [23]. Naturally, wheat and corn were contained 80% and 55% gluten of total protein, relatively. However, industries have developed the gluten-free corn starch for gluten allergic patient which gluten-free corn starch product has been also studied in this work. Wheat flour and corn starch are natural products containing with gluten. The comparison of relative current as illustrated in Figure 10 indicated that the gluten-MMIP is sensitive for gluten detection only in wheat flour and corn starch. But the gluten-free products showed low relative current which can be implied gluten-MMIP has selectivity for gluten detection.

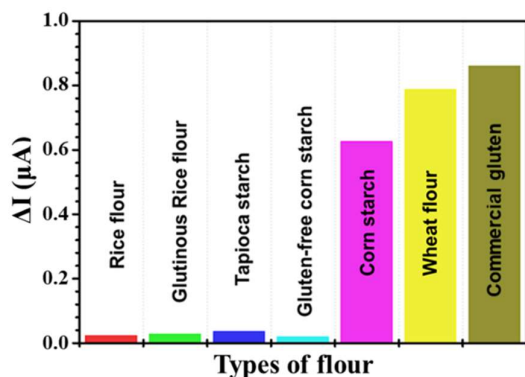


Figure 10. Determination results of gluten in real samples.

### 4. Conclusions

In this work, magnetic molecularly imprinted polymer was prepared using methyl methacrylate as a functional group for gluten detection. MMIP was applied on carbon paste electrode. The application of determination was tested gluten with amperometric method. Limit of detection of gluten-MMIP carbon paste electrode was 12.38 ppm. Thus, this work was presented gluten-MMIP carbon paste

electrode with low limit of detection, short time and easily for gluten detection. Gluten-MMIP could develop for gluten detection in food industry.

### 5. Acknowledgements

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