Effects of (monomer - crosslinker – initiator) composition during non imprinted polymers synthesis for catechin retention

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Abstract. MISPE (molecularly imprinted Solid Phase Extraction) is a separation technique using a solid adsorbent as a principle of MI (molecularly imprinted). Methacrylic acid (MAA) was used as a monomer, ethylene glycol dimethacrylate (EGDMA) as a crosslinker, benzoyl peroxide (BPO) as an initiator and acetonitrile (ACN) as a porogen. Catechin will be used as the template. Thermal and microwave methods were employed in the synthesis method. When analyzed using FTIR spectra, it was found that there were no significant differences between NIP (non-imprinted polymer) resulting from thermal method and that resulting from microwave method. Preparation of polymers by microwave method required 4 mins at 60-65 °C, significantly less than thermal method, that took 60 minutes at the same temperature. The variations of mole ratios of the monomer, the crosslinker, and the initiator were also performed. Based on the FTIR spectra, intensity of some peaks were changed due to the decreases of concentration. The optimum composition for NIP synthesis was MAA: EGDMA: BPO ratio of 5:30:0.5 (in mmole). The TGA curve showed that the NIP synthesized using microwave method experienced mass loss of around 98.50% at 604.8 °C.

1. Introduction

Steeping tea leaf has become one of the non-alcoholic and widely consumed drink in the world [1]. Tea (Camellia sinensis) is a plant that has a high polyphenol content. Tea can be grouped into several types of white tea, green tea, black tea and oolong tea. Almost all types of tea contain polyphenols in high enough quantities. Polyphenolic compounds contained in tea are catechins and its derivatives. Polyphenols that contained in tea are catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). Some studies show that consumption of tea good for health, including to reduce the risk of cholesterol, obesity, hypertension, antioxidants, antimicrobials, as well as protection against cardiovascular disease and cancer [2]. Figure 1 shows the chemical structure of major compounds which are contained in tea. In addition to derivative compounds catechin, in tea also has caffeine which adversely affects health such as insomnia, nervousness, irritability and malfunction condition during pregnancy and lactation [3]. To get the benefits of polyphenols and reduce the adverse effects of caffeine, required separation methods and chemical analysis accordingly. Levels of polyphenol compounds that are extremely low in the sample tea making separation and analysis of these compounds difficult. Complex matrix also makes these compounds are difficult to analyze. Various techniques have been carried concentration [4-6]. MIP
can be applied to liquid-liquid extraction, ion exchange, and dialysis, techniques concentration can also provide better results when using a sorbent solid [7,8] or molecularly imprinted polymer (MIP) [9,10].

Research on molecularly imprinted polymers (MIPs) have grown very rapidly in recent years. MIP has been widely used as chromatography media, sensors, artificial antibodies, catalysts and most are solid phase extraction. Affinity and excellent selectivity towards analyte molecule makes MIP one effective method of separating [11,12]. MIP has a very specific part of the analyte molecules in terms of both affinity and interaction as well as in terms of size. It can be formed in the polymerization process between functional monomer, crosslinker and molecular templates. The interaction is covalent, non-covalent or semi-covalent. After the polymerization process, the molecular prints will be released with the leaching process. Template formed on the polymer will provide the size, shape and appropriate interaction with the analyte molecules. The price is cheap, convenient storage, good mechanical strength, resistance to acids and bases, solvents and temperatures are some of the advantages possessed by MIP [13,14].

This study focuses on the synthesis of material in the separation and purification of catechin and derivatization of samples of tea leaves (Camellia sinensis). The initial phase comprise the synthesis of NIP (non-imprinted polymer). There were two main components in MIP, molecule templates and polymers. In this reported research, the main objective was to prepare the polymers. The ratios of the monomer, the crosslinker and the polymerization initiator were varied. Polymer synthesis using microwave method is a relatively new path to produce MIP and NIP, especially when using catechin as template molecule.

2. Methods
Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) were supplied by Sigma Aldrich. Acetonitrile (ACN) and benzoyl peroxide (BPO) were supplied by Merck. Catechin were supplied by Sigma. FTIR analysis were performed using Shimadzu IR Prestige 21. HPCL analysis were performed using infinity Agilent Technologies 1260 series HPLC with a column for the purposes Lichnosfer 100 RP 18 EC 5μm 12,5x0,4. Microwave synthesis were performed using Sineo Microwave Chemistry
Laboratories MAS-II. Thermal analysis was performed using NETZSCH STA 449 F1 Jupiter instrument.

The initial stage of this research was synthesis of NIP (non-imprinted polymer). The MIP molecules were synthesized by adding a template molecule at polymerization stage, the NIP was the polymer synthesized without the addition of template at the time of synthesis. NIP serves as a control and comparison of the performance of MIP. NIP was synthesized using two different methods, thermal method and microwave method. Thermal method used direct heating in oil bath, sand bath or electric heater. Methacrylic acid was used as monomer (M), ethylene glycol dimethacrylate as cross linking agent (Cr) and benzoyl peroxide as a polymerization initiator (I) and acetonitrile as porogen (P).

2.1. **Thermal Method**

In thermal method, a mixture of methacrylic acid (M): ethylene glycol dimethacrylate (Cr): benzoyl peroxide (I) with the ratio of 5 mmol : 30 mmole : 0.5 mmole (10:60:1), respectively and acetonitrile as porogen was placed in the reaction vesse. The mixture was sonicated for 15 minutes and then purged by nitrogen gas for 15 minutes to remove oxygen. The mixture was then heated using an oil bath at a temperature of 60-65 °C. At the beginning of the synthesis, the mixture was colorless. The mixture turned to white solids after 60 mins of heating process.

2.2. **Microwave Method**

A mixture of monomer, crosslinker, polymerization initiator and porogen with a specific composition was sonicated for 15 minutes. During the synthesis process, mixture was flowed with nitrogen gas. There were two stages of heating in microwave method. First stage lasted for 2 minutes at a temperature of 60 °C and the second stage takes place at a temperature of 65 °C for 2 minutes. The total time of 4 minutes was carried out in the microwave method. Table 1 shows the composition of the monomer, crosslinker and initiator used in the synthesis of NIP.

| Polymer | Methods     | M (mmole) | Cr (mmole) | I (mmole) |
|---------|-------------|-----------|------------|-----------|
| NIP A   | Thermal     | 5         | 30         | 0.5       |
| NIP B   | Microwave   | 5         | 30         | 0.5       |
| NIP C   | Microwave   | 5         | 5          | 0.5       |
| NIP D   | Microwave   | 5         | 50         | 0.5       |
| NIP E   | Microwave   | 5         | 30         | 0.1       |
| NIP F   | Microwave   | 5         | 30         | 2.0       |

2.3. **FTIR Analysis**

FTIR spectroscopy was performed to determine functional groups and spectral differences between reactants and reaction products. The method used was KBr pellet. The dried sample solids were mixed with KBr solids with a mass ratio of 1:10. This mixture was pressurized until a thin pellet is obtained.

2.4. **Thermogravimetric Analysis (TGA)**

The synthesized material was expected to have a good thermal resistance. Samples of less than 50 mg were placed in a container. The crucible used is aluminum oxide (Al₂O₃). The gas used was nitrogen with flow rate of 40 mL minute⁻¹. Maximum temperature being measured was 600 °C with the temperature increment rate of 30 °C per minute.

3. **Results and Discussions**

Precursor mixture with a ratio of methacrylic acid (M): ethylene glycol dimethacrylate (Cr): benzoyl peroxide (I) = 5 mmol : 30 mmol : 0.5 mmol = 10: 60: 1 was a composition that was used in both methods. Figure 2 shows the physical appearances NIP resulted from the two methods. Based on the
FTIR spectra, there were no fundamental differences between the NIP synthesized using the thermal and microwave methods (Figure 2). The list of important peaks in the FTIR spectra for NIP was presented in Table 2. FTIR spectra confirmed that the microwave method may also be used to synthesize the NIP, with shorter synthesis time.

![Figure 2](image2.png)

**Figure 2.** NIP synthesized using thermal method (a) and microwave method (b)

![Figure 3](image3.png)

**Figure 3.** FTIR spectra of NIP, EGDMA and MAA

**Table 2.** Wavenumber data for FTIR spectra of NIP thermal and microwave
The mole ratio of monomer, crosslinker and initiator was varied to obtain the optimum composition resulting NIP with best appearances and thermal stability. The compositions of monomer (MAA), crosslinker (EGDMA) and initiator (BPO) were presented in Table 1. The NIP compositions of B, C and D were prepared by variation of crosslinker without changing the amount of monomer and initiator. The NIP compositions of B, E and F have the amount of monomer and crosslinker were the same and the amount of initiator varied.

Table 3 shown the mass of products. The smallest mass was produced by NIP C while the amount of crosslinker has the least. NIP B and D have no significant difference by its mass. Number of initiator has no significant effects on the mass of the product.

| Functional group | Wavenumber (cm⁻¹) |
|------------------|------------------|
|                  | NIP thermal      | NIP microwave    |
| C-O stretch      | 1265.30          | 1261.45          |
| O-H stretch      | 3558.67          | 3610.74          |
| C-H stretch      | 2974.23          | 2956.67          |
| C=O stretch      | 1726.29          | 1730.15          |

Figure 4. FTIR spectra for NIP synthesized using microwave method

FTIR spectra of NIP B-F showed no differences (Figure 4). This indicated that the composition of the different crosslinker does not have a significant effect on the interactions between monomers, crosslinker and initiator [15].

Figure 5 shown the TGA curve for NIP synthesized using microwave method. The thermal decomposition can be divided into three stages. Stage 1 (T≤100 °C) mass loss about 2.04% due to the
mass loss caused by evaporation of volatile substances, mainly evaporation of water. Stage 2 (160°C≤T≤360°C) mass loss about 51.59% due to the weightlessness caused by decomposition of organic substance and stage 3 (360°C≤T≤440°C) mass loss about 44.87% largely resulted from the carbonization of organic matters [16]. NIP that did not have template molecule in its structure had a low ability in adsorbing the target molecule. HPLC analysis were performed using Agilent 1260 Infinity HPLC column RP 18 EC Lichnosfer 100 12.5x0.4 5μm to determine the ability of the molecular adsorption of catechins. Catechin standar was dissolved in water and analyzed using HPLC with eluent methanol:water = 1:1.

Based on the UV spectra obtained, the maximum wavelength measurements were at 280 nm. For initial research, 20 mL catechin feed solution mixed into sealed containers which have contained as much as 0.5 grams of material NIP. The mixture was stirred and allowed to stand for 30 minutes. After 30 minutes, the mixture was filtered to obtain catechin solution. This solution will be analyzed using HPLC to determine the levels of catechins (Figure 6). Concentration of catechin standard was 100 mg.L⁻¹. As shown in figure 6, catechin standard solution and extraction solution of NIP have different signal in same retention time. Catechin standard solution have higher signal than extraction solution. It showed that catechin concentration in extraction solution was lower than 100 mg.L⁻¹. NIP have ability to absorb catechin in solution [17].

![Figure 5. TGA and differential thermal curve of NIP B](image-url)
4. Conclusions
This study aimed to synthesize material NIP (non-imprinted polymer) for the retention of catechin. NIPs were successfully synthesized by two different methods, thermal method and microwave method. Thermal method takes relatively longer than the microwave method. At the same temperature, the thermal method took 60 minutes, while the microwave method took only 4 minutes. Physically, the method of microwave and thermal methods do not provide very significant difference. Variations crosslinker composition and the initiator does not give significant differences based on FTIR spectra. Percentage mass results (% yield) was also affected by the amount of crosslinker and initiator used. The best composition for NIP synthesis was 5.0 mmole MAA, 30 mmole EGDMA and 0.5 mmol BPO. TGA curve showed that NIP has thermal stability. For the preliminary analysis, the NIP has been contacted with catechin solution. The percentage decrease in peak chromatograms of standard catechins still not good although it has been decreasing. Further research will be focused on optimizing the NIP and synthesize catechin imprinted polymers (CIPs).

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