

## Synthesis of molecularly imprinted polymer (MIP) by radiation-induced polymerization and separation of ferulic acid from rice oil using MIP-packed column

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**Abstract:** A molecularly imprinted polymer (MIP) was synthesized by radiation-induced polymerization (RIP), where the ferulic acid was used as a template molecule, 4-vinylpyridine as a monomer and ethylene glycol dimethacrylate (EGDMA) as a cross-linking monomer. The MIP was packed in a glass column using a slurry method for use in medium pressure liquid chromatography (MPLC). The MPLC column was tested for separation and purification of ferulic acid from the rice oil. When repeated three times, the MPLC separation/purification yielded the ferulic acid with the purity higher than ~99%. The chemiluminescence of the luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) measured on a potato disc slide (5.0 mm thick) was enhanced in the presence of ferulic acid, while, without the ferulic acid, the chemiluminescence of luminol on the potato slice disc was not observed, which suggests the ferulic acid obtained from the rice oil can be useful for immunoassay.

**Key words:** Molecularly imprinted polymer (MIP)/Radiation-induced polymerization (RIP)/Ferulic acid/Rice oil /Chemiluminescence/Potato

### 1. Introduction

Molecularly imprinted polymer (MIP) is commonly used as a custom-made chromatographic support for separation of organic materials of similar structure.<sup>1-3</sup> MIP can also be used in drug assaying, taking advantage of their ability to mimic antibody,<sup>4,5</sup> to control the directions of reactions as designed catalyst,<sup>6,7</sup> or as substrate-selective sensors, simulating enzyme/antibody binding sites.<sup>8</sup> Since the pioneering work on MIP by Wulff and Sarhan<sup>9</sup>, a great deal of

research activities have been made in order to improve the MIP synthesis procedure and to obtain polymer-based adsorption materials with high molecular recognition capabilities.

Typically the synthesis of MIP proceeds in three steps: (1) spatial arrangement of the monomer molecules around the template molecules; (2) polymerization; (3) extraction of the template molecules from the polymer. The first step can be achieved by either covalent or non-covalent interactions between the template and the monomer molecules. Both

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approaches have been well established and approved by using polymers based on styrene, acrylate, and silica.

Ferulic acid can be extracted in a large amount from rice bran. It is well known that the ferulic acid acts as an antioxidant,<sup>10</sup> and are widely used in cosmetic formulations for continuous protection as a photo-protectant or for delaying the premature aging of skin. In addition, some of its lipophilic esters are now under screening in several tumor cell lines for prevention of photo-induced skin tumors.<sup>11</sup>

Polymerization is generally initiated either photochemically (e.g., UV light) or thermally (elevated temperature at 35-80°C) in the presence of specific polymerization-initiators. Both initiation techniques yield imprinted polymers of satisfactory qualities, although the selectivity of the polymers strongly depends on the reaction temperature: the lower the temperature during polymerization, the higher the separation factors.<sup>12</sup> This phenomenon can be attributed to the thermal destabilization of the complexes between the template molecules and the functional monomers.

The radiation-induced polymerization (RIP) carries some merits that it can be completed at room temperature, and it does not require the presence of initiators. RIP has been employed for the synthesis of various types of materials including polymer beads<sup>13</sup>, polymer-clay nanocomposites<sup>14</sup> and the polymer-metal nanocomposites.<sup>15,16</sup> Still, little has been reported on the synthesis of MIP by RIP.

In this paper, synthesis of MIP by RIP is reported with 4-vinylpyridine as a functional monomer, EDGMA as a cross-linking monomer in the presence of the ferulic acid as a template molecule.

## 2. Experimental

### 2.1. Materials

The chemicals used in this work are: ethylene glycol dimethacrylate (EGDMA) and 4-vinylpyridine (4-VPy) (obtained from Sigma-Aldrich Korea (Korea); chloroform, n-hexane and methanol (obtained from Samchun Chemical Co., Korea); Ferulic acid (Juno-

Rice Co., Japan). The rice oil was obtained from Searimhyunmi Co. (Korea). All chemicals were used as received, without further purification.

### 2.2. Preparation of MIP and MIP column

Ferulic acid (template molecule, 3.3 g), 4-VPy (functional monomer, 5.8 g), EDGMA (cross-linking monomer, 94.0 g) were mixed in chloroform (250 ml), and sonicated in a sealed glass vessel at room temperature for 15 min. In above mixture, the molar ratio of the template molecule, the functional monomer and the cross-linking monomer was maintained as 1/3.2/22.8. This solution was bubbled with nitrogen gas in order to remove dissolved oxygen for 30 min. Finally, the solution was irradiated with  $\gamma$ -radiation (Co-60 source) at a dose rate of 27.7Gy min<sup>-1</sup> for 180 min, giving the total dose of 5.0 kGy. A blank polymer was prepared in the same manner with only the template molecule (ferulic acid) missing. After the reaction was completed, the reaction mixture was filtered through a filter paper (Whatman filter paper No. 2) and dried in a vacuum oven at 60°C for 7 hr.

The dried MIP was crushed and ground with a ball-mill for 10 hr at the stirring speed of 600. The MIP particles were sieved through a 250-mesh screen, and then were packed into a glass column (200 mm × 80 mm ID) using a slurry method for use in medium pressure liquid chromatography (MPLC). The MPLC column packed with MIP particles was tested for separation and purification of ferulic acid from rice oil. The procedure for the preparation of a MIP-packed MPLC column is schematically shown in *Fig. 1*.

### 2.3. Liquid chromatography (MPLC and HPLC)

The phenolic acids (including the ferulic acid) were extracted from the rice oil using a liquid extraction. A MPLC (Prep. UV-10V, Yamazen, Osaka, Japan) equipped with a MIP-packed glass column described above was employed for separation and purification of ferulic acid from the phenolic acids extracted from the rice oil. Both the phenolic acids and the ferulic acid were analyzed using a HPLC

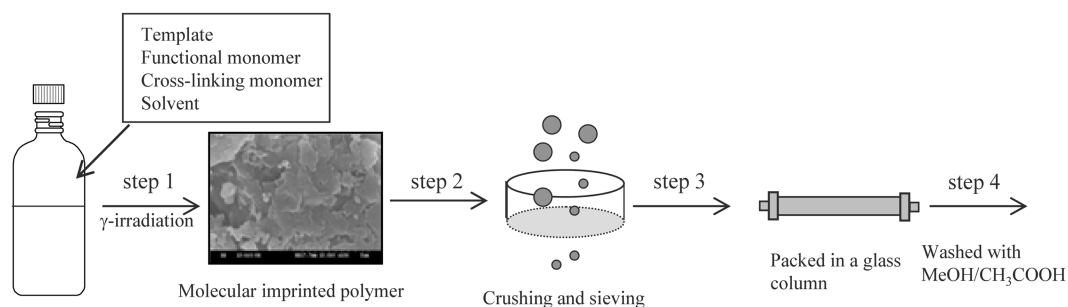


Fig. 1. Preparation procedure of a MPLC column packed with MIP synthesized by RIP.

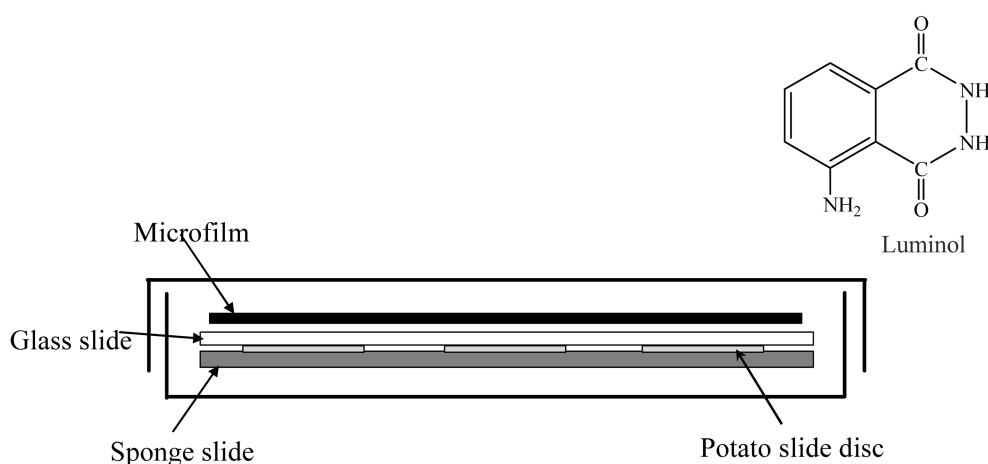


Fig. 2. Measuring device of chemiluminescence of luminol on a potato disc slide.

(Series 1100, Hewlett Packed Co., Boeblingen, Germany) equipped with a ODS column (250 mm  $\times$  4.6 mm ID). Details on the extraction of phenolic acids from rice oil will be explained later.

#### 2.4. Chemiluminescence of luminol in potato slide in the presence of ferulic acid

The chemiluminescence of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) was measured on a potato disc slide having the thickness of 5.0 mm in the presence of ferulic acid in a device shown in Fig. 2. The potato disc slide was placed on the wetted sponge slide in a glass dish, and then covered with a glass slide. A microfilm was finally placed on the glass slide in order to measure the chemiluminescence.

#### 2.5. Characterization of MIP

The MIP prepared by RIP was analyzed by a FT-

IR (Spectrum 1000, Perkin Elmer, USA), a Solid state  $^{13}\text{C}$ -NMR (DSX 400MHz, Bruker Analytische GmbH, Germany), a thermogravimetric analysis (TGA, SDT Q600, TA Instruments, USA), and BET analysis (KICT-SPA3000S Gas Adsorption Analyzer, Korea). The thermogravimetric analysis of the MIP (7-8 mg) was carried out under an argon atmosphere in the temperature range of 50-700°C. The heating rate was 20°C min $^{-1}$ .

### 3. Results and Discussion

#### 3.1. Synthesis of MIP

In a previous study [13], the radiation-induced polymerization (RIP) of ethylene glycol dimethacrylate (DEGMA) in organic solvent was reported, which yielded polymeric microspheres. As mentioned earlier, one of advantages of RIP is that it does not

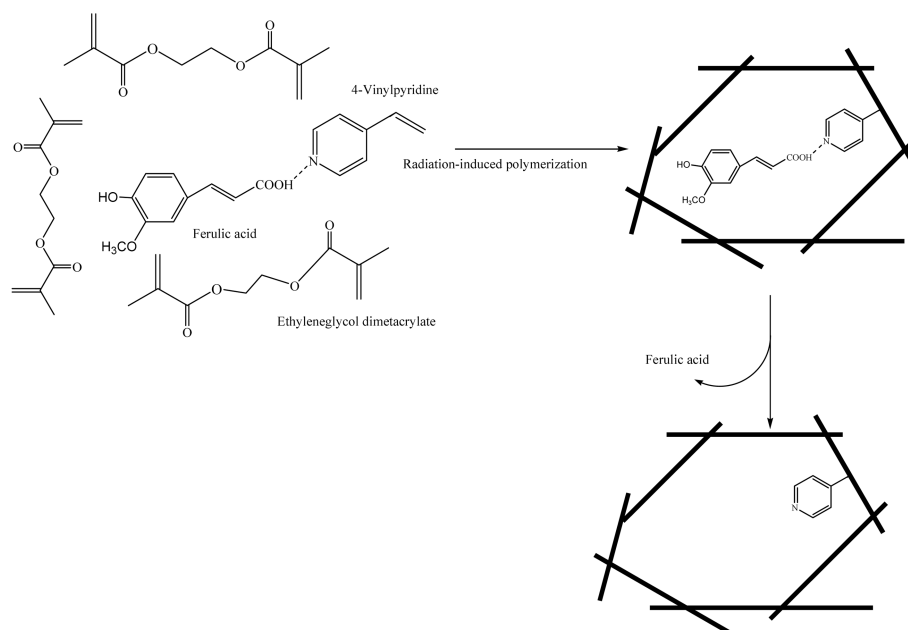


Fig. 3. Preparation of molecularly imprinted polymer (MIP) by radiation-induced polymerization using ferulic acid as a template molecule and 4-vinyl pyridine as a functional monomer.

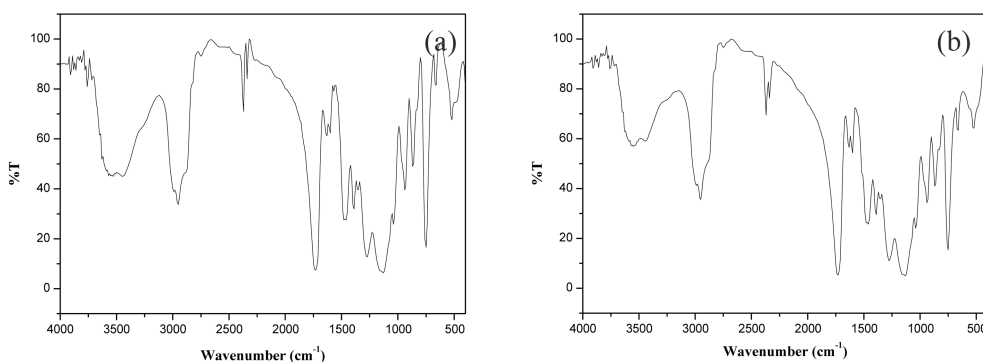


Fig. 4. FT-IR spectra of (a) blank polymer (without ferulic acid) and (b) MIP (with ferulic acid) prepared by RIP.

require a surfactant (or a stabilizer) and an initiator. A mixture of only the monomer and solvent yielded monodispersed microspheres. A MIP polymer can be prepared by RIP by the same manner. Fig. 3 shows the procedure for molecular imprinting of ferulic acid using 4-VPy as a functional monomer and EGDMA as a cross-linking monomer in chloroform. Pyridine is intrinsically a rather weak base ( $pK_a=5.4$ ), but forms sufficiently strong hydrogen bonding adducts with ferulic acid as shown in Fig. 3.

### 3.2. Characterization of MIP

Fig. 4(a) shows FT-IR spectrum of the blank polymer prepared without the template molecule (ferulic acid), and Fig. 4(b) that of the MIP prepared with the template molecule by RIP, respectively. In Fig. 4(b), the absorption bands specific to the ferulic acid are not noticed, probably because the amount of the ferulic acid is much lower than that of EGDMA. As mentioned earlier, the mol ratio of the ferulic acid to EGDMA is about 1/23.

Fig. 5(a) shows solid state <sup>13</sup>C-NMR spectrum of

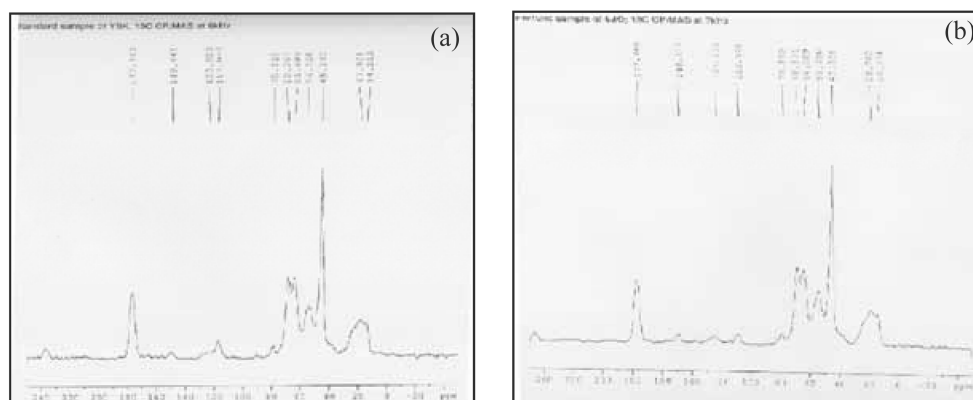


Fig. 5. Solid state  $^{13}\text{C}$ -NMR spectra of (a) blank polymer (without ferulic acid) and (b) MIP (with ferulic acid) prepared by RIP.

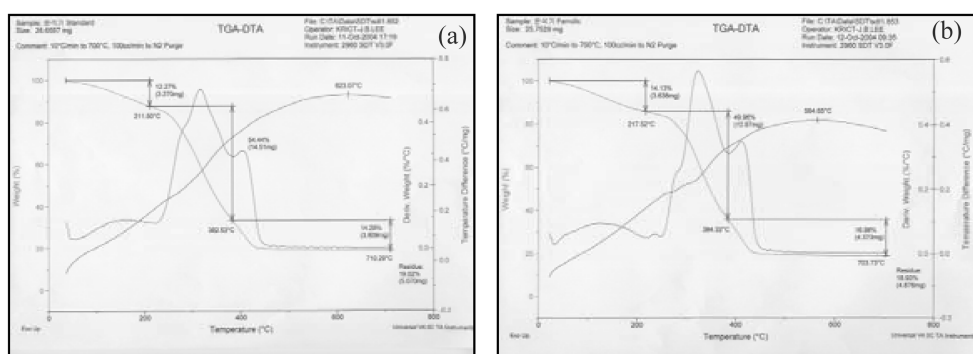


Fig. 6. TGA/DTA curves of (a) blank polymer (without ferulic acid) and (b) MIP (with ferulic acid) prepared by RIP.

the blank polymer and Fig. 5(b) that of the MIP prepared with the template molecule by RIP, respectively. Observed in both Fig 5(a) and 5(b) are the  $\text{CH}_3$ - peak at  $\sim 17$  ppm,  $-\text{CH}_2$ - peaks at  $\sim 45$  ppm,  $-\text{CH}_2\text{-O}$ - peaks at  $\sim 70$  ppm, a unreacted  $\text{C}=\text{C}$  peak at  $\sim 120$  ppm, and a carbonyl peak ( $\text{C}=\text{O}$ ) at  $\sim 177$  ppm at the ester linkage. A signal was observed at  $\sim 243$  ppm, which may be due to the triplet carbon that is formed during radiation-induced polymerization.

Fig. 6(a) shows TGA/DTA curves of the blank polymer and Fig. 6(b) that of the MIP prepared with the template molecule by RIP, respectively. In Fig. 6(a), the 1<sup>st</sup> weight-loss in the range of  $100\sim 200$  °C can be attributed to the vaporization of the unreacted monomers, and the 2<sup>nd</sup> weight loss in the range of  $200\sim 430$ °C to the decomposition of the polymer backbone. Unlike in Fig. 6(a), a weight-loss was found at  $230$ °C in Fig. 6 (b), seemingly due to the

vaporization of the template molecule (ferulic acid).

Finally, both the blank polymer and the MIP were analyzed using a single point BET method using nitrogen. The specific surface areas measured for the MIP and the blank polymer were  $15.6$  and  $25.3$   $\text{m}^2/\text{g}$ , respectively. This difference in the specific surface area suggests the template molecule has a certain influence on the propagation of polymerization in the space.

### 3.3. Extraction of phenolic acids from rice oil

Although the phenolic acids are insoluble in water, its salts (e.g., sodium phenolate) are soluble in water.  $20$  mL of rice oil was dissolved in  $100$  mL of hexane, and mixed with  $200$  mL of  $0.5\text{M}$  NaOH for  $24$  hr while stirring at room temperature. This is to change all phenolic acids (including the ferulic acid)

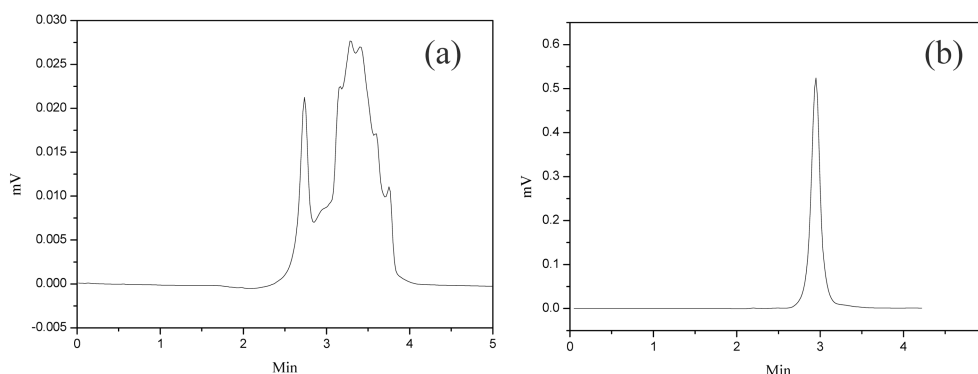


Fig. 7. HPLC chromatograms of (a) phenolic acid obtained by 'salt-nation' and (b) ferulic acid obtained from separation by MPLC with the MIP-packed column.

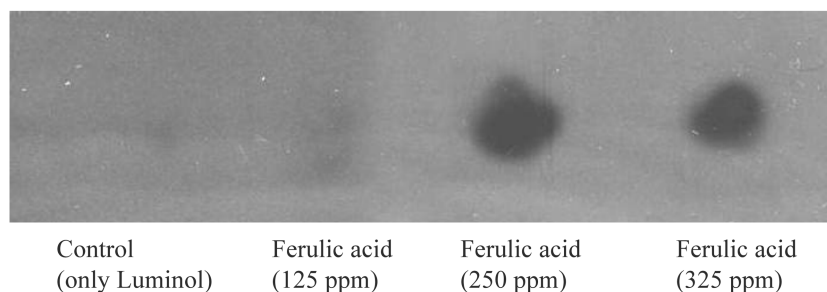
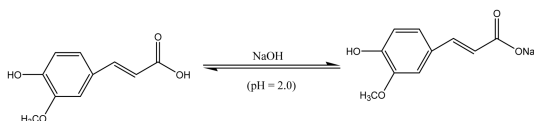


Fig. 8. Chemiluminescence of luminol measured on a potato disc slide in the presence of ferulic acid.

in the rice oil to their salt forms, which are soluble in water. Only the aqueous layer was taken using a separatory funnel (pH=13). Finally, 6M HCl solution was added into the aqueous layer until pH becomes 2.0, which will make the salts change back to acids as shown in the following scheme.



The phenolic acids precipitated as white powder in acidic solution, and were recovered by centrifugation.

#### 3.4. Separation and purification of ferulic acid by MPLC with a MIP-packed column

The ferulic acid was separated and purified using a MPLC by the following procedure. First, the phenolic acids obtained by the 'salt-nation' above

was dissolved in MeOH, and then passed through the MIP-packed column. Due to its strong 'interaction' with MIP, the ferulic acid will be retained in the MIP-packed column, while the other phenolic acids pass through the column. The ferulic acid retained in the column was eluted from the column with a mixed solvent of methanol and acetic acid in the volume ratio of 7/3. This procedure was repeated three times, and the ferulic acid with the purity higher than 99% was obtained from HPLC chromatogram results. The phenolic acids obtained by 'salt-nation' and the ferulic acid separated with the MIP-packed column were analyzed by a HPLC as shown in Fig. 7.

#### 3.5. Measurement of chemiluminescence on potato slide

Phenolic acids have a carboxylic acid group and one or more hydroxyl groups at the benzene ring. It is known that the chemiluminescence of the luminol-H<sub>2</sub>O<sub>2</sub>-horseradish peroxidase is either increased or decreased depending upon the position and the

nature of the substituents on the benzene ring and also upon the redox characteristics of the phenolic acid,<sup>17</sup> whose mechanism has been proposed by Thorpe *et al.* in 1987 and also by Lundin *et al.* in 1987.<sup>18,19</sup> Phenolic acids are natural products which influence the growth of plants by interacting with vegetable hormones.<sup>20</sup> The chemiluminescence of luminol has been measured on a potato disc slide in the presence of arachidonic acid and salicylic acid.<sup>21</sup> The chemiluminescence of luminol in the presence of ferulic acid obtained from rice oil has not been reported yet.

Fig. 8 shows the chemiluminescence of luminol measured on a potato disc slide (5.0 mm thick) with or without the presence of the ferulic acid. The chemiluminescence was observed with the presence of ferulic acid, while it was not observed without, and the intensity of chemiluminescence increases with increasing concentration of the ferulic acid. This result suggests that the ferulic acid can be useful for immunoassay.<sup>22</sup>

#### 4. Conclusions

A molecularly imprinting polymer (MIP) has been successfully synthesized by a radiation-induced polymerization (RIP) rather easily at room temperature without an initiator. Unlike in other polymerization, there is no need for an initiator in RIP, since the radiation provides a continuous supply of free radicals. The ferulic acid was separated and purified (higher than 99%) from the rice oil by MPLC with a glass column packed with the MIP. The chemiluminescence of luminol measured on a potato disc slide was enhanced in the presence of the ferulic acid, which suggests the ferulic acid can be useful for immunoassay.

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