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Simultaneous extraction of organic and inorganic compounds using molecularly/ion imprinted polymer

Yelin Lee, Hyeyoung Jung, Soomi Park, and Sunyoung Bae★

Department of Chemistry, Seoul Women's University, Seoul 01797, Korea

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Abstract: 5-Hydroxymethyl-2-furaldehyde (5-HMF) is considered one of the main quality indexes of various food products. Its metabolism in humans can potentially lead to carcinogenic compounds. Metallic ions such as Zn, Mg, Mn, and Fe have been reported to enhance 5-HMF formation. Recently, studies on adsorbents that can extract specific organic and inorganic substances with one adsorbent have been conducted. However, simultaneous analysis of organic and inorganic materials typically requires distinct pre-treatment and analytical methods, which increase a lot of labor and cost. In this study, hybrid imprinted polymer (HIP) by mixing 5-HMF imprinted polymer (FIP) and zinc ion imprinted polymer (ZIIP) were generated to extract two analytes, Zn ion and 5-HMF, simultaneously. Physicochemical characterization of HIP was conducted by Fourier-transform infrared spectroscopy, scanning electron microscopy, and sorption tests. Extraction conditions including adsorbent mixing ratio, adsorbate mixing range, and equilibrium time were optimized. Freundlich adsorption model was as the best-fitting isotherm model to elucidate the adsorption mechanism. Affinity of Zn ion and 5-HMF on HIP was superior to non-HIP. In conclusion, HIP showed reasonable feasibility that could be used as an adsorbent to be used for simultaneous extraction of organic and inorganic compounds present in the sample.

Key words: molecularly/ion imprinted polymer, 5-Hydrocxymethyl-2-furaldehyde, zinc ion, simultaneous extraction

1. Introduction

5-Hydroxymethyl-2-furaldehyde (5-HMF) is considered one of the main quality indexes of different groceries. 5-HMF might be metabolized by humans to potentially carcinogenic compounds.[1] Number of metallic ions such as Zn, Mg, Mn, and Fe have been reported to increase 5-HMF formation.[2] Recently, studies on adsorbents that can extract specific analytes efficiently have been conducted. However, the analysis of organic and inorganic materials from a sample might require different pre-treatment and analytical methods, which becomes labor intensive and costly. Molecule imprinting technique offers high affinity and selectivity for specific target compounds and is easy to prepare with low cost.[3]

Molecularly/ion imprinted polymers (MIPs/IIPs) are synthetic materials designed to have specific

★ Corresponding author
Phone : +82-(0)2-970-5652 Fax : +82-(0)2-970-7712
E-mail : sbae@swu.ac.kr

binding sites for target molecules/ions recognizing similar to antibodies or receptors.[4] Target analyte as a template is mixed with functional monomer to bind through covalent or non-covalent bonds. This template-monomer complex is polymerized in the presence of a crosslinker to stabilize the structure and create a rigid three-dimensional network around the template. To imprint cavities specifically for target analytes, the template is removed by washing with solvents.[3,4] These cavities are complementary in shape, size, and functional group to the target analytes.[4] MIPs/IIPs can be applied to various platforms and fields including the detection of heavy metal ions, organic molecules, nucleotides, and many more.[5-7]

Previous research has primarily focused on developing polymers that target either organic or inorganic compounds individually.[2,3,5,6] Due to its limitation, its application is only available to simpler matrices.[3] Real sample often contain a complex mixture of both types of compounds making single-target extraction method insufficient for comprehensive analysis. By employing imprinted polymers to enable the simultaneous recognition and extraction of both compounds, this approach would overcome the limitations of traditional methods.

In this study, 5-HMF Imprinted Polymer (FIP) and Zinc Ion Imprinted Polymer (ZIIP) were synthesized and mixed to extract two analytes at the same time. Extraction conditions including adsorbent mixing ratio, adsorbate mixing range, and equilibrium time were optimized. Adsorption isotherm test was conducted to identify adsorption mechanism by mixing various concentrations of 5-HMF and Zn ion. Selectivity test was also performed using homologues of 5-HMF and Zn ions which have similar radius and charge.

2. Experimental

2.1. Reagents and materials

Zinc nitrate hexahydrate (98 %) as a template and 4-vinylpyridine (95 %) as a monomer were purchased from Sigma-Aldrich (Missouri, USA). Ethylene glycol dimethacrylate (>97.0 %) as a crosslinker and benzoyl peroxide (>75.0 %) as an initiator were obtained from TCI (Tokyo, Japan). Acetonitrile (99.9 %) as a porogen was purchased from Daejung Chemicals & Materials (Siheung, Korea). Dithizone (>98 %) as an indicator was purchased from Alfa Aesar (Massachusetts, USA). Acetic acid (>99.5 %) was obtained from Ducksan Reagents (Ansan, Korea) and methanol (99.9 %) was obtained from Samchun Pure Chemical (Pyeongtaek, Korea). Iron(II) chloride tetrahydrate (99.0 %) and iron(III) chloride hexahydrate (98.0 %) were purchased from Daejung Chemicals & Materials (Siheung, Korea). Lead(II) nitrate (97.0 %) was obtained from Samchun Pure Chemical (Pyeongtaek, Korea). Cadmium(II) nitrate tetrahydrate (> 99.0 %), cobalt(II) nitrate hexahydrate (98.0 %), and nickel(II) chloride (>98 %) were purchased from Sigma-Aldrich (Missouri, USA).

5-Hydroxymethyl-2-furaldehyde (97 %) and methyl 5-methyl-2-furoate (97 %) were purchased from Alfa Aesar (Massachusetts, USA). 5-Methyl-2-furfural was obtained from TCI (Tokyo, Japan). Syringe filter (polytetrafluoroethylene (PTFE) with glass fiber pre-filter (13 mm I.D. × 0.2 μm pore size) used for filtration of the supernatant was purchased from Echromscience (Daegu, Korea). Syringe filter (polyvinylidene fluoride (PVDF), 25 mm I.D. × 0.45 μm pore size) used for filtration of diluted honey sample was purchased from Hyundai Micro (Seoul, Korea). Double distilled water (18.2 MΩ·cm, PURE ROUP 50, Purewater, Kyeonggi-do, Korea) was used for entire experiments.

2.2. Synthesis of 5-HMF molecularly imprinted polymer and Zn ion imprinted polymer

Hybrid imprinted polymer in this study was synthesized to imprint the cavity structures of target compounds, Zn and 5-HMF, by bulk polymerization method as described in previous studies.[3,8] For Zn imprinted polymer, 0.4 mmol of zinc nitrate was dissolved in 1 mL of acetonitrile (ACN), then 1.2 mmol of 4-vinylpyridine (4-VP), 6.0 mmol of ethylene glycol dimethacrylate (EGDMA), and 0.017 mmol of benzoyl peroxide (BPO) were sequentially added at the various mole ratios.[3] In each step, the solution was thoroughly mixed by vortexing and then followed by purging with N_2 for 20 s. Then, the solution was

placed in the oven at 110 °C for 45 min to be polymerized (ZIIP_bulk). The final polymer was ground after cooling. To create the Zn imprinted cavity on the polymer, Zn ion and unreacted substances were removed by mixing with removal solvent, a mixture of acetic acid and methanol at the ratio of 1:9 (v/v). The mixture was stirred for 2 h with removal solvent at 1:2 ratio and replaced the removal solvent until the Zn ion was completely removed. After confirming the Zn removal completion, fresh methanol was added to the polymer and stirred for 12 h and dried at 65 °C for 12 h. The final Zn imprinted polymer (ZIIP) was generated and stored at room temperature before further use (*Fig.* 1(A)). Non-Zn ion imprinted polymer (NZIIP) was prepared in the same condition as Zn imprinted polymer synthesis as shown in *Fig*. 1(B).

5-HMF imprinted polymer was synthesized as described in the previous study.[8] After mixing 1 mmol of 5-HMF, 5 mL of ethyl acetate (EA) and 4 mmol of methacrylic acid (MAA), and allow them to stand for 6 h so that hydrogen bonding was sufficiently formed. Then, 20 mmol of EGDMA and 0.05 g of BPO were added and mixed thoroughly. An aliquot, 3 mL of this solution was transferred into 20 mL vial to polymerize in oven at 85 °C for 15 h. After cooling the polymer, the final polymer (FIP_bulk) was prepared via grinding process. To remove 5-HMF and impurities from the polymer, removal was conducted for 24 h using 5 M HCl. After the step was completed, the polymer was washed with methanol for 2 h and then dried in oven at 65 °C for overnight (FIP) presented in *Fig.* 1(C). Non-5 HMF imprinted polymer (NFIP) was synthesized in the same condition as 5-HMF polymer synthesis without 5-HMF. Its non-5-HMF imprinted polymer was also performed in the same condition as 5-HMF imprinted polymer without

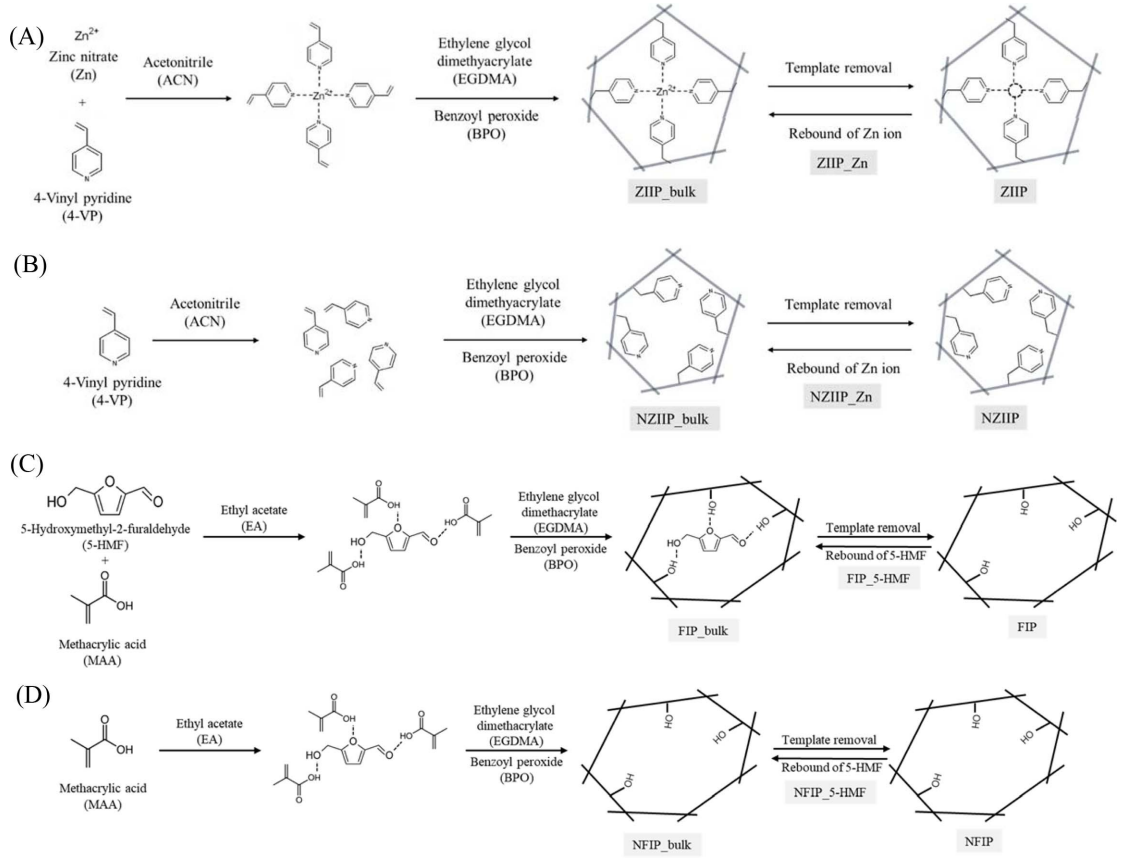

Fig. 1. Schematic representation for the synthesis procedures of (A) ZIIP, (B) NZIIP, (C) FIP, and (D) NFIIP.

template (*Fig.* 1(D)).

2.3. Characterization of synthesized imprinted polymers

The surface characteristics and particle size of the prepared polymers were analyzed using Fourier transform-infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), melting point measurement, and Thermogravimetric analyzer (TGA). The functional groups of imprinted polymer was analyzed using a KBr method using a FT-IR (Spectrum 100, Perkin Elmer, Waltham, Mass., USA) in the range of 400 cm^{-1} – 4000 cm^{-1}. The surface of HIP was conducted by Magellan 400 scanning electron microscope (FEI company, Hillsborough, Switzerland). A melting point measurement system (SMP3, Cole-Parmer, Staffordshire, UK) was used to verify the thermal properties of the synthesized polymer. The temperature of the polymer was increased from room temperature to 280 ºC at a rate of 5 ºC/min.

2.4. Hybrid Imprinted polymer (HIP) preparation and sorption test

The optimal ratio was investigated to prepare hybrid imprinted polymer (HIP) by mixing ZIIP and FIP and the amount of Zn and 5-HMF sorbed on the HIP was measured. ZIIP and FIP imprinted polymers as adsorbent were combined at the various mixing ratios (9:1, 8:2, 7:3, 5:5, and 1:9) while the amount of total adsorbent was fixed to 0.1111 g. The same experiment was performed to non-HIP (NHIP) which was a mixture of NZIIP and NFIP.

To investigate the effect of adsorbate on the adsorption of Zn ion and 5-HMF, the amount adsorbed was investigated at various adsorbate concentrations added to solution. The total concentration of analytes was fixed, mole ratios of Zn ion and 5-HMF as adsorbates were varied to 0:9, 2:7, 3:6, 4.5, 6:3, 7:2, and 9:0. The mole ratio was presented as Eq. (1), where [Zn] is the concentration of Zn ion (mmol·dm^{-3}) and [5-HMF] is the concentration of 5-HMF (mmol·dm^{-3}).

$$\frac{\text{Moles of Zn}}{\text{Sum of moles of Zn and 5-HMF}} = \frac{[\text{Zn}]}{[\text{Zn}]+[5-\text{HMF}]} \quad (1)$$

The equilibrium time was optimized by performing kinetic test. 0.1111 g of HIP were mixed with 9 mL of Zn and 5-HMF standard solution. The mixtures were placed and shaken in the shaker at 250 rpm to measure adsorption while maintaining the temperature at 25 ºC.

A selectivity test was also conducted using compounds with a similar shape and size to 5-HMF and the same charge as the Zn ion including 5-hydroxymethyl-2-furaldehyde, 5-methyl-2-furfural, methyl 5-methyl-2-furoate, Fe^{2+} and Co^{2+}. In the previous study, solid:solution ratio of ZIIP was optimized at 1:80 (0.1 g: 8 mL), and FIP at 1:90 (0.0111 g: 1 mL).[3,8]

Sorption and selectivity tests were conducted as follows. 0.1111 g of HIP was mixed with 9 mL of a mixture of Zn and 5-HMF standard solution. The mixtures were shaken in the shaker at 250 rpm for 24 h while the temperature maintained at 25 ºC. At the end of sorption experiment, the supernatant after centrifugation was filtered by 0.2 μm pore size syringe filter (PVDF, 25 mm I.D. × 0.45 μm). Zn ion and 5-HMF left in the solution were analyzed by UV-Vis spectrophotometer and ultra-performance chromatograph (UPLC) coupled with UV-Vis spectrometer, respectively.

2.5. Determination of 5-HMF and Zn ion in real samples

The real sample was tested using acacia honey commercially available (Dongseo company, Korea). It was homogenized by hands for 1 min before dilution. To make 25 % (w/v) of honey stock solution, 12.5 g of honey was dissolved in 50 mL of methanol. A stock solution was diluted after filtration using a syringe filter (PVDF, 25 mm I.D. × 0.45 μm). To minimize the matrix effect, real sample was diluted to 0.01 % (w/v) for method validation. The real samples were prepared by spiking the Zn ion and 5-HMF standard solutions. Quantitative analysis of the real sample was performed by UV-Vis spectroscopy for Zn ion after forming a red complex with dithizone[3] and ultra-performance liquid chromatography-UV/Vis detector (UPLC Nexera XR series equipped with SPD-20A UV/VIS detector, Shimadzu, Kyoto, Japan) for 5-HMF

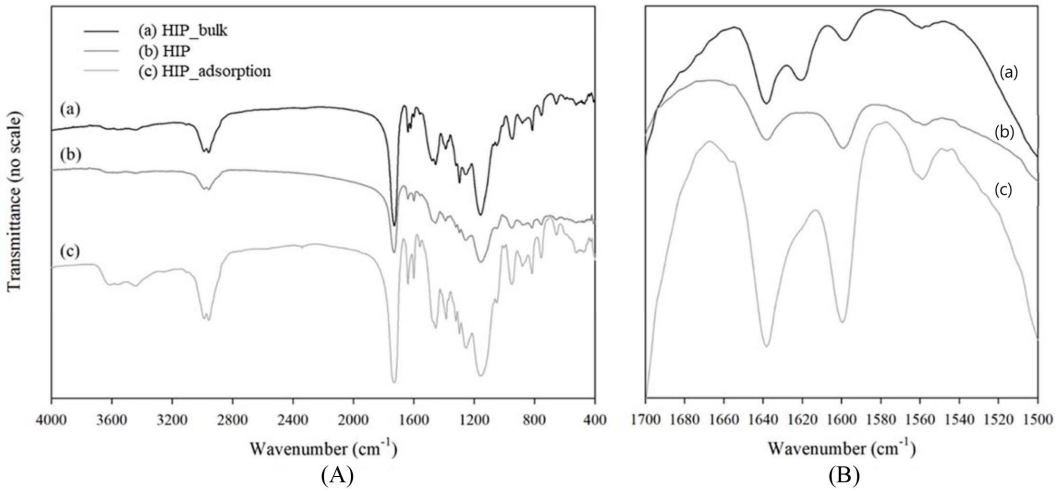

Fig. 2. FT-IR spectra of (a) HIP_bulk, (b) HIP, and (c) HIP_adsorption. (A) between 400 cm^{-1} and 4000 cm^{-1} and (B) between 1500 cm^{-1} and 1700 cm^{-1}.

determination. Chromatographic separation was utilized to enhance sensitivity and precision to measure 5-HMF from the complex matrices. It was achieved on an ACE® C18-PFP column (150 mm × 2.1 mm, 3 μm particle size). The mobile phase consisted of acetonitrile and water with 0.5 % formic acid in a ratio of 3:7 (v/v) and delivered at a flow rate of 0.2 mL/min. The injection volume was 10 μL, and the detection was carried out at 277 nm. The mass spectrometer was operated in electrospray ionization (ESI) positive ion mode using selected ion monitoring (SIM) for detection. The interface voltage was set to 4.5 kV, with a desolvation line temperature of 250 °C and a drying gas flow rate of 5.0 L/min. The detector voltage was maintained at 1.05 kV.

3. Results and Discussion

3.1. Characterizations of HIP formation

The functional groups of ZIIP and FIP of HIP formation were analyzed using Spectrum 100 Fourier transform spectroscopy (Perkin Elmer, Waltham, USA) with the KBr pellet method to confirm the polymer synthesis. FT-IR spectra were expected to identify the shift of a specific peak during the polymerization process, which involves the binding of the template with the monomer.[3,9] Additionally, the spectra were anticipated to show the changes after the removal of template, where no binding between the template and monomer occurs. Regarding ZIIP synthesis, the presence of Zn binding with pyridine ring of 4-VP (HIP_bulk, HIP_adsorption) at 1620 cm^{-1} was used to confirm the completion of ZIIP polymerization[3] while no-binding of Zn to 4-VP (HIP) was found at 1602 cm^{-1} in *Fig.* 2. *Fig.* 2(B) is an enlargement of the spectrum around this region. Comparatively, the presence of 5-HMF was confirmed through an adsorption study instead of by FT-IR peaks because a specific peak shift caused by the binding of the template with the monomer was not found.

The SEM images were used to confirm that the pore structure of the HIP and the difference on the surface of polymerization, removal, and adsorption using HIP made of ZIIP:FIP = 9:1, and ZIIP:FIP = 5:5, respectively. The surface morphology of HIP polymerization, removal, and adsorption were analyzed by Magellan 400 scanning electron microscope (FEI company, Hillsborough, Switzerland). *Fig.* 3 shows the high-resolution images with secondary electrons and the difference in images with backscattered electrons (BSE). In *Fig.* 3, HIP_bulk (*Fig.* 3(A)), HIP (*Fig.* 3(B), and HIP_adsorption (*Fig.* 3(C)) surfaces were all formed in a spherical bead shape. This structure is essential for maximizing the surface area

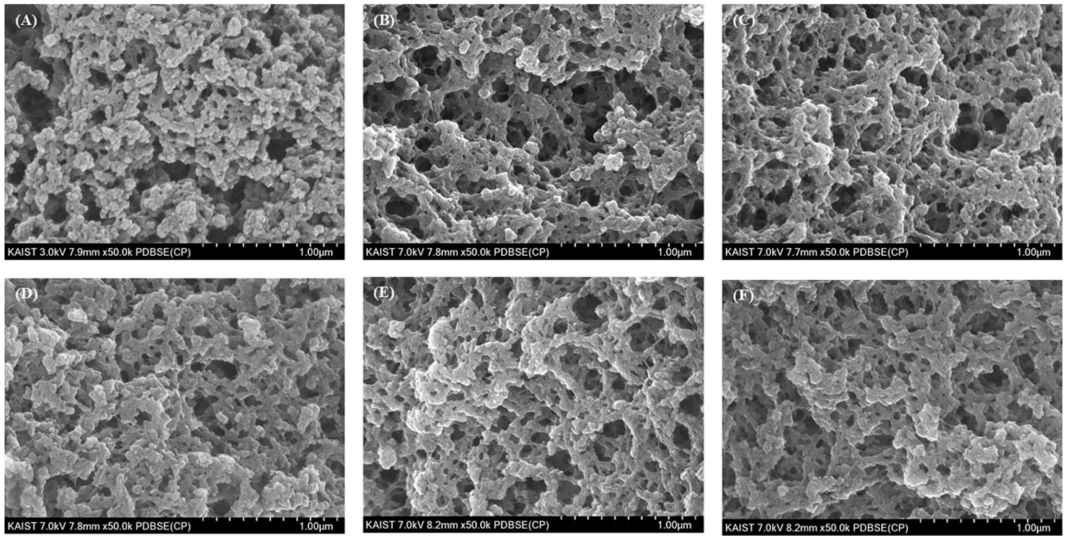

Fig. 3. SEM images of HIP at each adsorption step. (A) Polymerization, (B) removal, (C) adsorption of ZIIP:FIP = 9:1, (D) Polymerization, (E) removal, and (F) adsorption of ZIIP:FIP = 5:5.

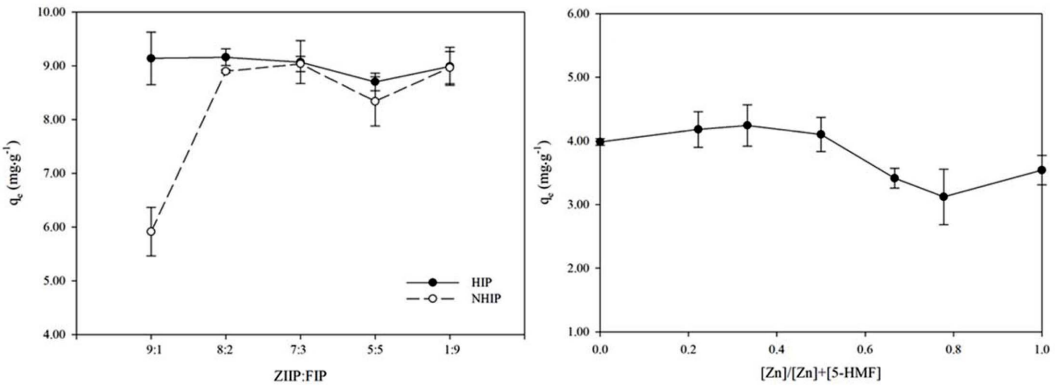

Fig. 4. The optimized adsorption conditions. (A) Optimized mixing ratio of adsorbent and (B) effect of mixing ratio of adsorbate.

of the adsorbent and for efficient adsorption. The type of porogen mainly influences on porosity of the polymer and pore diameter. Acetonitrile might create a better micropore structure and large surface area than other porogen such as dichloromethane and chloroform.[10] No significant difference between HIP and HIP_adsorption after target compounds removal was observed on the SEM image because the atomic radius of Zn ion and molecule size of 5-HMF might be smaller than that of SEM would detect. HIP was thermally stable up to the temperature of 200 °C.

3.2. Optimization of extraction conditions

For simultaneous extraction of Zn ion and 5-HMF, ZIIP and FIP were mixed. FIP and ZIIP were combined at the optimized amount of adsorbent at ratio of 9:1 as shown in *Fig.* 4(A). Most of the ratios have the similar specific adsorption amount, q_e, of HIP and NHIP, except 9:1 which is determined as the optimal condition of mixing ratio. When the proportion of FIP added to HIP exceeds 10 %, it may result in increased non-specific binding of Zn ion and 5-HMF.

To determine the effect of analytes ratios in solution, the q_e of HIP was investigated at a various mixing ratio

of adsorbate (*Fig.* 4(B)). The adsorption amount remained similar up to a ratio of 0.5. However, when the proportion of Zn exceeded half of the total amount, the q_e decreased. Thus, HIP could be applicable to a sample which has more 5-HMF than Zn ratio in solution. 5-HMF, predominantly found in honey, is an indicator used to determine its freshness.[2,11] Although Zn ion can be present in trace amount in honey,[12] HIP might be effectively utilized for the majority of honey samples due to its reliable adsorption capacity.

Equilibrium time was determined by the kinetic model. *Fig.* 5 shows the plot of HIP applied to pseudosecond-kinetic model (*Eq.* (2)).

$$\frac{t}{q_t} = \frac{1}{V_0} + \frac{1}{q_e} t \tag{2}$$

The adsorbed amount at specific time, q_t (mg·g^{-1}), is a function of time, t (min). The equilibrium amount, q_e, can be calculated using Eq. (2). The initial adsorption rate, V_0, represents the starting rate of adsorption. The linear equation of the model had a slope of 0.1057 mg·g^{-1} and a y-intercept of -2.0926 min·g·mg^{-1}. At equilibrium, the Zn ion adsorbed on HIP was calculated to be 9.46 mg·g^{-1}. Based on the raw data (not shown), the equilibrium time was optimized to 1440 min, as the adsorbed amount reached equilibrium at this time.

3.3. Adsorption isotherm

Langmuir, Freundlich, Temkin, Dubinin–Radushkevich, and Elovich isotherms models were applied to the adsorption of Zn ion and 5-HMF on HIP to determine the best-fitting isotherm.[13] The optimum isotherm is defined as the one that best models the experimental data characterized by a high value of the coefficient of determination, R.[2,14] The Freundlich isotherm (Eq. (3)) was found to provide an excellent fit for the adsorption process with an R^2 value of 0.9658. In comparison, the R^2 values for the Langmuir, Temkin, Dubinin-Radushkevich, and Elovich isotherms were 0.3635, 0.8075, 0.2935, and 0.5540, respectively.

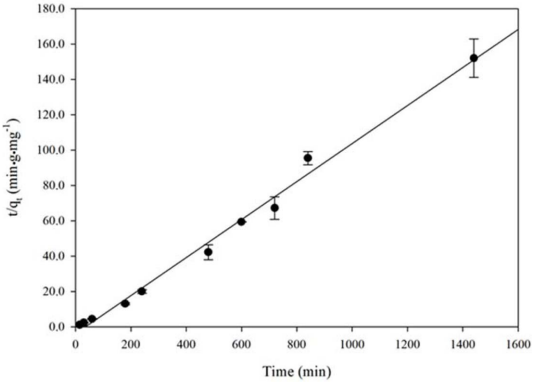

Fig. 5. The kinetic test result of HIP applied to pseudo-second-kinetic model.

$$q_e = K_F C_e^{1/n} \tag{3}$$

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \tag{4}$$

Where K_F is Freundlich isotherm constant (mg·g^{-1}), C_e equilibrium concentration (mg·L^{-1}), and n is adsorption intensity.

Freundlich isotherm explains the non-ideal and reversible adsorption process that occurs on heterogeneous surfaces.[15,16] It describes multi-layer sorption, and adsorption heat and affinities were distributed to surface non-uniformly.[16] Since the analytes were a mixture of Zn ion and 5-HMF, they might be adsorbed to the adsorbent surface to multilayer. When n approaches 1, it indicates that the surface on which adsorption occurs is relatively uniform and has a high adsorption capacity. If $n < 1$, it indicates a chemical adsorption process and $n > 1$, it implies a physical adsorption process. The values of n between 1 and 10 mean good adsorption.[17] The value of n for HIP was 1.17 and 0.166 for NHIP. It indicates a strong interaction between Zn ion and 5-HMF on the adsorbent, HIP.

K_F was computed from the linear equation (Eq. (4)) by plotting logq_e versus lnC_e. It corresponds quantitative affinity between the adsorbent and adsorbate. Higher K_F indicates a greater adsorption capacity. K_F for HIP was 0.222 (mg·g^{-1})·(L·g^{-1})$^{1/n}$ and for NHIP was 3.52×10^{-13} (mg·g^{-1})·(L·g^{-1})$^{1/n}$. It clearly shows that the adsorption of Zn ion and 5-HMF on HIP was favorable and adsorption capacity (KF) was greater than NHIP.

Table 1. The amounts of molecules and ions (q_e) adsorbed on HIP or NHIP at equilibrium, and selectivity coefficient (k_{HIP}) on HIP

Molecule	q_e (mg·g^{-1})		k_{HIP}
	HIP	NHIP	
Methyl 5-methyl-2-furoate	0.688 (±0.102)	0.659 (±0.124)	0.0579 (±0.0086)
5-Methyl-2-furfural	8.96 (±0.04)	4.83 (±0.26)	0.754 (±0.004)
5-Hydroxymethyl-2-furaldehyde	11.9 (±0.0)	1.64 (±0.33)	1.00 (±0.00)
Fe^{2+}	<LOD	<LOD	0
Co^{2+}	<LOD	<LOD	0
Zn^{2+}	5.24 (±0.29)	3.88 (±0.67)	1.00 (±0.08)

3.4. Selectivity test

To evaluate the binding affinity of HIP for specific target analytes, Zn ion and 5-HMF, in the presence of competing substances, a selectivity test was conducted. This test would be utilized to enhance adsorption including the sorption efficacy, optimization of adsorption process, and application of adsorption process in various fields.[18] To conduct the selectivity test, similar charges for ions or similar shape and size of molecules were selected. Co^{2+} (70 pm), Fe^{2+} (70 pm), and Zn^{2+} (74 pm) were selected for their low selectivity coefficients among Cd^{2+} (95 pm), Co^{2+} (70 pm), Pb^{2+} (119 pm), Ni^{2+} (70 pm), Fe^{2+} (70 pm), and Fe^{3+} (60 pm), which have the same charge or a similar ionic radius to Zn^{2+}. Molecules were selected which have a similar size or functional groups as 5-HMF including methyl 5-methyl-2-furoate (furoate), and 5-methyl-2-furfural (5M2F).

The q_e^T and selectivity coefficients of HIP (k_{HIP}) were computed and compared using Eq. (5). Selectivity coefficients (k) for Zn ion or 5-HMF relative to other metal ions or molecules in the solution are defined as follows.

$$k_{HIP} = \frac{q_e^T}{q_e^O} \quad (5)$$

where, q_e^T is amount of Zn ion or 5-HMF adsorbed on HIP or NHIP at equilibrium (mg·g^{-1}), q_e^O is amount of other ions or molecules adsorbed on HIP or NHIP at equilibrium (mg·g^{-1}), and k_{HIP} is selectivity coefficient of Zn or 5-HMF on HIP (unitless).

Results on HIP and NHIP were summarized in *Table* 1. The k_{HIP} for Fe^{2+} and Co^{2+} were 0 indicating that no adsorption occurred between these adsorbates and both HIP and NHIP. By setting the selectivity coefficient of 5-HMF to 1, that of furoate was 0.06 and of 5M2F was 0.754, respectively. Amount of furoate adsorbed on HIP was the lowest because the size of furoate was the largest and it might have no site to hydrogen bonding with HIP. 5M2F might have no site to interact with monomer as well, but its size was small enough to be entrapped in the cavity. Since 5M2F was also adsorbed on NHIP approximately half the amount observed for HIP, it could be inferred that some sorption of 5M2F on HIP could be considered to occur through non-specific binding such as van der Waals forces and physical entrapment.[19]

The selectivity coefficients reveal that 5-HMF is highly preferred over both 5M2F and furoate in the adsorption process. The selectivity of the HIP may be attributed to the size of analyte and its interaction with the monomers. The HIP exhibited a high recognition ability for Zn ion and 5-HMF compared to other ions or molecules investigated in this study.

3.5. Validation of analytical method

Validation process was conducted to ensure that the developed method was reliable, accurate, and suitable for its intended purpose. The adsorption experiment was performed under the optimized conditions. The real sample was prepared as described in section 2.5. Application to real sample and method validation were proceeded by UV-Vis spectroscopy for Zn ion and UPLC-UV for 5-HMF.

Detection of Zn ion over the entire working range of 5-points calibration curves (triplicate measurements)

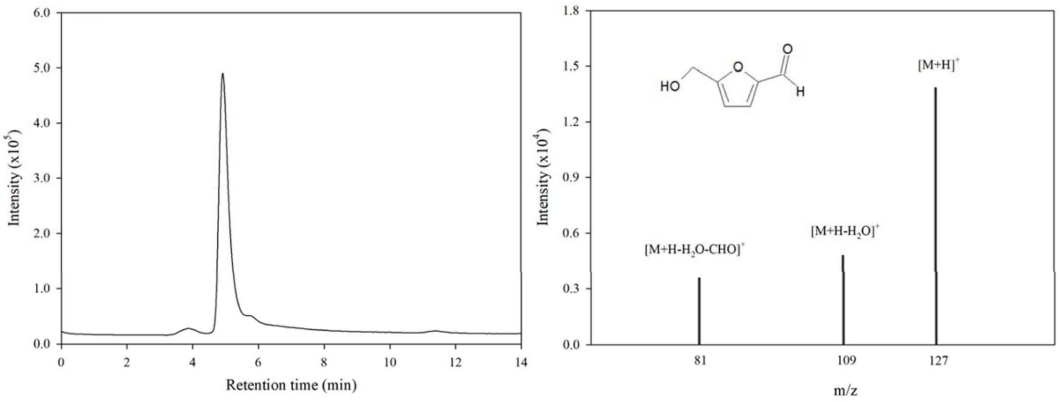

Fig. 6. The LC chromatogram (A) and mass spectrum (B) of 5-HMF in real sample.

showed good linearity with the regression coefficient of the linear equation (R^2 = 1.0000). The limit of detection (LOD) was determined by multiplying three times the standard deviation of the blank measurements by the slope of the calibration curve, resulting in 0.097 mg·L^{-1}. The limit of quantitation (LOQ) was calculated by multiplying ten times the standard deviation of the blank measurements by the slope of the calibration curve, yielding 0.324 mg·L^{-1}. The dynamic range was 0.324 mg·L^{-1} – 10 mg·L^{-1}.

5-HMF detection was conducted by UPLC/UV. High linearity was obtained over the entire working range of 7-points calibration curves by an internal standard method (triplicate measurements). The value of R^2 of the linear equation was 0.9997. The values of LOD and LOQ were 0.218 mg·L^{-1} and 0.726 mg·L^{-1}, respectively. The dynamic range was 0.726 mg·L^{-1} – 100 mg·L^{-1}. The accuracy was investigated to determine the recovery by spiking 15 mg·L^{-1} of Zn ion and 8 mg·L^{-1} 5-HMF standard solution to 0.01 % (w/v) honey sample. Analytical recoveries were 70.6 (± 2.4)% in 3 replicate adsorption experiment.

The reproducibility for the precision of the analytical method was investigated using mixture of Zn ion and 5-HMF standard solution. The values of relative standard deviation (RSD) for the intra-assay (run to run) in 3 replicate adsorption experiment was 2.8 % for low concentration, and 2.2 % for high concentration while RSD for inter-assay (day to day) in 3 replicate adsorption experiment was 2.1 % for low concentration and 5.0 % for high concentration.

3.6. Application to real sample

HIP was applied to the honey sample with 5-HMF spiked. The HPLC chromatogram and mass spectrum of 5-HMF in the honey sample were shown in *Fig.* 6. The concentration of 5-HMF in the honey sample was determined to be 69.6 (± 6.81) mg·kg^{-1}. The signals of m/z = 127, 109, and 81 were detected for 5-HMF. The protonated molecule $[M+H]^+$ (m/z = 127) was the base peak while the fragment ion $[M+H–H_2O]^+$ (m/z = 109) and $[M+H–H_2O\text{-}CHO]^+$ (m/z = 81) were present at low relative abundance.[20,21]

4. Conclusions

Simultaneous extraction conditions of Zn ion and 5-HMF for HIP were optimized, and the adsorption isotherm was identified to follow the Freundlich isotherm model. It employed to describe multilayer adsorption of adsorbate on the adsorbent surface, which effectively explained the sorption of both Zn ion and 5-HMF in this study. The maximum sorption capacity and intensity of HIP were higher than those of NHIP. HIP demonstrated high selectivity for Zn ion and 5-HMF up to 17 times greater than for homologs and other ions. Finally, validation of the proposed extraction method using HIP combined with

UV-Vis spectrometer and UPLC-UV was conducted using 0.01 % (w/v) real honey sample.

Simultaneous extraction of organic and inorganic compounds shows great potential in various industries such as environmental monitoring, drug safety, and industrial waste treatment, and food products. Its high selectivity and efficacy would allow more accurate detection and separation of the harmful substances in complex matrices. Despite these promises, some limitations could be addressed including scalability of HIP synthesis to precisely generate molecular or ion-binding sites achieved on an industrial scale, reusability of HIPs as binding site may degrade or become saturated over time, regeneration of HIPs after multiple extraction, and stability of HIP utilized in complex matrices as real-world samples. Overcoming these challenges would enable the simultaneous analysis of organic and inorganic substances in complex samples using HIP to facilitate the practical application in future studies.

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Authors' Positions

Yelin Lee	: Graduate Student	Soomi Park	: Graduate Student
Hyeyoung Jung	: Graduate Student	Sunyoung Bae	: Professor