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Evaluation of molecularly imprinted anion-functionalized poly(ionic liquid)s by multi-phase dispersive extraction of flavonoids from plant

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ABSTRACT

Molecularly imprinted anion-functionalized poly(ionic liquid)s (MAPILs) were prepared by radical polymerization for the multi-phase dispersive extraction (MPDE) of flavonoids from plants. Poly(ionic liquid)s were functionalized with different anions via anion metathesis to enhance their separation efficiency, called anion-functionalized poly(ionic liquid)s (APILs). A molecularly imprinting technique was introduced to produce specific recognition sites by forming complexes between the template molecules and anion-functionalized ionic liquid monomers to reduce the interactions with the interference substances and increase the selectivity. Multi-phase dispersive extraction (MPDE) was applied for separation instead of the traditional solid phase extraction method. The target compounds were first extracted by three-phase (sample–solvent–sorbent) dispersive extraction and cleaned up after removing the sample matrix. This method significantly decrease in the interference and analysis cost. A suitable sorbent for MPDE could be identified based on the adsorption behaviors of flavonoids on different MAPILs. The mean recovery yields of quercitrin, myricetin, and amentoflavone from *Chamaecyparis obtusa* under the optimized conditions were 88.07, 93.59, and 95.13%. This is a promising method for the extraction, separation and determination of flavonoids or other polyphenolic compounds from natural and other sources.

1. Introduction

Ionic liquids (ILs), which consist of organic cations associated with inorganic or organic anions, have attracted widespread recognition in chemistry for their unique properties including negligible vapor pressure, good thermal stability, non-flammability, high ionic conductivity and miscibility with water and organic solvents [1]. Moreover, they interact with target compounds via anion exchange, hydrogen bonding, π–π, or hydrophobic interactions, and are used in analytical chemistry for extraction and separation [2–4]. Recently, a special class of packing called IL-based materials was developed for gas chromatography (GC) [5,6], high-performance liquid chromatography (HPLC) [7,8] and solid-phase extraction (SPE) [9,10], etc. Of these IL-based materials, poly(ionic liquid)s (PILs) have attracted attention because they have the properties of both ionic liquids and polymers, and have a number of innovative applications [10,11]. On the other hand, there are limited reports on the use of facile anion-exchange to tune the properties of PILs [12–14] compared to the modification of cations to enhance the separation efficiency. Different to the modification of cations, the modification of anions can be easily achieved by anion metathesis for different purpose and target compounds without complicate reactions. The development and evaluation of PILs with different functional anions is necessary to extend and facilitate the application of PILs to separation. Accordingly, these proposed anion-functionalized PILs (APILs) were evaluated by the separation and determination of flavonoids (quercitrin, myricetin and amentoflavone) (Fig. 1) from *Chamaecyparis obtusa* (*C. obtusa*).

Flavonoids are a large class of compounds found widely in nature with most possessing a range of biological and pharmacological activities, such as antihypertensive, anti-inflammatory and antiviral [15–17]. Quercitrin, myricetin and amentoflavone are the main flavonoids in *C. obtusa*, which is distributed mainly in Korea, Japan, and the north eastern part of China. On the other hand, flavonoids need to be separated from interference prior to analysis due to the complexity of the sample matrix. The separation of high-value-added flavonoids with a molecularly imprinted polymer (MIP) is a useful method [18–20] because of the special properties of MIP such as predetermination, specific recognition, and practicability. Accordingly, a molecular imprinting technique can be used to increase the selectivity of APILs. In addition, the specific binding capacity and selectivity of MIPs make them suitable sorbents for solid-phase extraction (SPE), which is a well established technique for cleaning of many different classes of compounds in a range of matrices. However, conventional SPE can be only used to clean-up the extract of sample. Multi-phase dispersive extraction (MPDE)

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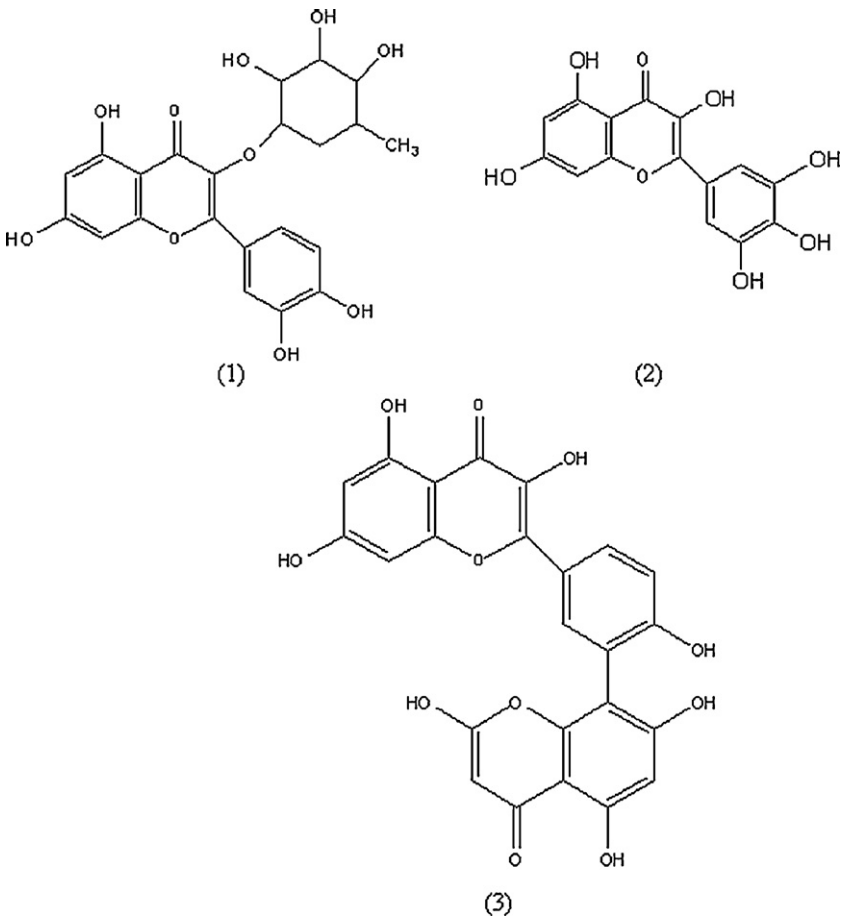

Fig. 1. Chemical structures of quercitrin (1), myricetin (2), and amentoflavone (3).

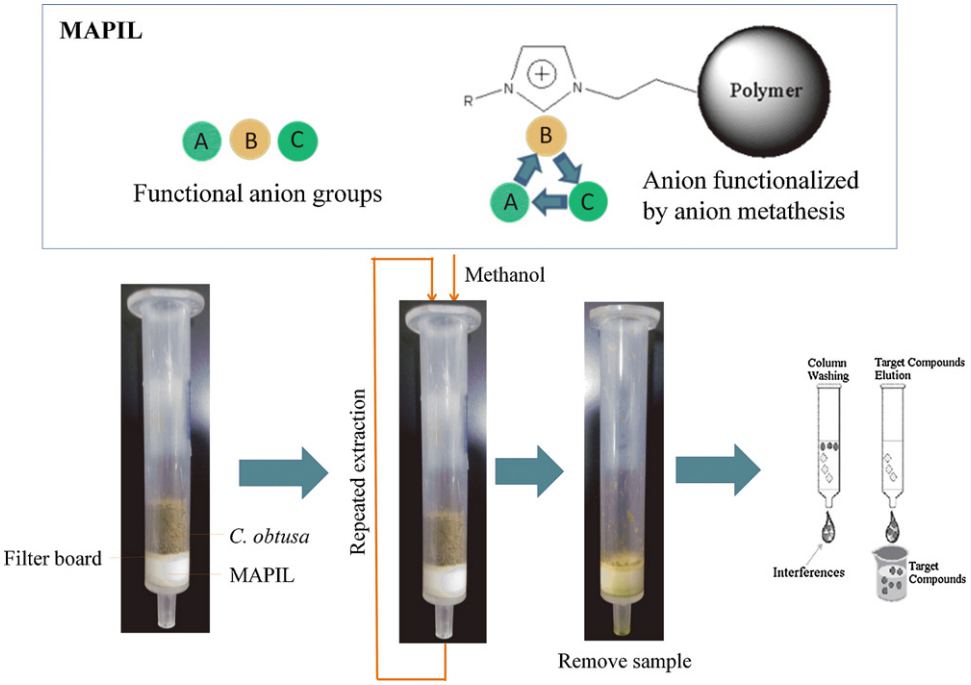

Fig. 2. Scheme of multi-phases dispersive extraction (MDPE).

was therefore developed for the simultaneous disruption, extraction, and clean-up of solid samples based on traditional SPE. As shown in Fig. 2, the sample matrix and sorbent were separated by a filter board and packed into a SPE cartridge. The sample was first extracted with a solvent and cleaned up after removing the sample matrix. This method eliminated most of the complications associated with classical solid–liquid (only for extraction) or solid-phase extractions (only for clean-up) of solid samples by allowing extraction and clean-up using a simple procedure with the recoverable use of sorbents. Moreover, SPE generally involves loading, washing, and eluting with loading being the most time-consuming part of the process, because it requires time to achieve adequate adsorption of the target compounds onto sorbent. MPDE method combined extraction and loading steps to decrease the time consumption, and to reduce the use of solvents during washing.

By thorough consideration, molecularly imprinted anion-functionalized PILs (MAPILs) were developed for the MPDE of flavonoids from *C. obtusa*. The MAPILs were prepared using different functional ILs, porogens, crosslinkers and templates, and optimized by comparing the specific adsorption capacities. The optimal polymer was used as a sorbent in MPDE for the extraction and separation of quercitrin, myricetin and amentoflavone from *C. obtusa*. All necessary conditions were investigated systematically to optimize the process. The proposed MAPIL-MPDE method has potential applications to the fast, convenient, and efficient isolation of a range of flavonoids or other polyphenolic compounds from plants.

2. Experimental

2.1. Chemicals

2,2′-Azobisisobutyronitrile (AIBN) (>99%) was from Junsei Chemical Co. (Tokyo, Japan). 1-Vinylimidazole (98%), ethyl bromide (>98%), *n*-butyl bromide (>97%), *n*-hexyl bromide (>95%), *n*-octyl bromide (>98%) and 3-aminopropyl bromide hydrobromide (>97%), sodium benzenesulfonate (>96%) and sodium dodecyl sulfate (>95%) were obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Quercitrin, myricetin (>99%), amentoflavone (>99%), lactic acid (>85%), sodium lactate (>99%) and ethyleneglycol dimethacrylate (EDGMA) (>98%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA) was acquired from Kanto Chemical Co., Inc. (Tokyo, Japan). Methanol, ethanol, *n*-propanol, *n*-butanol and other organic solvents were from DUKSAN PURE CHEMICALS Co., Ltd. (Ansan, Korea). Distilled water was filtered using a vacuum pump (Division of Millipore, USA) and filter (HA-0.45, Division of Millipore, USA) prior to use. All other solvents were of HPLC or analytical grade. All samples were filtered (MFS-25, 0.2 μm TF, WHATMAN, USA) before being injected into the HPLC system.

2.2. HPLC analysis and characterizations

The chromatography system consisted of a Waters 600s Multi solvent Delivery System, a Waters 616 liquid chromatography (Waters Associates, Milford, MA, USA), a Rheodyne injector (20 μL sample loop) and variable wavelength 2487 UV dual channel detector. Data processing was carried out using a Millennium 3.2 consisting of a HP Vectra 500PC. HPLC analysis was performed with a commercial C_{18} column (4.6 mm × 250 mm, 5.0 μm) purchased from RStech (Daejeon, Korea). The mobile phase was acetonitrile/water/trifluoroacetic acid (35/65/0.1, v/v/v) at a flow rate of 0.5 mL min^{-1}, and detection was performed at a UV wavelength of 375 nm.

The FT-IR spectra were obtained on a Vertex 80 V (Bruker, Billerica, MA, USA) between 400–4000 cm^{-1} at a scan rate of 20 scans min^{-1} using KBr pellets. Field emission-scanning electron microscopy (FE-SEM) was conducted on an S-4300 microscope (Hitachi, Ontario, Canada). The Brunauer–Emmertt–Teller (BET) surface areas (N_2 atmosphere at −195.85 °C) were measured using an ASAP2020 surface area and porosimetry analyzer (Micromeritics, Norcross, GA, USA).

2.3. Preparation of APIL and MAPIL

The general synthetic scheme for the ionic liquid monomers is as follows [21]. A 100.0 mL reactor was loaded with 0.1 mol of 1-vinylimidazole, 0.1 mol of *n*-alkyl bromide (or 3-aminopropyl bromide) and 30 mL of methanol. The mixture was stirred at 60 °C for 15 h. After cooling, the reaction mixture was added dropwise to 1.0 L of diethyl ether. The white precipitate was filtered and dried at room temperature until a constant weight was reached. 1-Vinyl-3-butylimidazolium lactate was synthesized using the procedure reported elsewhere [22].

Flavonoid-imprinted MAPILs were prepared by thermal-initiated polymerization in 4.0 mL glass vials [10]. The polymerization mixture contained 0.1 mmol quercitrin, 0.3 mmol IL monomers, 1.5 mmol EDGMA (cross linker), and 1% (w/w) AIBN, dissolved in a suitable porogen. The solution was sonicated for 10.0 min, sparged with helium for 5.0 min to remove oxygen, and polymerized at 70.0 °C in a water bath for 24.0 h. After polymerization, the polymers were ground and suspended repeatedly in acetone to remove any small particles. The polymer particles were dried under vacuum, placed into a column, and washed with 6.0 mL methanol and 6.0 mL methanol/HCl (90:10, v/v) to remove the templates. Finally, the polymer particles were anion-functionalized by ion-exchange with lactic acid. After balancing with methanol, they were dried in an oven (50 °C) for 24.0 h. The normal APILs were similarly prepared, treated and anion-functionalized [23,24]. Table 1 lists the synthesized polymers.

2.4. Adsorption

10.0 mg of the sorbents were stirred with 1.0 mL of each flavonoid standard solution at 0.025 mg mL^{-1} in vials at room temperature until the concentration of free flavonoids stopped decreasing and the equilibrium adsorption was obtained. The amounts of adsorbed flavonoid on the polymers were calculated by subtraction. The repeatability was assessed by performing the adsorption of flavonoids five times.

2.5. Procedure of MPDE

Fig. 1 shows a schematic diagram of the procedure of the MPDE. The miniaturized MPDE procedure was achieved using a small amount of sample and proportionately less support or solvent. 0.15 g of *C. obtusa* powder and 0.1 g of MAPIL sorbent were placed into an empty cartridge (5.0 cm × 8.0 mm I.D.), rinsed repeatedly with 1.0 mL of methanol, washed with 2.0 mL of water and eluted with 2.0 mL of methanol/HCl (90/10, v/v). The eluent was evaporated and re-dissolved in 1.0 mL of the mobile phase for further HPLC analysis. In order to obtain the total extracted amounts of flavonoids in power of *C. obtusa*, the sample was extracted several times by methanol assisted extraction until no flavonoids were detected by HPLC. The sum of extracted amounts of flavonoids in each extract was assigned as the total amounts of flavonoids in *C. obtusa*. According to the reference [25] and previous researches, 5.0 g of powdered *C. obtusa* leaves were immersed in 50.0 mL methanol for 4 h, and the extract was stored for further use. The recoveries for MPDE were calculated from the equation: Recovery

Table 1
Studied polymers in this experiment.

	Cation	Type of porogen	Amount of progen (mL mmol^{-1})	Monomer/cross linker ratio (mmol mmol^{-1})	Anion	Template/monomer ratio (mmol mmol^{-1})
APIL1	1-Vinyl-3-ethylimidazolium					
APIL2	1-Vinyl-3-butylimidazolium					
APIL3	1-Vinyl-3-hexylimidazolium	Ethanol				
APIL4	1-Vinyl-3-octylimidazolium		0.5			
APIL5	1-Vinyl-3-proylamine-imidazolium					
APIL6		Methanol		1/3		
APIL7		*n*-Propanol				
APIL8		*n*-Butanol				
APIL9			0.125		Br^-	
APIL10			0.25			
APIL11			0.75			–
APIL12			1.0			
APIL13				1/2		
APIL14	1-Vinyl-3-butylimidazolium			1/4		
APIL15		Ethanol		1/5		
APIL16				1/6		
APIL17			0.5		BF_4^-	
APIL18					PF_6^-	
APIL19				1/5	Tf_2N^-	
APIL20					Lactate	
APIL21					Benzenesulfonate	
APIL22					Dodecyl sulfonate	
MAPIL1						1/1
MAPIL2						1/2
MAPIL3	1-Vinyl-3-butylimidazolium	Ethanol	0.5	1/5	Lactate	1/3
MAPIL4						1/4
MAPIL5						1/5

(%) = Recovered amount of flavonoid by MPDE (mg g^{-1})/Total amount of flavonoid (mg g^{-1}) × 100%.

3. Results and discussion

3.1. Characterization

The FT-IR spectra of APIL17-22 (Fig. 3) show peaks at 1634 cm^{-1} and 1570 cm^{-1}, characteristic of imidazolium groups [26], and 1730 cm^{-1}, attributable to the C=O group of EDGMA. Band at 554 cm^{-1} and 624 cm^{-1}, 838 cm^{-1}, 1050 cm^{-1}, and 1325 cm^{-1} in Fig. 3 are attributed to the characteristic bond of S=O, P–F, B–F, and lactate, respectively; these confirms the formation of PILs with different anions after the anion-exchange reaction. These data indicate the successful preparation of the intended polymers.

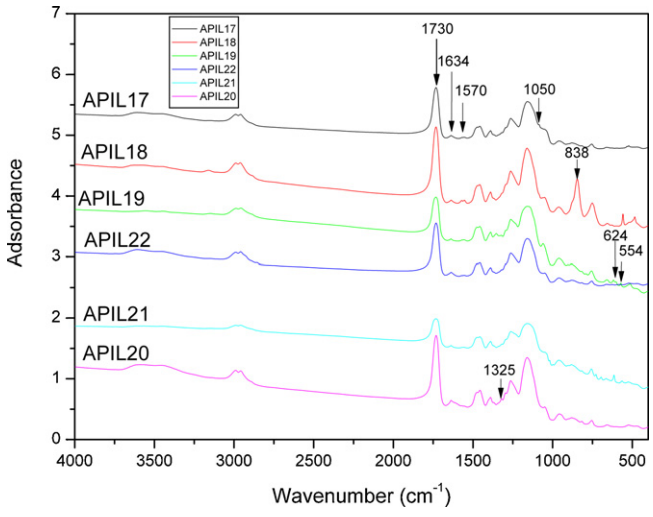

Fig. 3. FT-IR spectra of APIL17, APIL18, APIL19, APIL20, APIL21 and APIL22.

The structures of APIL20 and MAPIL3 were studied by SEM and BET. Fig. 4 show monolithic structures (Fig. 4A) that likely arose due to their similar synthesis of monolithic columns. When the polymers were magnified to ca. 500 nm (Fig. 4B), MAPIL3 showed clearer sub-porous structure than that of APIL20. These porous structures of APIL20 and MAPIL3 allowed investigation by BET, which found surface areas of 39.28 m^2 g^{-1} and 133.12 m^2 g^{-1} and average pore sizes of 111.08 Å and 49.448 Å, respectively. This was due to the molecular imprinting forming cavities that increased the surface area of MAPIL as average pore size decreased.

3.2. Optimization of APILs

Sorbent selection is essential for efficient MPDE. Several factors can affect the preparation of APILs, which can influence the selectivity and adsorption capacity of the target analytes. The amounts adsorbed indicate the performance of each material because MPDE is involved the adsorption of flavonoids on sorbents.

ILs, as functional groups, were evaluated for their ability to adsorb quercitrin, myricetin, and amentoflavone. An IL structure, including cations and anions, has a significant effect on their physicochemical properties and thus adsorption capacity. The anions of IL were later optimized because a change in anions complicates the synthesis of APILs. Therefore, APIL1, APIL2, APIL3 and APIL4, each with Br^- anions, were evaluated with respect to their cations (Fig. 5A). Adsorption was affected by increasing the alkyl chain length, with APIL2 showing the highest efficient. Although hydrophobic interactions increased from ethyl to octyl at the 1-position of the imidazolium ring, long alkyl chains decreased the other interactions because of steric hindrance and the blocking of pores in polymer. The adsorption behaviors of these polymers (PM and APIL5) were tested because MAA and amino-imidazolium IL are also used as functional groups in polymers for the separation of flavonoids [19,25]. Both of these two polymers were less efficient than APIL2. Therefore, APIL2 was selected for further optimization.

The pore size affects the arrangement of the functional groups, located within the pores [27]. Several alcohols (methanol,

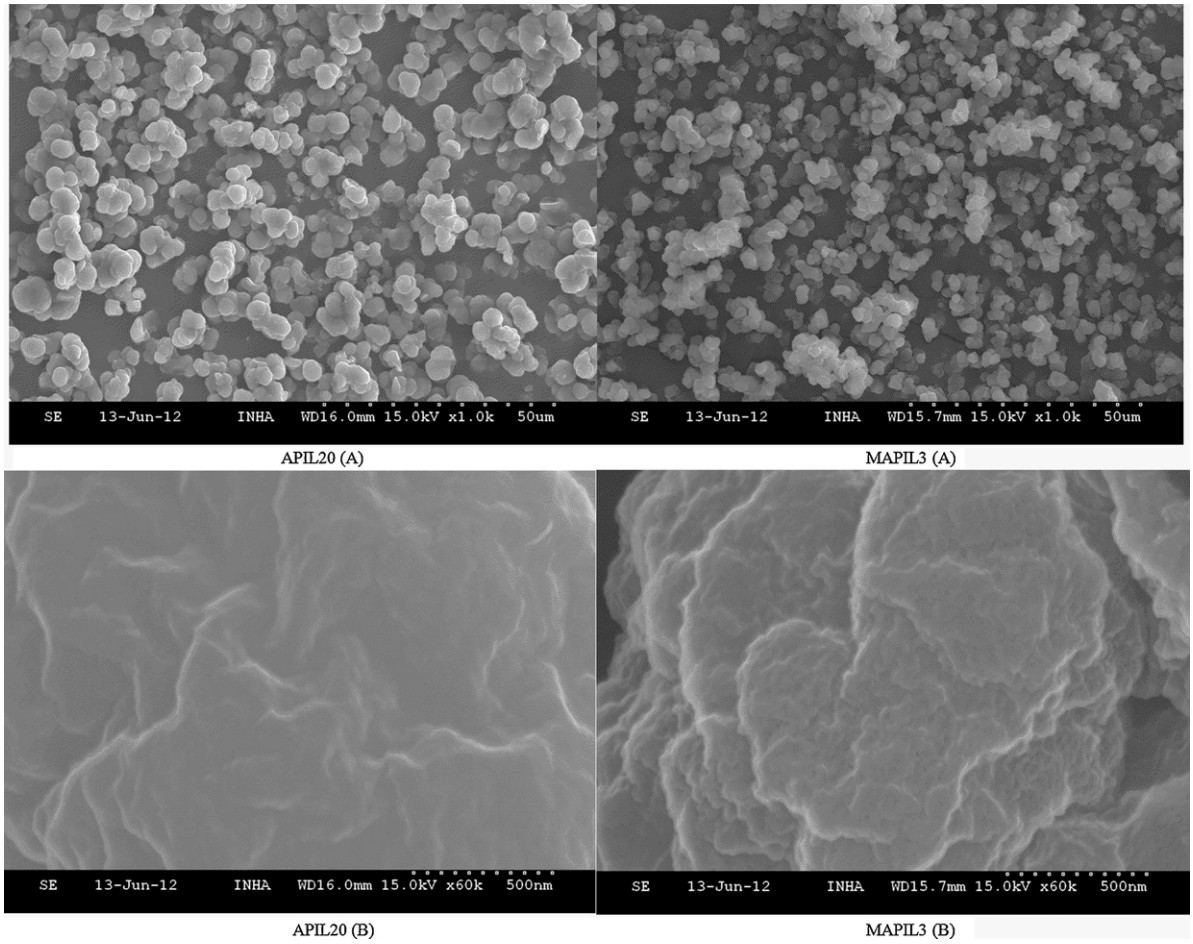

Fig. 4. SEM images of APIL20 and MIAPL3.

ethanol, *n*-propanol and *n*-butanol), which can dissolve quercitrin, myricetin and amentoflavone, were chosen as porogens considering the properties of the target compounds (Fig. 5B). The adsorption of flavonoids improved greatly with increasing alkyl chain length from methanol to ethanol. A significant decrease in adsorption was observed when *n*-propanol and *n*-butanol was used as porogens. The volume of porogen per amount of monomer and crosslinker was examed in the range of 0.125–1.0 mL mmol^{-1} (Fig. 5C). The amount adsorbed increased with increasing amount of porogen between 0.125 and 0.50 mL mmol^{-1} but decreased with further increases in porogen. This can be explained by the different pore sizes in the polymers arising as the type and amount of porogen. Generally, increasing the volume and alkyl chain length of the porogen will lead to larger pores with reduced surface area. The use of less and porogen with a shorter alkyl chain will produce smaller pores and a larger surface area. Small pores prevent the target compounds from penetrating the polymer, whereas large pores with a low surface area reduce the adsorption capacity. Therefore, APIL2 is still considered to be the best sorbent.

The pore size of polymers decreased with increasing amount of cross linker. The cross linker also affected the distribution of active sites in the APILs. If the functional groups are too close to each other, the target compounds cannot interact effectively with the functional groups due to steric hindrance. In the series of APILs prepared at different monomer/cross linker ratios (1/2, 1/3, 1/4, 1/5, and 1/6 mmol mmol^{-1}, Fig. 5D), the amounts adsorbed were highest at a monomer/cross linker ratio of 1/5 (mmol mmol^{-1}). Overall, APIL15 was considered optimal for the maximized adsorption of quercitrin, myricetin, and amentoflavone.

As mentioned above, when the morphology of the polymer is fixed, the identity of the anions has a significant effect on the properties of the ILs. Therefore, APILs with different anions (Br^-, BF_4^-, Tf_2N^-, PF_6^-, lactate, benzenesulfonate, and dodecyl sulfonate) were assessed. As shown in Fig. 5E, lactate was more efficient than the other anions. Because of the different distribution of flavonoids between the APILs and methanol phases, the adsorption behaviors were quite different. Especially for lactate, benzenesulfate and dodecyl sulfonate anions can provide hydrogen bonding, π–π and hydrophobic interactions, respectively. APIL with extra hydrogen bonding interactions provided by lactate was found to be more efficient. Overall, APIL20 was considered optimal for the maximum adsorption of flavonoids.

The template/monomer ratio can also affect the recognition and selectivity. An excess of functional monomer relative to the template will cause in steric mismatch in the imprinted polymer and excessive binding sites. Insufficient monomer will reduce self-assembly and selectivity. The template: monomer ratio was tested between 1/1 and 1/5 (mmol mmol^{-1}) (Fig. 6). In contrast to the previous tests, the polymer with the lowest adsorption capacity was considered most suitable. Multiple interactions between the template and functional groups of the ILs would result in one template molecular being bound to several functional groups, thereby reducing the adsorption capacity. The lowest amounts of flavonoids adsorbed were obtained at a template: monomer ratio

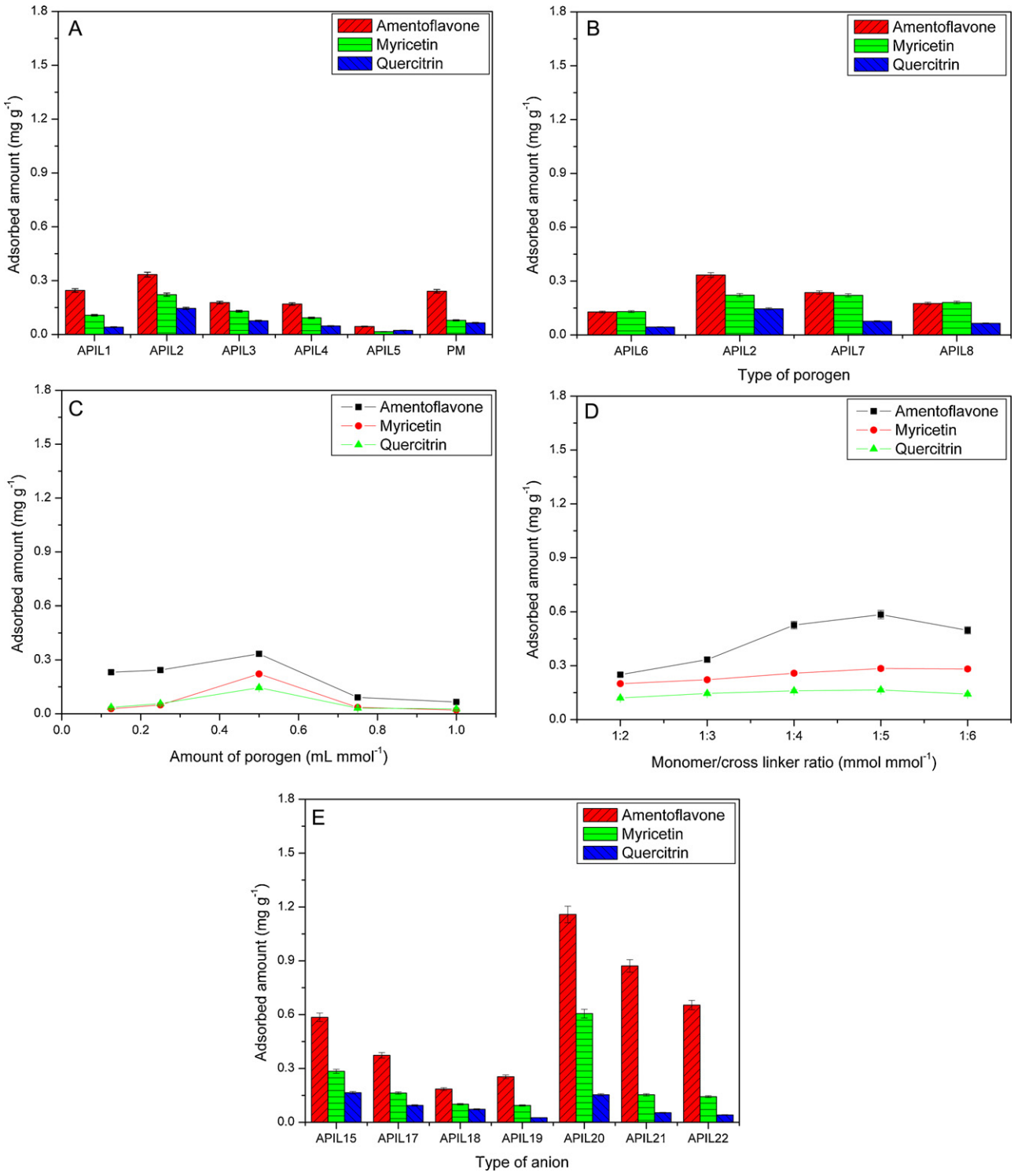

Fig. 5. Effect of cations (A), types (B) and amounts (C) of porogen, monomer/cross liker ratios (D), and anions (E) of APIL on adsorbed amounts of flavonoids on APILs.

of 1/3 (mmol mmol^{-1}) (MAPIL3). Therefore MAPIL3 was considered to have the highest selectivity.

3.3. Optimization of the MPDE procedure

As with MSPD, one of the outstanding advantages of MPDE is that extraction and clean-up can be carried out in a single simple procedure. In this study, 1.0 mL of solvent could be used repeatedly as the extraction solvent with a proportionate MAPIL to reduce the level of solvent consumption and simultaneously separate the target analytes from interference. The main parameters that affect the washing and elution steps were investigated systematically to optimize the MPDE process.

According to a previous study [18,20], methanol is used widely as a solvent in the extraction of flavonoids. Therefore, different methanol/water solution (50–100%) proportion of methanol/water solution was tested as the extraction solvent (Fig. 7A). To achieve the best extraction and adsorption, 100.0 mg of *C. obtusa* was

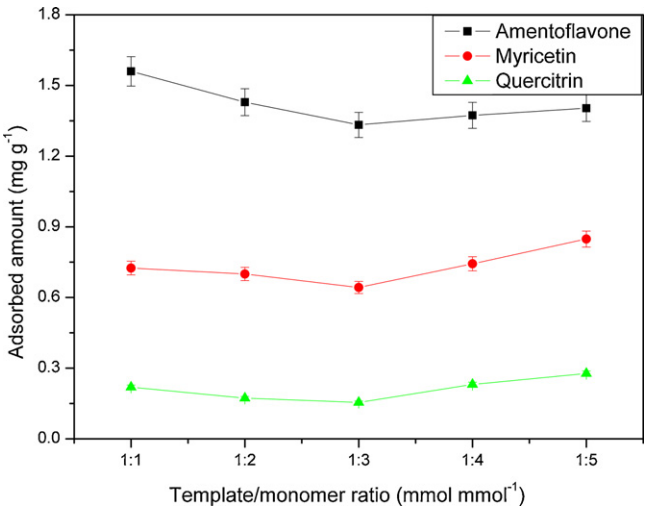

Fig. 6. Effect of template/monomer ratio on adsorbed amounts of flavonoids on MIAPILs.

packed into a SPE cartridge with 100.0 mg of packing and rinsed with 1.0 mL of solvent. After removing the sample matrix, the adsorbed flavonoids were eluted using a 1.0 mL of methanol/HCl solution (90/10, v/v). Pure methanol showed the best performance.

After optimizing the extraction solution, 1.0 mL of methanol was used for MPDE with five repeated extractions. A suitable sample/sorbent ratio can increase the interface area between the target compounds and sorbent, and allow complete adsorption of the sample components to facilitate their transfer to the sorbent. An insufficient amount of sorbent cannot provide complete adsorption of the extracted flavonoids, which can reduce the recovery of the target compounds. On the other hand, an excessively large proportion of sorbent decreases the extraction efficiency. An excessively large proportion of sorbent increased the dead volume of SPE cartridge with increasing the volume of extraction, washing and elution solvent. Therefore, this situation was not efficient for extraction. Therefore, sample/sorbent ratios ranging from 1/2 to 5/2 (mg mg^{-1}) were evaluated. The ratio of 3/2 provided the largest amounts of myricetin and amentoflavone adsorbed (Fig. 7B). Although the recovery yields of quercitrin decreased at the ratio of 3/2, the sample/sorbent ratio of 3/2 was optimal due to economical consideration.

The repetition times of extraction by methanol in MPDE was optimized to extract the flavonoids from *C. obtusa* adequately. Accordingly, different repetition times (up to 5 times) were evaluated. The flavonoids extracted increased with increasing repetition times from 1.0 to 3.0 times, and remained constant thereafter (Fig. 7C). Therefore, three repetitions of extraction were selected considering the extraction efficiency and time consumption.

After removing the *C. obtusa* powder, a solvent was needed to wash the weakly interacting interference. According to previous studies [20], water was used as the washing solvent to avoid unnecessary interference of the sample and environment by organic solvents. Most of the interference was removed with 2.0 mL of water. Because the main interaction was hydrogen bonding interaction between target compounds and MAPILs, addition of acid (HCl) can not only breaks the hydrogen bonding interaction between imidazolium group and target compounds, but also changes the anion of MAPILs by anion metathesis for decreasing the hydrogen bonding interaction between anion of ILs and target compounds. Therefore, the flavonoids were eluted with different volumes of a methanol/HCl (90:10, v/v). The use of more than 2.0 mL did not increase the elution of flavonoids significantly. Therefore, 2.0 mL of methanol/HCl was considered optimal. To validate the efficiency

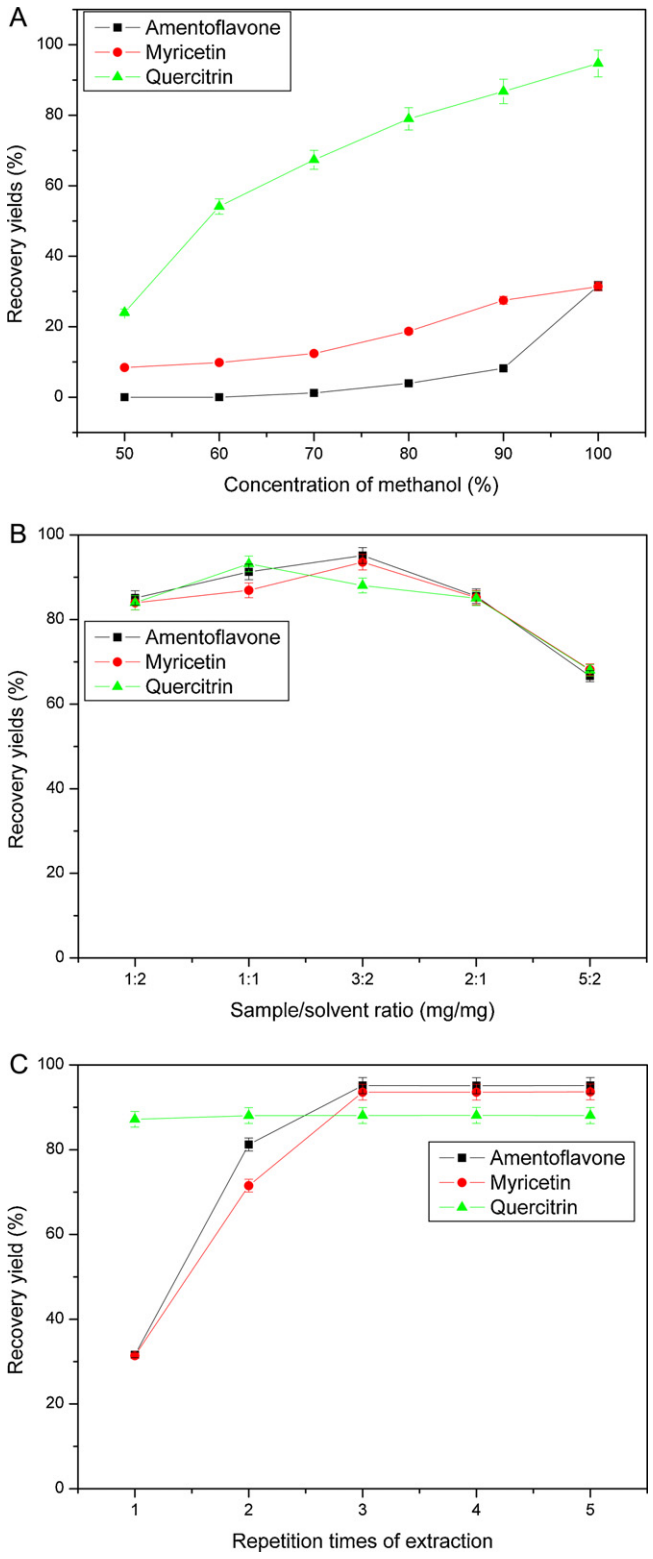

Fig. 7. Effect of methanol concentration (A) and sample/sorbent ratio (B) repetition times of extration (C) on recovery yields of flavonoids.

of the method, the level of extraction from *C. obtusa* was compared with that of methanol dipping extraction. Overall, based on the flavonoids extracted by methanol, 88.07, 93.59, and 95.13% recovery yields of quercitrin, myricetin, and amentoflavone were obtained from *C. obtusa* by MPDE. The analytical results of the *C.*

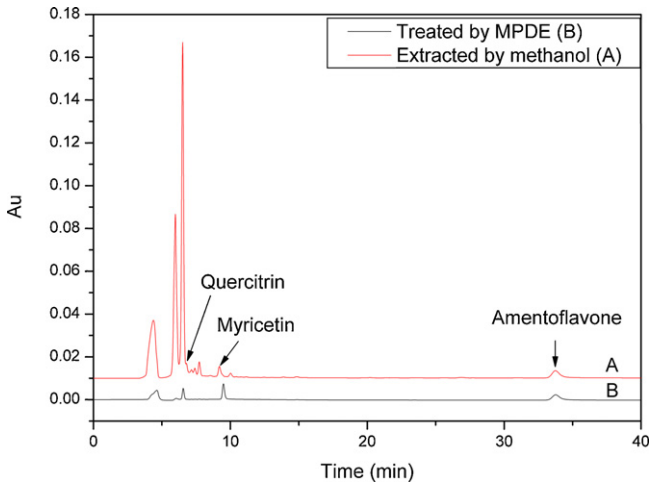

Fig. 8. Chromatograms of the sample extracted by methanol (A) and treated by MPDE (B).

Table 2
Linear range, LODs and repeatability of the method.

Analyte	Linear range ($mg\,mL^{-1}$)	r	RSD (%)	LODs ($ng\,mL^{-1}$)
Quercitrin	5.0×10^{-3}–0.5	0.9997	2.89	60.0
Myricetin	5.0×10^{-3}–0.5	0.9991	2.66	75.0
Amentoflavone	5.0×10^{-3}–0.5	0.9996	3.16	55.0

obtusa samples extracted by methanol and treated by MPDE were compared (Fig. 8).

3.4. Regeneration and recycling of MAPIL

MAPIL was recycled for the further separation of quercitrin, myricetin, and amentoflavone from the *C. obtusa* extract by MDPE. The regeneration of MAPIL was achieved by rinsing with 1.0 mL of methanol/HCl solution (90/10, v/v), 2.0 mL of methanol and subsequently 2.0 mL of 2.0 mol/L lactic acid. The recovery yields of quercitrin, myricetin, and amentoflavone by MAPIL over four cycles from the *C. obtusa* extract were 88.07–86.24%, 93.59–91.13% and 95.13–92.36%, respectively. The recovery yields decreased lightly, demonstrating the stability of the sorbent. In this experiment, we use MAPILs for over four cycles. However, this material can be reused several times which were according to the different requirement of recovery yields.

3.5. Analytical performance

To evaluate the proposed MAPIL-MPDE method, a series of experiments were designed to assess the linearity, precision, detection limits and other characteristics under optimized conditions. All the analytes had good linearity, with correlation coefficients (r), between 0.9991 and 0.9997 (Table 2). The precision was determined over five MPDE of quercitrin, myricetin and amentoflavone from the extract, and the RSD was 2.66–3.16%. Based on a signal-to-noise ratio of 3, the limits of determination (LODs) of the three flavonoids range from 55.0 to 75.0 $ng\,mL^{-1}$. This demonstrates the stability of the proposed method and its potential applications in the determination of other medicinal products.

4. Conclusion

MAPIL was developed for the MPDE of flavonoids (quercitrin, myricetin and amentoflavone) from *C. obtusa*. The APILs employed anion metathesis for functionalization of the anion to enhance the adsorption capacity and selectivity of PILs. With the introduction of molecularly imprinting technology, the APIL was upgraded to MAPIL with structure recognition. This MPDE method including simultaneous extraction and separation was combined used with MAPIL to pretreat the *C. obtusa* sample for analysis. Overall, the proposed MAPIL-MPDE method combining the advantages of MAPIL and MPDE is simple and efficient, and can be applied to other flavonoids and polyphenolic compounds from other complex biological samples. Although this method was original developed for sample preparation of analysis, it can be potentially applied for large quantity samples.

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