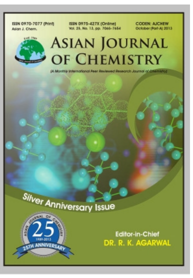
Asian Journal of Chemistry; Vol. 25, No. 13 (2013), 7257-7262

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14529



Adsorption of Flavones from *Chamaecyparis obtusa* by Ionic Liquid-Immobilized Polymers

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(*Received*: 5 September 2012; *Accepted*: 24 June 2013) AJC-13685

The adsorption isotherms of three flavones (quercitrin, myricetin and amentoflavone) on five different ionic liquid polymers were compared. The interaction between the polymer and target compounds were examined. The experimental parameters in the equilibrium isotherms were estimated by linear and nonlinear regression analysis. The adsorption equilibrium data of the three flavones were examined using three different models and the best model for these experimental data was the competitive Langmuir-Freundlich. The regression coefficients of adsorption isotherms were myricetin (0.908 in methylimidazole polymer), quercitn and amentoflavone (0.937 and 0.919 in carboxy-imidazole polymer, respectively).

Key Words: Ionic liquid, Adsorption, *Chamaecyparis obtusa.*

INTRODUCTION

Flavones, such as quercitrin, myricetin and amentoflavone are the other major active compounds in the *Chamaecyparis obtusa* (Fig. 1)[1]. These compounds exhibit antioxidant[2], antidiarrhoeic[3] and anti-inflammatory[4] properties. Sorbents are required to purify them. The sphere of ionic liquids is developing at a very rapid rate, as the many valuable properties of these liquids are identified and employed[5]. Ionic liquids are considered probable solvents to substitute volatile organic solvents in a wide range of chemical reactions and separation. Ionic liquids have attractive properties, such as hydrophobicity, miscibility with several inorganic/organic solvents and π-π interactions between analyte and functional groups of the ionic liquids[6]. For example, ionic liquids have been considered in the following areas: as solvents for reactions, absorption media for gas separations, separating agents in extractive distillation, heat transfer fluids, for processing biomass[7], for sample preparation, liquid-phase extraction, chromatographic separations, as a porogen in polymer synthesis and as the working fluid in a range of electrochemical applications. Some researchers have investigated the application of obtained ionic liquid-modified materials to separate intimate organic compounds[8] and extract bioactive components from natural products[9]. In addition, porous polymers with large surface areas and special porous structures have been introduced[10]. When the modifiers have specific functional groups that can form strong interactions with bioactive compounds, they can be introduced to replace nonspecific or less bioconjugation groups[11].

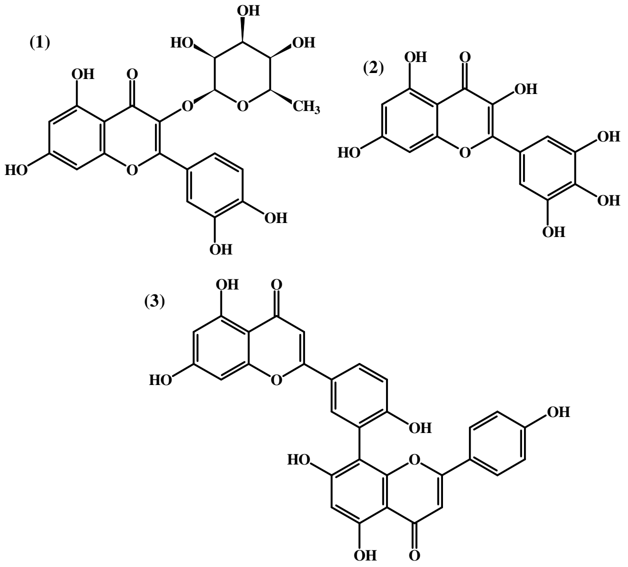


Fig. 1. Structures of (1) quercitrin, (2) myricetin and (3) amentoflavone

The adsorption isotherm is normally introduced to detect the separation efficiency. The thermodynamic theories developed for adsorption isotherms have been used to examine the equilibrium relationship between the mobile phase and stationary phase[12]. By fitting the experimental adsorption isotherm to a mathematical model, it is possible to evaluate the properties of a system by using the corresponding fitting coefficients. After calculating these mathematical models, the most suitable model for the experimental data is obtained. Using this method, the individual band profile of separate samples can be predicted under a range of conditions and conditions can be optimized[13]. The experiments and modeling are related to the adsorption isotherms and their parameters. The success of the experiments and modeling is related to the precision of the adsorption isotherms and their parameters[14]. The Langmuir equation is the most popular and widely used equilibrium isotherm among the many nonlinear isotherm models. Primarily, the Langmuir model was designed for monolayer adsorption with no interactions between the adsorbed molecules. Therefore, its ability to correlate the experimental data is limited. Other models with more parameters and higher correlation accuracy have been proposed[15]. Competitive Langmuir isotherms for liquid chromatography have been studied by Juza and Huthmann[16, 17], Guiochon[14], and Khattabi *et al.*[18]. The nonlinear behaviour of liquid chromatography under nonlinear conditions should be considered properly in an equilibrium isotherm. The successes of the experiments and modeling are related to the precision of the adsorption isotherms and their parameters[19]. Radically the Langmuir model was purposed for monolayer adsorption with no interaction between adsorbed materials. Therefore its ability to correlate experimental data is limited[20]. The Langmuir and the Freundlich isotherm equation were used as a convenient representation of experimental data[21].

Several extraction and purification molecule have been researched. But remain no selectivity of the target compounds. In this study, one blank polymer and five imidazoles with different functional groups (imidazole, methylimidazole, carboxyl-imidazole, aminoimidazole and cyano-imidazole) were used as surface modifiers. Because of ionic liquid-modified porous polymers with large surface area, numbers of functional groups and high selectivity of target compounds. The adsorption isotherms of three flavones on five different ionic liquid polymers were compared and the interactions between the polymer and target compounds were examined.

EXPERIMENTAL

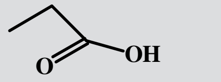
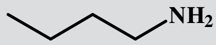
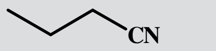
Quercitrin, myricetin and amentoflavone were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Methanol, acetonitrile and trifluoracetic acid were obtained from Duksan Pure Chemical Co. Ltd., (Ansan, Korea). 2,2'-Azobisisobutyronitrile was supplied by Junsei Chemical Co. (Tokyo, Japan). 3-Aminopropyl bromide hydrobromide (97 %), 4-(chloromethyl)-styrene (90 %), divinylbenzene (50 %) and polyvinylpyrrolidone (PVP, K 30), imidazole (98 %), 1-methylimidazole (99 %), 1-imidazole acetic acid (98 %), 1-(3-aminopropyl)imidazole (97 %), 1-(2-cyanoethyl)-2-methylimidazole (98 %) and 9,10-phenanthrenequinone (99 %) were purchased from Tokyo Chemical Industry Co. Ltd., (Tokyo, Japan). All chemicals and reagents were of HPLC grade. Distilled water was filtered through a vacuum pump (Division of Millipore, Waters, USA) and filter (HA 0.45, Division of Millipore, USA) prior to use.

Synthesis of ionic liquid polymers: Ionic liquid polymer was synthesized following the proecess: A solution of 5.65 mL of 4-(chloromethyl)styrene(monomer), 16.2 mL of divinylbenzene (crosslinker), 5.0 mL of heptanes (porogen) and 0.22 g of AIBN (initiator) in 100.0 mL PVP was prepared with stirring under a nitrogen atmosphere. The substrate mixture was then heated at 343 K for 24 h. After the reaction, the obtained blank polymer was filtrated and washed with ethanol to remove the excess of unreacted organic reactants. Then blank polymer (2 g) and different ionic liquid modifiers (2.0 mmol) were added to 35 mL of ethanol and after 12 h reflux, the ionic liquid polymers were obtained.

Chromatography conditions: Chromatography was performed using a waters 600s multi solvent delivery system, a waters 616 liquid chromatography and waters 2487 variable wavelength, dual-channel UV detector (Waters Associates, Milford, MA, USA). A syringe with a 25 μL injection volume and 20 μL sample loop were used. Data processing was performed with Millennium 3.2 software. The HPLC analysis was performed on a commercial C_{18} column (4.6 × 250 mm, 5 μm) purchased from RStech Co. (Daejeon, Korea). Acetonitrile/water/trifluoracetic acid (35:65:0.1, v/v/v) was used as the mobile phase at a flow rate of 0.5 mL/min and detection was carried out at a wavelength of 372 nm.

Preparation of the absorption isotherm: The sorbent was prepared from the synthesized ionic liquid-based porous polymers in the laboratory. Table-1 shows the structure of the ionic liquid-based porous polymers (imidazole polymer (PIm); methylimidazole polymer (PImM); carboxyl-imidazole polymer (PImCOOH); amino-imidazole polymer ($PImNH_2$); cyano-imidazole polymer (PImCN)). The polymers (2 mg) were placed into micro tubes and mixed with 1 mL of the standard flavone solutions (0.5, 1, 1.5, 2, 2.5, 3, 5 and 10 μg/mL) at room temperature for 72 h. The supernatant was then collected and filtered (0.2 μm). The experimental adsorption isotherms were fitted to the linear, Langmuir, competitive Langmuir and Freundlich models. This process was accomplished using the solver function in Origin Pro 7.5 software (Origin Lab Corporation, MA, USA) by varying the fitting parameters to reach a value of 1 for the squared correlation coefficient (r^2).

TABLE-1
STRUCTURES OF ALL IONIC LIQUID-BASED POROUS POLYMERS

Name of polymer	Structure
Blank polymer	No ionic liquid group
PIm	R= no additional group
PImM	R = – CH_3
PImCOOH	R = 
$PImNH_2$	R = 
PImCN	R = 

RESULTS AND DISCUSSION

Ionic liquid polymer characterized: SEM image of carboxy-imidazole polymer (Fig. 2) shows the porous structure was shaped and the surface area (blank polymer, 44.23 m^2/g; carboxy-imidazole polymer, 30.52 m^2/g) was determined by BET. After synthesis, the pores was blocked by functional groups of -COOH. Thus, the carboxy-imidazole polymer surface area is smaller than blank polymer[22]. The ohter ionic liquid-based porous polymers also surace area is smaller than blank polymer because of functional groups. But the interaction between three flavones and functional groups of ionic liquid-polymer affects shows the difference adsorbed amount.

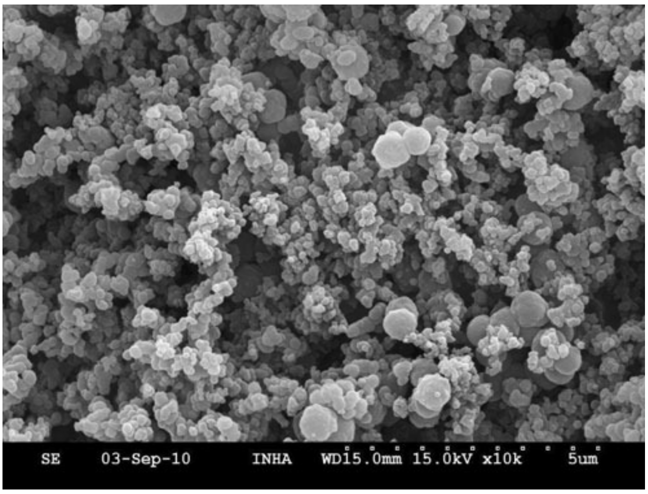


Fig. 2. SEM image of PImCOOH

Determination of the concentrations of flavones: Initially, the calibration curves (y: peak area, x : concentration) were obtained using the chromatographic peak areas measured at five concentrations (0.5, 1, 1.5, 2, 2.5, 3, 5 and 10 µg/mL) with methanol. As a result, linear regression equation of the three target compounds were $Y = 261479x-78386$ ($r^2 = 0.9981$) for quercitrin, $Y = 418195x-11409$ ($r^2 = 0.9993$) for myricetin and $Y=176626x=85939$ ($r2=0.9987$) for amentoflavone. The adsorption isotherm as a power tool was introduced to examine the interactions between the target compounds and sorbents. The adsorbed amounts (Q) of the three flavones on all the sorbents was calculated using the following equation:

$$Q = \frac{(C_o - C)V}{m} \quad (1)$$

where Q (µg/g) is the absorbed amount, C_0 (µg/mL) is the initiator concentration, C (µg/mL) is the unabsorbed concentration, V (mL) is the volume of the sample solvent and *m* (g) is the mass of the sorbent. Q was calculated using this procedure and the experimental parameters were then compared with the equilibrium isotherms by linear and nonlinear regression analysis. Table-2 showed the adsorption of target compounds on differences sorbent. Due to the different ionic liquid groups on polymers, they had some different chromatographic behaviours on adsorbents.

TABLE-2
ADSORPTION OF TARGET COMPOUNDS
ON DIFFERENCES SORBENTS

Polymer	Concentration (µg/mL)	Adsorbed amount (Q, µg/g)		
		Quercitrin	Myricetin	Amentoflavone
Blank polymer	0.5	0.0271	0.2763	0.3817
	1.0	0.1041	0.5807	0.5279
	1.5	0.1268	0.5659	0.6742
	2.0	0.6206	1.4578	0.6299
	2.5	1.3290	1.6028	0.9776
	3.0	3.5102	2.1988	1.0058
	5.0	4.2628	2.5831	1.9249
	10.0	4.3299	2.5291	1.8955
PIm	0.5	0.0354	0.7444	0.4998
	1.0	0.1695	0.7780	0.9600
	1.5	0.1613	1.3703	1.5110
	2.0	0.5652	1.4655	2.2475
	2.5	1.7298	1.5349	2.2555
	3.0	1.9902	1.4866	2.3280
	5.0	2.0659	1.8276	3.6155
	10.0	2.0598	1.8486	3.4247
PImM	0.5	0.1298	1.2652	0.2569
	1.0	0.4794	1.3668	0.9255
	1.5	0.1485	2.3418	1.2170
	2.0	0.5705	2.3638	2.5780
	2.5	1.5724	2.2988	2.2208
	3.0	2.1210	2.9243	2.8548
	5.0	3.2394	3.2198	3.1902
	10.0	3.3756	3.2285	3.0948
PImCOOH	0.5	0.9915	1.4261	1.4050
	1.0	1.3889	1.4802	1.3482
	1.5	1.8963	1.7331	1.9569
	2.0	2.1705	1.8418	2.3110
	2.5	3.2713	2.0827	3.1293
	3.0	3.5343	2.0719	3.2569
	5.0	4.8908	2.1429	4.1906
	10.0	4.6214	2.2165	4.1612
$PImNH_2$	0.5	0.2627	0.4074	0.3497
	1.0	0.5282	0.6241	0.5688
	1.5	0.8670	0.9547	0.5728
	2.0	0.7210	0.9189	0.9722
	2.5	0.8500	1.0663	1.2348
	3.0	1.1081	1.2350	1.8222
	5.0	1.7543	1.3600	1.7047
	10.0	1.7538	1.3510	1.7682
PImCN	0.5	0.3969	0.5441	0.2892
	1.0	0.6200	0.6183	0.3653
	1.5	0.5075	0.9010	0.4038
	2.0	0.6396	0.9722	1.0943
	2.5	1.8145	1.2274	1.2630
	3.0	1.6126	1.1954	1.4168
	5.0	1.9897	1.4697	1.9454
	10.0	1.9612	1.4625	1.9190

Analysis of the adsorption isotherms: The effects of different functional groups in polymer were evaluated. Fig. 3 shows the absorbed amounts of the three target compounds on different polymers. The best polymer was PImM and PImCOOH. The force of interaction between three flavones and functional groups affects the adsorbed amount on sorbent, such as the ionic liquid-polymer. Amino-imidazole polymer had hydrophobic interactions with the three flavones and PImCN showed hydrogen-bonding and dipole-dipole interactions. On the other hand, the long carbon chain reduced the pore size and frustrated the interactions between the imidazolium groups and three flavones. Therefore, the $PImNH_2$ and PImCN exhibited low adsorbed capabilities. Methyl imidazole polymer exhibited a large pore size because of the short carbon chain other than the ionic liquid-polymer as well as feeble π-π and hydrophobic interactions between the methyl imidazolium groups and myricetin. Hydrogen-bonding interaction occurred in PImCOOH. For PImCOOH, the -COOH group exhibited substantial hydrogen-bonding interaction with the carbonyl group. In this case, PImM had the highest adsorbed amount

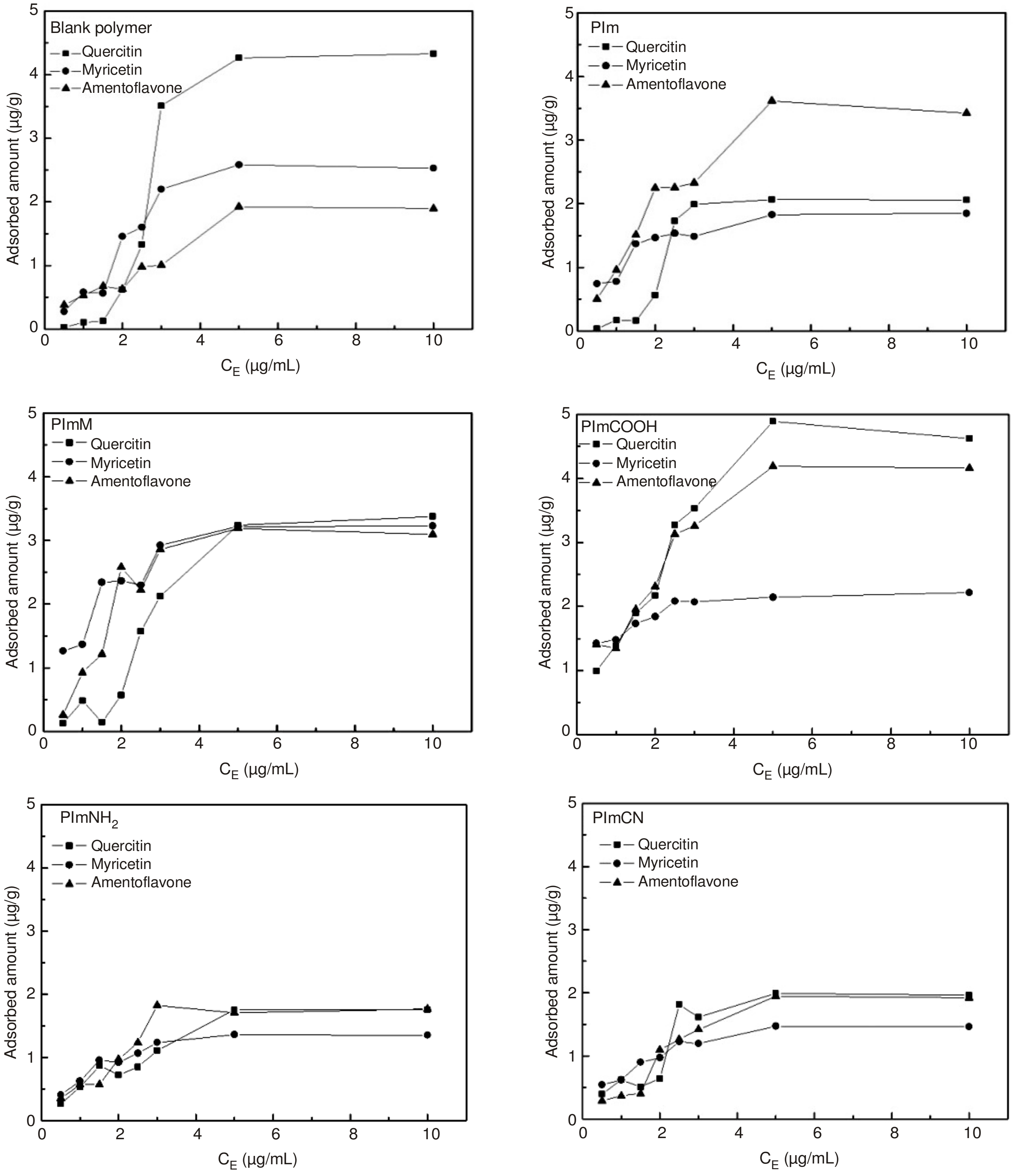


Fig. 3. Adsorbed amounts of three flavones on different sorbents

from myricetin and PImCOOH had the highest adsorbed amount from quercitin and amentoflavone.

The experiment data of three flavones were fitted to the following adsorption isotherm models:

$$Q = aC_E + b \tag{1}$$

$$Q = \frac{aC_E}{1 + bC_E} \tag{2}$$

$$Q = aC_E^{1/c} \tag{3}$$

$$Q = \frac{aC_E^c}{1 + bC_E^c} \tag{4}$$

where a, b and c are parameters and C_E (µg/mL) is the concentration of the three flavones in the solution samples. These adsorption isotherm models are the linear (2), Langmuir (3), Freundlich (4) and Langmuir-Freundlich (5) equations, respectively. The numerical coefficient 'a' in competitive Langmuir isotherm model was derived from the experimental values of the retention factors. The numerical coefficients 'b' and 'c' were obtained by fitting the experimental band profiles and the profiles given by the calculations of experimental data. The influence between the two compounds was obtained through the numerical coefficients 'b' and 'c'. Table-3 lists the adsorption parameters by three adsorption isotherm models. Among these adsorption isotherm models, the Langmuir equation is a more commonly used equation owing to its simplicity and strong theoretical background. Three essential propositions of the Langmuir isotherm[23] are the monolayer coverage,

TABLE-3
PARAMETERS IN ADSORPTION ISOTHERM OF TARGET CMOPUNDS ON THE DIFFERENCE SORBENTS

Sorbent	Compound	No. of adsorption isotherm	Parameters			r^2
			a	b	c	
Blank polymer	Quercitrin	(2)	0.51753	0.13918		0.83036
		(3)	0.86471	0.07863		0.75624
		(4)	0.79940		1.25117	0.72027
		(5)	0.00022	0.00005	10.1587	0.99068
	Myricetin	(2)	0.23618	0.72147		0.78987
		(3)	1.03858	0.26518		0.85856
		(4)	0.90630		1.94088	0.76465
		(5)	0.38935	0.14555	2.80866	0.95555
	Amentoflavon	(2)	0.17263	0.45193		0.88907
		(3)	0.59209	0.19043		0.89270
		(4)	0.57581		1.78361	0.85978
		(5)	0.53024	0.20633	1.25372	0.89702
PIm	Quercitrin	(2)	0.22274	0.38713		0.72875
		(3)	0.69300	0.19098		0.70920
		(4)	0.61828		1.65243	0.63076
		(5)	0.00037	0.00018	11.10031	0.99231
	Myricetin	(2)	0.10256	1.05509		0.75424
		(3)	1.99651	0.94073		0.91100
		(4)	1.08325		3.63319	0.80278
		(5)	2.04906	1.00905	1.13910	0.91360
	Amentoflavon	(2)	0.28938	1.18287		0.81614
		(3)	1.66109	0.34234		0.84234
		(4)	1.37206		2.18417	0.82936
		(5)	1.35985	0.35547	1.65718	0.95508
PImM	Quercitrin	(2)	0.37707	0.25268		0.86857
		(3)	0.70616	0.09023		0.83513
		(4)	0.67503		1.32419	0.79649
		(5)	0.03049	0.00901	4.82315	0.97976
	Myricetin	(2)	0.18885	1.77418		0.76991
		(3)	3.17748	0.84204		0.90706
		(4)	1.83338		3.43513	0.81391
		(5)	3.20978	0.87129	1.06577	0.90768
	Amentoflavon	(2)	0.25535	1.22832		0.71322
		(3)	1.81189	0.41724		0.84003
		(4)	1.38557		2.35150	0.70588
		(5)	1.06588	0.33041	2.59940	0.94667
PImCOOH	Quercitrin	(2)	0.39050	1.6009		0.82454
		(3)	2.17843	0.32667		0.91251
		(4)	1.84770		2.17836	0.82933
		(5)	1.76330	0.32864	0.67440	0.93704
	Myricetin	(2)	0.07494	1.63555		0.75849
		(3)	5.73324	2.49626		0.88775
		(4)	1.64116		6.38244	0.84782
		(5)	4.53946	1.78747	1.12436	0.91103
	Amentoflavon	(2)	0.30706	1.74113		0.83188
		(3)	2.45963	0.45267		0.9127
		(4)	1.89504		2.56141	0.85110
		(5)	2.41493	0.4717	1.12436	0.91892
$PImNH_2$	Quercitrin	(2)	0.15770	0.4937		0.87416
		(3)	6.80046	2.60957		0.91362
		(4)	0.59977		1.96055	0.86814
		(5)	0.61908	0.27292	1.20755	0.91752
	Myricetin	(2)	0.08341	0.72384		0.75422
		(3)	1.23789	0.75769		0.94732
		(4)	0.75107		3.23457	0.81381
		(5)	1.26340	0.8577	1.35126	0.96241
	Amentoflavon	(2)	0.14503	0.66187		0.94984
		(3)	0.92912	0.37395		0.82785
		(4)	0.75002		2.29069	0.72573
		(5)	0.57636	0.30187	2.14358	0.88568

Sorbent	Compound	No. of adsorption isotherm	Parameters			r^2
			a	b	c	
PImCN	Quercitrin	(2)	0.17119	0.64709		0.74329
		(3)	0.88601	0.30428		0.75950
		(4)	0.76204		2.10190	0.67973
		(5)	0.40339	0.18995	2.46556	0.81554
	Myricetin	(2)	0.09131	0.7577		0.79838
		(3)	1.26308	0.72018		0.94330
		(4)	0.79078		3.18167	0.85319
		(5)	1.28162	0.7521	1.07341	0.94468
	Amentoflavon	(2)	0.17916	0.51602		0.81742
		(3)	0.73397	0.24446		0.87687
		(4)	0.65600		1.88975	0.79465
		(5)	0.31544	0.15545	2.56627	0.95431

adsorption site equivalence and independence. Langmuir-Freundlic isotherm equation has three parameters than other equation and higher correlation accuracy have been proposed. The competitive Langmuir-Freundlich isotherm was used to better compare the competitive adsorption. The regression coefficients (r^2) of competitive Langmuir-Freundlich of myricetin was 0.90768 in PImM and quercitrin and amentoflavone was 0.93704 and 0.91892 in PImCOOH, respectively.

Conclusion

The adsorption isotherms of three flavones, quercitrin, myricetin and amentoflavone on different five ionic liquid polymers were compared and the interactions between the polymer and target compounds were examined. Methyl imidazole polymer showed the highest adsorbed amount from myricetin and carboxy-imidazole polymer has the highest adsorbed amount from quercitrin and amentoflavone. The hydrogen-bonding interaction between the three flavones and functional groups affects the adsorbed amount on the sorbent due to the force of the interaction, such as the large pore size, feeble p-p and hydrophobic interactions. The regression coefficients (r2) of competitive Langmuir-Freundlich of myricetin were 0.90768 in methyl imidazole polymer and quercitne and amentoflavone was 0.93704 and 0.91892 in carboxy-imidazole polymer, respectively.

ACKNOWLEDGEMENTS

This study was supported by Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology (2012-0005250).

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