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Evaluation of Ionic Liquid-Modified Mesoporous Silica Particles for Separation of Polysaccharides

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A group of ionic liquid-modified mesoporous silica particles was synthesized and characterized. A suitable sorbent with a suitable imidazole group and anion was selected according to its adsorption behaviour to the two polysaccharides on various synthesized particles. To confirm the conditions for the optimal adsorption capacity of the proposed sorbent, the standard/sorbent ratio, time and pH were investigated. The adsorption behaviour of these polysaccharides allowed the purification and separation of one compound from another and might find applications in extraction from natural plants in further studies.

Keywords: Ionic liquid, Mesoporous silica particles, Alginate, Fucoidan, HPLC.

INTRODUCTION

Alginate or alginic acid is a gelling polyuronide that is composed of β-D-mannuronic acid (M-block) and α-L-guluronic acid (G-block) arranged in a block wise, non-regular order along the chain [1]. Alginate is used widely as a food ingredient owing to its colloidal properties and has been applied in medicine for the treatment of various diseases, such as allergic diseases, hypertension and tumors and in drug delivery systems [2-5]. Fucoidan, a sulfated polysaccharide, essentially contains fucose and sulfate, with a small proportion of xylose, mannose, galactose, uronic acids and sometimes even proteins [6,7]. Fucoidans have exceptional bioactivities, such as antibacterial and antiviral, anticoagulant, antithrombotic, antiinflammatory and antitumoral activities [8]. Therefore, polysaccharides, such as fucoidan and alginate have attracted increasing attention.

Many studies have reported that the molecular weight plays an important role in both the function of polysaccharides and the analytical solution. Size exclusion chromatography (SEC) is the most common way of distinguishing and detecting a variety of polysaccharides according to their molecular weight. The molecular weights of fucoidan, alginate and even other polysaccharides from different sources vary widely, which will lead to overlap [9]. In addition, in the extraction procedure, pH, time and temperature can affect the length of the macromolecules, which will reduce the accuracy for further analysis. Considering these factors, the extracted polysaccharides should be purified and separated before being detecting by size exclusion chromatography to avoid the overlapping molecular weights between different polysaccharides.

Mesoporous siliceous particles (MSP) with well-defined pore structures and excellent stability have been applied widely to catalysis and the adsorption process for its specific selectivity [10]. To increase the adsorption capacity of mesoporous siliceous particle, a method for modifying functional groups on mesoporous siliceous particle was proposed. Modifiers with specific functional groups can strengthen the interactions. The ionic liquid (IL)-modified mesoporous siliceous particle exhibits multiple interactions, such as ion exchange, electrostatic and hydrophobic interactions.

This study provides a simple and efficient solution to the purification and separation of biomacromolecules with similar molecular weights based on the polysaccharides adsorption discrepancy on ionic liquids-modified mesoporous siliceous particle. The optimal modification of mesoporous siliceous particle by ionic liquids was investigated and the adsorption isotherm that is associated with the adsorption capacity was used to evaluate the feasibility of the material for further applications. The potential application of the ionic liquids-modified mesoporous siliceous particle are also examined.

EXPERIMENTAL

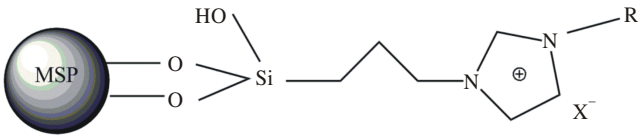
Fucoidan, alginic acid sodium salt, sodium tetrafluoroborate (98 %) *bis*(triflоromethane)sulfonimide lithium salt (≥ 99 %), poly(ethylene glycol)-block-poly(propylene glycol)-

block-poly(ethylene glycol) (PEG-PPG-PEG) and ammonium fluoride (NH_4F, 98 %) were obtained from Sigma Aldrich (St. Louis, MO, USA). Tetraethoxysilane (TEOS, 98 %) was acquired from Alfa Aesar (Heysham, England). Methanol, toluene and ethanol were supplied by Duksan Pure Chemical Co. Ltd. (Ansan, Korea). Hydrochloric acid was purchased from Daejung (Gyonggi-do, South Korea). Imidazole (99 %), 1-methylimidazole (99 %) and 3-chloropropyltriethoxysilane (95 %) were obtained from Aldrich (Milwaukee, WI, USA). 1-Ethylimidazole (98 %) and 1-butylimidazole (98 %) 1,3,5-trimethylbenzene (TMB, 99 %) were supplied by Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Distilled water was filtered using a vacuum pump (Division of Millipore, Waters, USA) and a filter (HA-0.45, Division of Millipore, Waters, USA). The samples were filtered through a filter (Minisart RC 15, 0.5 µm, Goettingen, Germany) before being injected into the HPLC system.

Preparation of ionic liquid-modified mesoporous siliceous particle: Based on previous studies, a convenient and simple method for synthesizing mesoporous siliceous particle was used [11-13]. 4 g of PEG-PPG-PEG was dissolved in a HCl solution and 5 g of 1,3,5-trimethylbenzene was then added to the reactor. The mixture was stirred at 40 °C for 2 h. Subsequently, 9 mL of tetraethoxysilane was added drop wise and the mixture was transferred to an autoclave at 40 °C for 20 h. Ammonium fluoride (45 mg) was then added to the autoclave with increasing temperature to 150 °C for 24 h. After aging, the white precipitate was filtered, washed sequentially with water and ethanol and dried at 60 °C. The white powder was then calcined at 900 °C in a muffle furnace for 6 h. After cooling, the white particles were the desired mesoporous siliceous particle.

To synthesize ionic liquid-modified mesoporous siliceous particle, the mesoporous siliceous particle powder was first immersed in hydrochloric acid for 24 h, washed with deionized water and dried at 100 °C for 8 h. The activated mesoporous siliceous particle (5 g) was suspended in 50 mL of dry toluene and an excess of 3-chloropropyltriethoxysilane (5.0 mL) was added. The suspension was stirred and heated under reflux for 12 h. After reflux, the reaction was stopped when the modified mesoporous siliceous particle was cooled to room temperature and washed sequentially with toluene, ethanol and methanol. The synthesized chloropropyl mesoporous siliceous particle (MSPprCl) was dried at 60 °C for 10 h.

Subsequently, the chemically-bonded chloropropyl group on the mesoporous siliceous particle surface was reacted with imidazole, 1-methylimidazole, 1-ethylimidazole and 1-butylimidazole, respectively. Briefly, 5 g of dry chloropropyl mesoporous siliceous particle was added to a reaction flask containing 50 mL of toluene and an excess of imidazoles (5 g). The mixture was heated under reflux with continuously stirring for 24 h. After cooling to room temperature, the modified mesoporous siliceous particle was washed sequentially with toluene, ethanol and methanol. The mesoporous siliceous particle bonded chemically with imidazolium was dried at 60 °C for 10 h. The mesoporous siliceous particle-modified materials bonded with imidazole, 1-methylimidazole, 1-ethylimidazole and 1-butylimidazole are called MSPprImCl, MSPprMImCl, MSPEImCl and MSPprBImCl, respectively (Fig. 1).



MSPprImCl: R = no additional group

MSPprMImCl: R = —CH_3; X = Cl^-

MSPprEImCl: R = —CH_2—CH_3; X = Cl^-

MSPprBImCl: R = —$(CH_2)_3$—CH_3; X = Cl^-

Fig. 1. Chemical structure of all the ionic liquid-modified mesoporous siliceous particle sorbents

Adsorption: The static method was performed on different synthesized particles to examine the adsorbent capacity. The particles (10 mg) and 1 mL of alginate and fucoidan with concentrations ranging from 0.3 to 1 mg mL^{-1} were added to the vials with constant stirring at room temperature for 3 h to obtain the equilibrium adsorptions. When the equilibrium adsorption was obtained, the amounts of adsorbed polysaccharides on the MSP-ILs were calculated by subtracting the concentration of unadsorbed polysaccharides from the initial amounts of those polysaccharides.

HPLC analysis: The HPLC system consisted of a YL9112 Isocratic pump (Young Lin Co., Anyang, Korea), RI detector (RI750F, Young Lin Instrument Co., Anyang, Korea) and integrated data system (Clarity Chromatography Software, version 2.3, DateApex, EU). Injection valves with 20 µL sample loops were used. HPLC was performed using a Waters Ultrahydrogel™ WATO 11530 size exclusion column (300 × 7.8 mm i.d.) and a Waters Ultrahydrogel™ WATO 11565 guard column (40 × 6.0 mm i.d.) from Waters (Milford, MA, USA). The mobile phase was water. The flow-rate and injection volume was set to 0.6 mL min^{-1} and 10 µL, respectively.

Characteristic analysis: The synthetic mesoporous siliceous particle was characterized by field emission-scanning electron microscopy (FE-SEM, S-4200, Hitachi, Ontario, Canada), transmission electron microscopy (TEM, CM200, Philips, Netherlands) and Brunauer-Emmett-Teller (BET) analysis. The BET surface area (N_2 atmosphere at -195.8 °C) was measured using an ASAP2020 surface area analyzer (Micromeritics, Norcross, GA, USA).

Fourier transform infrared (FT-IR, Vertex 80 V, Bruker, USA) spectroscopy was performed in the range of 4000-400 cm^{-1} with a scan rate of 20 scans min^{-1}. A KBr pellet was used for FT-IR analysis. Thermogravimetric analysis (TGA, SCINCO thermal gravimeter S-1000) was performed at a heating rate of 20 °C min^{-1} under nitrogen.

RESULTS AND DISCUSSION

Performance evaluation: The mesoporous siliceous particles were synthesized using the methodology reported elsewhere [14,15]. The SEM image of mesoporous siliceous particle (Fig. 2a) indicated the uniform nanospheres with a mean particle size of 3-5 µm. According to Fig. 2b (TEM) and the N_2 sorption data, the mesoporous siliceous particles exhibited a uniform pore distribution and porosity on the particle surface. As a result, the mean pore size, surface area

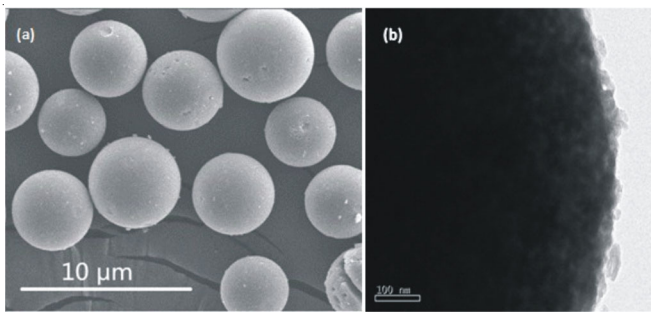


Fig. 2. (a) SEM and (b) TEM images of mesoporous siliceous particle

and porous volume was 140 Å, 625 m^2 g^{-1} and 2.15 cm^3 g^{-1}, respectively, which showed stability and homogeneity.

Fig. 3 shows the FT-IR spectra of the ionic liquid-modified mesoporous siliceous particles. The asymmetric Si-O stretching and Si-O bending modes of silica were observed at about 470 and 810 cm^{-1}, respectively [16]. The peak at 1593 cm^{-1} represents the imidazole groups [17]. The hydrogen in O-H stretching vibration bonded to water can be reflected on the broad band at 3442 cm^{-1}. The weight loss observed by TGA between 200 to 800 °C was associated with the loss of organic groups in synthesized sorbents, which can be used to determine the thermal stability of the modified sorbents [18]. Fig. 4 shows the TGA curves of the ionic liquid-modified mesoporous siliceous particles. Overall, the modified sorbents applied in this study had been successfully synthesized.

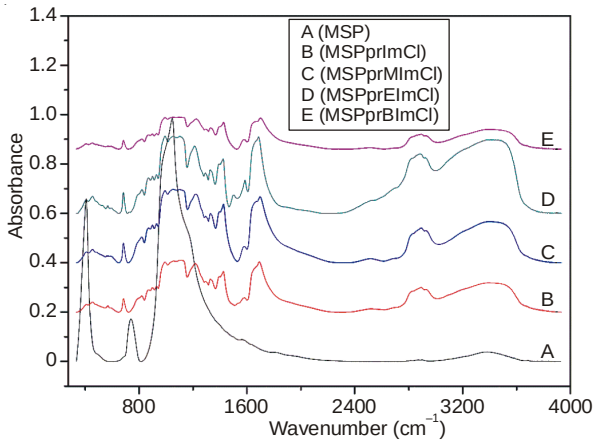


Fig. 3. FT-IR spectra of ionic liquids modified mesoporous siliceous particles

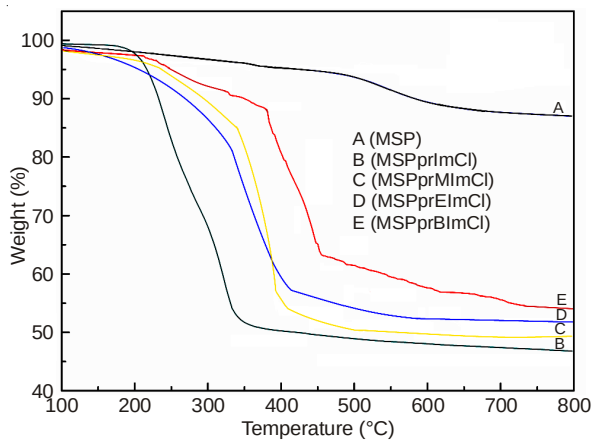


Fig. 4. TGA curves of all the ionic liquid-modified mesoporous siliceous particle sorbents

Optimization of ionic liquids modified sorbents: The calibration curves of the polysaccharides were constructed at six increasing concentrations, ranging from 0.3 to 1.2 mg mL^{-1}. The linear correlation equations were Y = 99.925X - 20.242, R^2 = 0.9974 for fucoidan and Y = 100.43X - 13.112, R^2 = 0.9967 for alginate, where Y is the peak area (mV) and X is the concentration of the injected solution (mg mL^{-1}).

The amounts of fucoidan and alginate adsorbed on the modified sorbents in the flasks were measured after preparing different concentrations of equilibrium adsorptions. The adsorption capacity of the sorbents was considered, which provides a method to examine the interactions between the target compounds and modified sorbents. The amounts of fucoidan and alginate adsorbed onto the different sorbents were obtained using the following equation:

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

where Q (mg g^{-1}) is the amount adsorbed, m (g) is the mass of the sorbent, V (mL) is the volume of the sample solvent, C_o (mg mL^{-1}) is the initiator concentration and C (mg mL^{-1}) is the unabsorbed concentration.

As shown in Fig. 5a, the amounts of both fucoidan and alginate adsorbed on the different ionic liquid-modified mesoporous siliceous particles increased in the following order: MSP-26 < MSPprImCl < MSPprMImCl < MSPprEImCl < MSPprBImCl. The adsorbed amounts of the two polysaccharides on mesoporous siliceous particle were lower than that on mesoporous siliceous particle with the modified ionic liquids, which showed that the functional groups of the sorbents affect the selectivity towards and the adsorption capacities of the target compounds. According to the data, the adsorption capacities increased with increasing length of alkyl groups. This can be explained by the variation in the pore size in mesoporous siliceous particle. The proper pore size should not only allow the target compounds to enter, but also makes them easier to capture the target compounds. Therefore, the pore size of mesoporous siliceous particle after being modified using butylimidazole appears to have satisfied the demands that can improve the adsorptions. The molecular weight of alginate is larger and appears to have difficulty in entering the pores. In this case, the amounts of alginate adsorbed onto the modified mesoporous siliceous particle were much lower because of the effect of size exclusion. The adsorption capacity discrepancy makes it possible to purify and separate one compound from another.

Ionic liquid-modified silica was also investigated to confirm the utility of the ionic liquid-modified mesoporous siliceous particles. As shown in Fig. 5b, the ionic liquids modified mesoporous siliceous particles exhibited stronger affinity and larger adsorption capacity discrepancy to both fucoidan and alginate compared to ionic liquid-modified silica. This phenonmenon was attributed to the pores on mesoporous siliceous particles. As a result, MSPprBImCl was better than others and was used in further experiments.

Ionic liquids normally are composed of organic cations and anions with unique chemical and physical properties. The properties of both the cation and anion are useful tools for fine-tuning the properties of the synthesized ionic liquids. The

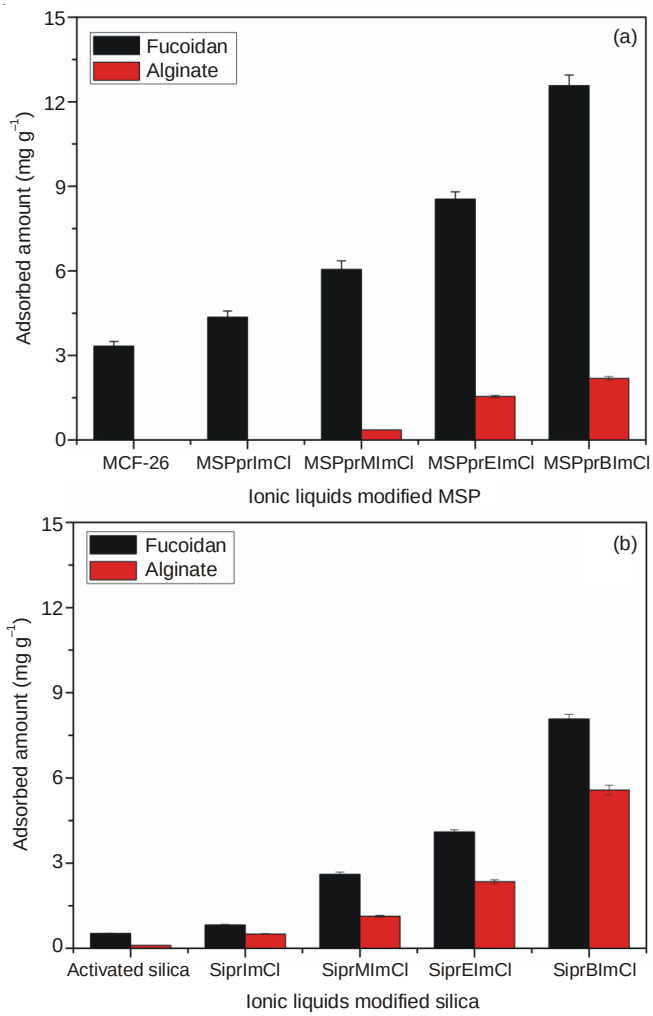


Fig. 5. Adsorbed amounts of fucoidan and alginate on (a) ionic liquids modified mesoporous siliceous particles and (b) ionic liquids modified silica

ionic liquids resulting from the cations and anions can have hydrophilic, hydrophobic, super acidic, basic, water miscible and water immiscible properties [19,20]. Currently, the cation can affect the hydrogen bonding ability or hydrophobicity, whereas the anion is used to control the water miscibility [21,22]. Several hydrophobic and hydrophilic salts of *bis*(trifluoromethylsulfonyl)imide Tf_2N^-, PF_6^-, BF_4^-, Cl^- were prepared. The results suggested that the hydrophobicity of the anions increases from Cl^-, BF_4^-, PF_6^- to Tf_2N^- [23]. The major interactions between fucoidan and the particles was ion exchange interaction and the capture ability of the pore size in the mesoporous siliceous particles, which means that Cl^- anions has larger adsorption capacity of fucoidan. On the other hand, the amount of alginate adsorbed decreased with increasing hydrophobicity of the anions (Fig. 6). Overall, the use of MSPprBImCl was preferred due to the obvious adsorption discrepancy of fucoidan and alginate.

Effect of concentration on adsorbed efficiency: An inadequate amount of sorbent cannot provide complete adsorption of the target compounds, but an excessive proportion of the sorbent can also decrease the efficiency by increasing the required solvent. A suitable standard/sorbent ratio is crucial for providing a thorough adsorption of the compounds to

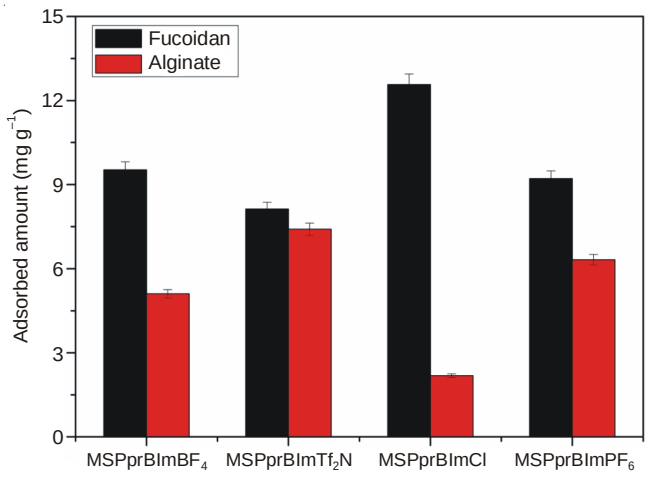


Fig. 6. Adsorbed amounts of fucoidan and alginate on MSPprBIm with different anion groups

facilitate transfer to the sorbent. As shown in Fig. 7, the maximum adsorption appeared when the standard concentration was 0.5 mg mL^{-1} for 20 mg of the MSPprBImCl. In addition, the amounts of alginate adsorbed were much lower than those of fucoidan at certain concentrations, which indicated that most of the alginate remained in the extract. Therefore, fucoidan and alginate can be separated easily from one another.

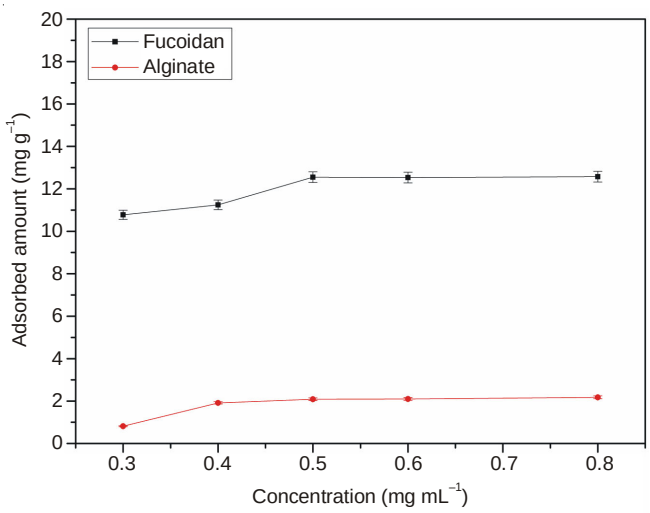


Fig. 7. Effect of concentration on adsorbed efficiency (time: 3 h; temperature: room temperature; pH: 7)

Effect of time on adsorbed efficiency: The time for the polysaccharides and sorbent contacting with each other is another essential factor that contributes to the adsorbed amount. The adsorbed amounts of both fucoidan and alginate were investigated as a function of the immersion time, ranging from 2 to 8 h. Fig. 8 shows that the amounts of fucoidan and alginate adsorbed on MSPprBImCl increased with increasing time, whereas the increased amounts were negligible when the time was longer than 4 h. On the other hand, regardless of the time, the amount of fucoidan adsorbed was much higher than that of alginate. As a result, the time for mixing polysaccharides and sorbent was set to 4 h.

Effect of pH value on adsorbed efficiency: As shown in Fig. 9, the effects of pH ranging from 5 to 10 on the adsorption

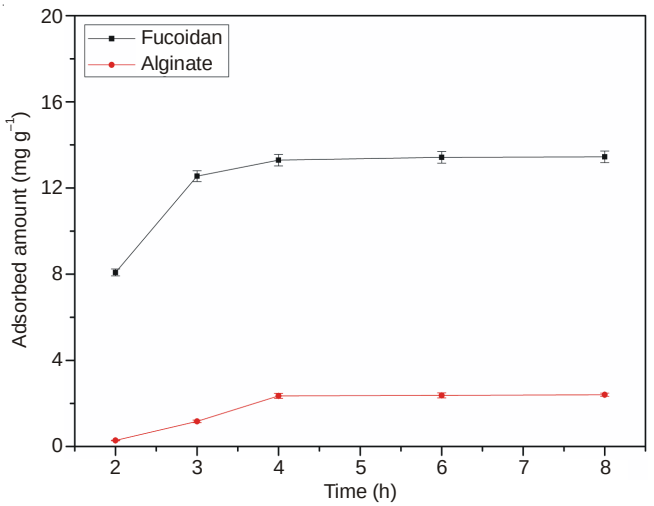


Fig. 8. Effect of time on adsorbed efficiency (Standard compound concentration: 0.5mg mL^{-1}; temperature: room temperature; pH: 7)

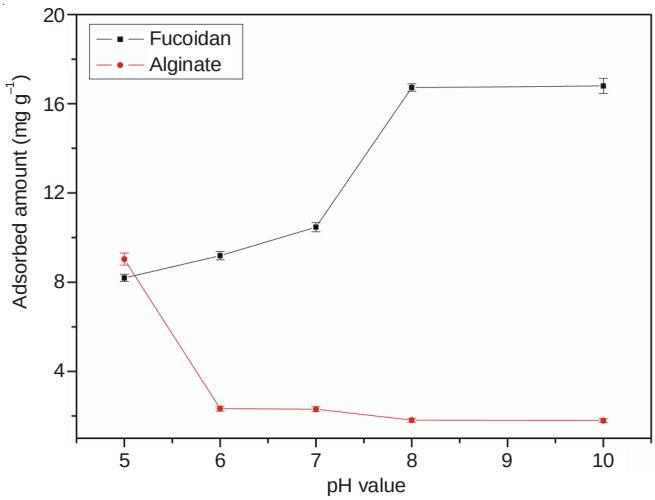


Fig. 9. Effect of pH value on adsorbed efficiency (Standard compound concentration: 0.5 mg mL^{-1}; time: 4 h; temperature: room temperature)

efficiency were investigated. The amount of fucoidan adsorbed on MSPprBImCl increased with increasing pH, which means that the alkaline condition made it easier for fucoidan adsorption. On the other hand, the amount of alginate adsorbed decreased with increasing pH. This completely opposite trend suggests that the adsorption discrepancy is more obvious under alkaline conditions. In this situation, alkaline solutions were found to be the optimal mixing condition for the purification and separation of polysaccharides.

Analytical performance: A series of experiments was designed to examine the linearity, precision, limit of detection (LOD) and other properties. Both targets showed good linearity ($R^2 > 0.99$). The LODs of fucoidan and alginate based on a signal-to-noise ratio of 3 were 0.23 and 0.36 mg mL^{-1} respectively. Repeatability assays were conducted according to the relative standard deviations (RSDs), which were determined from five injections of the two polysaccharides over a five-day period. The RSDs of fucoidan and alginate were 2.1 and 3.2 %, respectively. These results confirm the acceptable accuracy and precision of the proposed method as well as its potential applications in polysaccharides separation.

Conclusion

Ionic liquid-modified mesoporous siliceous particles with the expected pore size, high concentration of functional groups and highly selective properties were synthesized for the purification and separation of polysaccharides. Compared to the ionic liquids modified silica, MSPprBImCl showed better affinity to the analytes, which have potential applications for the separation of polysaccharides in natural plants. In addition, the proper linked anion, standard concentration, time and pH value were determined. The proposed method based on the difference in the adsorption capacity between fucoidan and alginate showed that purification and separation fucoidan and alginate can be separated and purified from natural sources.

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