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Application of Ionic Liquid Modified Silica for Solid-Phase Extraction of Polysaccharides from *Laminaria japonica*

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Silica-confined ionic liquids were synthesized for solid-phase extraction of polysaccharides, which was determined by high-performance liquid chromatography coupled with RI detection. The sorbent with amino-imidazolium groups was found to be the optimal material by a comparison of the adsorption capacity of fucoidan and laminarin onto different synthetic sorbents. The proper elution solvents for both laminarin and fucoidan were decided from practical tests. The sorbent performed stably and selectively, demonstrating potential applications in the separation of hydrophilic biomacromolecules, such as polysaccharides.

**Keywords**  Solid-phase extraction; Ionic liquid modified sorbents; Laminarin; Fucoidan

**INTRODUCTION**

*Laminaria japonica*, which is one of the most important marine medicinal foodstuffs, is an abundant and widely distributed source of biologically active compounds. As the typical biological components, water-soluble polysaccharides, such as laminarin and fucoidan, and alkali-soluble polysaccharide alginic acids have attracted considerable attention. To predict and evaluate the biological activities of polysaccharides, their separation and purification processes, which
are the foundations for further study, occupy an important position. A variety of methods have been applied to separate and purify polysaccharides, including size exclusion chromatography, ion-exchange chromatography, ethanol precipitation, salting out, and ultrafiltration membrane separation. Solid-phase extraction (SPE) is a high-performance technology for separating bioactive compounds from plants, but little attention has been paid to its usage in polysaccharide separation.

As an emerging alternative to liquid–liquid extraction for the separation, purification, and even solvent exchange of solutes from another solution, SPE overcomes some of the problems associated with liquid–liquid extraction, namely, the use of expensive solvents, disposal of organic solvents, incomplete phase separation, less than quantitative recovery, and complex procedures. The selection of sorbents that are closely related to the analytical parameters, such as the affinity, selectivity, and capacity, is crucial in an SPE process. In view of the importance of sorbents for SPE, the traditional SPE materials were modified using a range of methods. Among these, some modified sorbents have been increasingly attractive.

Ionic liquids (ILs) are considered to be a greener alternative owing to their novel chemical and physical characteristics, such as nonvolatility, wide electrochemical window, tunable viscosity, thermal stability, wide liquid range, and nonflammability. IL-modified silica materials (Si-ILs) are widely used in separation work because of their provided interactions including hydrophobic, π-π, hydrogen bonding, and ionic interactions. A range of Si-ILs have been synthesized and applied as HPLC stationary phases and SPE sorbents to separate low-molecular-weight bioactive compounds. On the other hand, there are few reports describing the application of IL-modified silica to the separation of biomacromolecules.

In this study, Si-ILs were synthesized and used to pack cartridges for SPE separation of laminarin and fucoidan from Laminaria japonica. The adsorption isotherms were examined to evaluate the adsorption capacity of the synthesized materials, and the proper elution solvents for both target compounds were determined experimentally.

RESULTS AND DISCUSSION

Synthesis of Si-ILs

Si-ILs were synthesized according our previous method. Silica was first activated by reacting with an excess of 3-chloropropyltriethoxysilane to get chloropropyl silica (SilprCl). The chloropropyl group–bonded silica (SilprCl) was then reacted with imidazole, 2-propylimidazole, and 1-(3-aminopropyl)
imidazole to produce modified silica materials bonded with imidazole, 1-butylimidazole, 2-propylimidazole, and 1-(3-aminopropyl) imidazole, which are called SilprImCl, SilprBImCl, SilprPImCl, and SilprImNH2Cl (Fig. 1), respectively.

Figure 2: TGA curves for (A) SilprCl and (B) SilprImNH2Cl.
Table 1: Elemental analysis of IL-modified silica

<table>
<thead>
<tr>
<th>Materials</th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
<th>Coverage (μmol/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SilprCl</td>
<td>7.04</td>
<td>1.62</td>
<td>—</td>
<td>3.96</td>
</tr>
<tr>
<td>SilprImNH₂Cl</td>
<td>8.21</td>
<td>1.67</td>
<td>1.42</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Performance Evaluation

The weight loss observed between 200°C and 800°C is generally associated with the loss of organic groups attached to the material surface. Therefore, thermogravimetric analysis (TGA) can be applied to determine the thermostability of the chemically modified silica materials. Figure 2 shows the TGA curves of SilprImCl and SilprImNH₂Cl. SilprImNH₂Cl shows greater loss because of the extra combination of amino groups. In comparison with SilprCl, elemental analysis (Table 1) of SilprImNH₂Cl revealed the appearance of nitrogen, showing that immobilization on the surface had been successful. According to the percentage of carbon (C%), the concentration of organic groups bonded to the silica surface was 3.96 μmol/m² of chloropropyl groups for SilprCl. And from the percentage of nitrogen (N%), the concentration of imidazolium groups attached to the chloropropyl silica is calculated as 0.71 μmol/m² for SilprImNH₂Cl. The calculation formulas of the surface coverage are listed as follows:

\[
\text{SilprCl (μmol/m²)} = \frac{C\%}{36 \times (1 - C\% - H\%) \times S} = 3.96 \quad (1)
\]

\[
\text{SilprImNH₂Cl (μmol/m²)} = \frac{N\%}{42 \times (1 - C\% - H\% - N\%) \times S} = 0.71 \quad (2)
\]

where H%, C%, N%, and S represent the percentage of hydrogen, carbon, nitrogen, and the specific surface area of the silica support (m²/g). As a result, immobilization of the amino group was also successful.

Figure 3 shows the FT-IR spectra of SilprCl and SilprImNH₂Cl. The asymmetric Si—O stretching and Si—O bending modes of silica correspond to the lower-frequency bands at approximately 470 and 810 cm⁻¹. The band at 1593 cm⁻¹ represents the imidazole groups. The band of the N—H group was observed from 3300 to 3600 cm⁻¹. Therefore, the SilprImNH₂Cl was synthesized successfully.
Adsorbed Amount on Synthesized Sorbents

The calibration curves of the two polysaccharides were first constructed according to the measurements of the chromatographic peak areas at six increasing concentrations, ranging from 0.25 to 2.0 mg/mL. The linear correlation equations were \( y = 86.766x - 3.2675 \) (\( r^2 = 0.997 \)) for fucoidan, and \( y = 103.19x - 9.6704 \) (\( r^2 = 0.991 \)) for laminarin \((y \) is the peak area and \( x \) is the concentration of the polysaccharides).

The adsorption capacity of these sorbents was introduced not only to examine the interactions between the target compounds and the sorbent but also to choose the optimal sorbent for separating the two different polysaccharides. The amounts of fucoidan and laminarin adsorbed onto the different sorbents were determined using the following formula:

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

where \( Q \) (mg/g) is the adsorbed amount, \( C_o \) (mg/mL) is the initiator concentration, \( C \) (mg/mL) is the unabsorbed concentration, \( V \) (mL) is the volume of the sample solvent, and \( m \) (g) is the mass of the sorbent.

As a result, the order of the amounts of polysaccharides adsorbed by different Si-ILs was SilprImNH₂Cl > NH₂ (commercial NH₂ cartridge) > SilprImCl > SilprBImCl > SilprPImCl for fucoidan, and SilprBImCl > SilprPImCl > NH₂ > SilprImCl > SilprImNH₂Cl for laminarin (Table 2). Fucoidans are composed mainly of sulfated esters of fructose. In addition, they also contain...
other monosaccharides, such as mannose, galactose, glucose, xylose, and uronic acid, and even acetyl groups and proteins.\textsuperscript{[17]} Laminarin is composed of glucose monomers joined by $\beta$-1,3-glycosidic bonds with $\beta$-1,6-branching.\textsuperscript{[18]} According to the order, no direct relationship was observed for the absorption capacity between the length of the alkyl groups and fucoidan. On the other hand, when the length of the alkyl groups increased, the adsorbed laminarin amounts were increased. Hydrogen bonding and $\pi$–$\pi$ and ionic interactions were available in SilprImCl. The adsorption capacity of SilprImCl was relatively low for both polysaccharides. The amount of fucoidan adsorbed onto SilprImNH$_2$Cl was the highest because SilprImNH$_2$Cl exhibited extra hydrogen bonding and ionic interactions from amino groups compared to other Si-ILs.\textsuperscript{[16]} Moreover, the amount of laminarin adsorbed onto SilprImNH$_2$Cl was the lowest due to its high hydrophilicity, which made it easier to elute and separate one target from another. Thus, SilprImNH$_2$Cl had the best selectivity.

Figure 4: Adsorbed amounts of fucoidan and laminarin by SilprImNH$_2$Cl.
Based on the above optimization, SilprImNH$_2$Cl was selected for further studies. As shown in Figure 4, the adsorption isotherms were introduced to investigate the interactions between polysaccharides and SilprImNH$_2$Cl. According to the data, the adsorbed amounts of polysaccharides onto SilprImNH$_2$Cl increased with increasing concentrations of fucoidan and laminarin, and the maximum adsorption appeared when the concentration was 0.80 mg/mL. Another phenomenon was also observed in the experiment. The adsorbed amounts of laminarin were found to be much lower than that of fucoidan at high concentrations, which indicated that most of the laminarin remained in the extract, and thus the fucoidan and laminarin can be easily separated from each other.

### Separation

A mixture of polysaccharides was prepared in water and 0.2 mL of aqueous solution was loaded onto SilprImNH$_2$Cl cartridges. The amounts of the polysaccharides in the eluents were detected by HPLC. Subsequently, 1 mL of different solvents was used to separate fucoidan and laminarin. The amount of laminarin adsorbed onto SilprImNH$_2$Cl was low, so it was easier to find the elution solvent for laminarin. First, solvents with different polarities were chosen to elute laminarin (Fig. 5). All these organic solvents had the ability to elute most of the laminarin and a little of the fucoidan out from the SilprImNH$_2$Cl cartridge. On the other hand, for 0.80 mol/L hydrochloric acid, a large amount of laminarin and fucoidan was eluted. Therefore, acidic or salt solutions need to be considered as the eluents for fucoidan. When acetonitrile was used, a
small amount of fucoidan was detected in the eluent from the SilprImNH₂Cl cartridge, but most of the laminarin had been eluted. Therefore, acetonitrile was considered to be optimal.

After using acetonitrile to elute laminarin, different salts and acids were arranged for eluting fucoidan. Considering that fucoidan contains a sulfonic group in its structure, the anion-exchange interaction between fucoidan and SilprImNH₂Cl is one of the major interactions. Sodium nitrate, nitric acid, hydrochloric acid, and sodium chloride solutions were dissolved in water at a concentration of 0.80 mol/L. By comparing the amounts eluted of fucoidan by different salts and acids (Fig. 6), the salts were found to be better than the acids. A sodium nitrate solution was found to be the best for eluting fucoidan.

**Effect of Sodium Nitrate Solution Concentration**

A sodium nitrate solution was selected as the optimal eluent for fucoidan, and a range of sodium nitrate concentrations was investigated with a loading of 2 mg/mL of the mixtures (Fig. 7). The amount of eluted fucoidan increased with increasing sodium nitrate concentration from 0.30 to 0.80 (mol/L), which means enhanced ionic strength. The eluted amount began to level off when the ratio was increased to 1 mol/L. In light of the performance of the different concentrations, a sodium nitrate solution of 0.80 mol/L was selected as the appropriate concentration for eluting fucoidan. The repetition times were also evaluated. The use of more than 2 mL did not improve the amounts of both

![Figure 6: Elution performance of fucoidan by different solvents.](image-url)
polysaccharides eluted significantly. Therefore, 2 mL of a 0.80-mol/L sodium nitrate solution was considered the best.

**Analytical Performance**

To evaluate the proposed method, its linearity, precision, limit of detection (LOD), and other properties were estimated. The LODs of laminarin and fucoidan based on a signal-to-noise ratio of 3 were 0.13 mg/mL and 0.18 mg/mL, respectively. Repeatability assays were conducted by five injections of standard mixtures of two polysaccharides after SPE over a 5-day period. The relative standard deviations (RSDs) of fucoidan and laminarin were 3.9% and 4.2%, respectively. This confirmed the stability of the proposed method and its further potential applications.

**Application of Proposed Method in Separation of Natural Plant Extracts**

After loading 0.2 mL of the natural extract of *Laminaria japonica*, the SilprImNH₂Cl cartridge was eluted sequentially with acetonitrile (2 mL) and a 0.8-mol/L sodium nitrate solution (2 mL). The two polysaccharides were separated successfully (Fig. 8). Approximately 54.18 mg/g of fucoidan and 255.10 mg/g laminarin were obtained.
CONCLUSION

IL-modified silica sorbents were developed for SPE of polysaccharides. Anion-exchange metathesis with other interactions improved the adsorption capacity for fucoidan, whereas the adsorption capacity for laminarin was very low. The adsorption discrepancy of fucoidan and laminarin onto the SilprImNH2Cl sorbent made it possible to develop a new way to separate them. The analysis of a real sample under optimized elution conditions revealed the proposed method to be a viable alternative for separating these two polysaccharides.

EXPERIMENTAL

Materials

The seaweed species, *Laminaria japonica*, was purchased from a local market in Korea. The fucoidan and laminarin standards were obtained from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile, methanol, chloroform, and sodium nitrate were supplied by Duksan Pure Chemical Co. Ltd. (Ansan, Korea). Hydrochloride acid was purchased from Daejung (Gyonggi-do, South Korea). Imidazole (99%) and 3-chloropropyltriethoxysilane (95%) were obtained from Aldrich (Milwaukee, WI, USA). 2-Propylimidazole and 1-(3-aminopropyl) imidazole were acquired from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Distilled water was filtered using a vacuum pump (Division of Millipore, Waters, Milford, MA, USA) and a filter (HA-0.45, Division of Millipore, Waters,
Milford, MA, USA). The samples were filtered (Minisart RC 15, 0.45 μm, Goettingen, Germany) before being injected into the HPLC system.

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 mm i.d.) from Waters (Milford, MA, USA). The mobile phase was water. The flow-rate and injection volume were set to 0.6 mL min⁻¹ and 10 μL, respectively.

Preparation of Si-ILs

Si-ILs were synthesized as in our previous report.[11] Silica was first immersed in hydrochloric acid for 24 h, washed with deionized water, and dried at 100°C for 8 h. The activated silica (5 g) was suspended in 50 mL of dry toluene, and an excess of 3-chloropropyltriethoxysilane (5 mL) was added. The suspension was stirred and heated under reflux for 12 h. Subsequently, the reaction was stopped when the modified silica was cooled to rt, and washed sequentially with toluene, ethanol, and methanol. The synthesized chloropropyl silica (SilprCl) was dried at 60°C for 10 h.

The chloropropyl group–bonded silica (SilprCl) was then reacted with imidazole, 2-propylimidazole, and 1-(3-aminopropyl) imidazole. Briefly, 5 g of dry SilprCl 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 continuous stirring for 24 h. After cooling to rt, modified silica was washed sequentially with toluene, ethanol, and methanol. The silica chemically bonded with imidazolium was dried at 60°C for 10 h to get SilprImCl, SilprBImCl, SilprPImCl, and SilprImNH₂Cl.

Adsorption

The static method was performed on the different synthesized particles to evaluate the adsorbent capacity. The particles (10 mg) and 1 mL of the standard mixtures of laminarin and fucoidan with a series of concentrations were added to vials with constant stirring at rt for 3 h to obtain the equilibrium adsorptions. When the equilibrium adsorptions were obtained, the amounts of adsorbed polysaccharides on the Si-ILs were calculated by subtracting the concentrations of unadsorbed polysaccharides from the initial amounts of these polysaccharides.
Procedure of SPE

Si-ILs (0.2 g) were packed into empty cartridges and pretreated with ethanol and methanol. Polysaccharide standard mixtures (0.2 mL) were loaded onto the packed cartridges. Different solvents, such as water, ethanol, acetonitrile, chloroform, sodium nitrate solution, and hydrochloride acid solution, were then assessed to identify the proper elution solvents for both polysaccharides. The filtrates were evaporated until dryness and reconstituted in water (0.2 mL) for further HPLC detection. Hydrochloric acid (0.10 mol L$^{-1}$) was used to extract fucoidan and laminarin from Laminaria japonica at a sample-to-solvent ratio of 1/30 g mL$^{-1}$ for 4 h at 80°C.

Characteristic Analysis

The hydrogen, carbon, and nitrogen contents were measured by elemental analysis performed on an EA1112 instrument (Italy). The Fourier transform infrared (FT-IR, Vertex 80 V Bruker, USA) spectra were measured in the range of 4000–400 cm$^{-1}$ with a scanning rate of 20 scans min$^{-1}$. A KBr pellet was used for the FT-IR analysis. TGA (SCINCO thermal gravimeter S-1000) was performed at a heating rate of 10°C min$^{-1}$ under nitrogen.

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