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Original Research Article

Synthesis, Characterization, and Application of Molecularly Imprinted Polymer - Modified Silica Gel for Andrographolide Purification from *Andrographis paniculata* (Burm.f.) Nees Methanol Extract

Wiwin Winingsih*, Adang firmansyah, Rayyan Ayesha, Purri A Novwanti, Lucia N.L.T. Bahy

Departement of Pharmacochemistry, Sekolah Tinggi Farmasi Indonesia Bandung, 40266, Indonesia

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ABSTRACT

Molecularly Imprinted Polymer- Modified silica is silica gel coated with Molecularly Imprinted Polymer (MIP) which has cavities with the same shape, size, and functional groups as the template molecule, allowing for selective absorption. Therefore, this study aimed to synthesize, characterize, and apply MIP-modified silica for purification of andrographolide from the *Andrographis paniculata* (Burm.f.) Nees methanol extract. To synthesize the MIP-modified silica, the surface imprinting method was employed, with APTES, TEOS, acetic acid, and andrographolide, as the functional monomer, cross-linking agent, catalyst, and template molecule, respectively. Modified silica was characterized using FTIR and SEM. Its performance was tested by determining the imprinting factor. The purity of purified methanol extract was tested using HPLC. FTIR and SEM characterization confirmed the presence of MIP polymer on the surface of silica gel. Its imprinting factor was 1.22. Purification results of the *Andrographis paniculata* (Burm.f.) Nees extract demonstrated that modified silica improved the purity of methanol extract from 1.46 % to 65%

Keywords: Modified Silica Gel, Andrographolide, Surface Imprinting, Silica gels

Introduction

Andrographolide, the primary compound discovered in the *Andrographis paniculata* (Burm.f.) Nees leaves, exhibits various biological activities, including anti-inflammatory, antidiabetic, antiplatelet, antibacterial, antiviral, and immunostimulant effects.[1-4] The pharmacological activity of andrographolide and the growing interest in natural treatments promote extensive study on its isolation from the *Andrographis paniculata* (Burm.f.) Nees plant. This compound relies on recrystallization and *chromatographic* methods for its purification.[5] However, multiple recrystallization processes are required to obtain a high-purity isolate, while chromatography employs environmentally hazardous organic solvents. Therefore, there is a need to develop purification method that is simpler and more environmentally friendly.

Silica gel is widely used as an adsorbent in column chromatography and solid-phase extraction. This is due to the excellent stability, superior thermal resistance, and high adsorption capacity of silica gel. However, the presence of OH groups on silica gel allows interactions with all polar and semi-polar compounds, making the purification of compounds more challenging. Therefore, for purification purposes, the interaction ability of silica gel is restricted to a single compound only, achieved by modifying the silica gel surface with Molecularly Imprinted Polymers (MIPs), simplifying the purification process.[6]

*Corresponding author. E mail: wiwinwidaningsih@stfi.ac.id
Tel: 062 -089662063583

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Modified silica refers to silica gel coated with MIP which is a polymer with specific and selective cavities designed to imprint the target or template molecule, in terms of its shape, size, and functional groups[5]. It comprises molecules or compounds to be imprinted (template), functional monomers, crosslinkers, initiators, and porogen solvents.[7]

The coating of silica with MIP aims to prevent the expansion of the polymer structure, which could alter the imprint of the target compound due to interactions with organic solvents. This modification enhances the specificity and selectivity of silica gel as an effective adsorbent by modifying silanol groups.[8] A previous study conducted by Yang *et al*.[9] on the Bisphenol A compound, synthesized MIP-modified silica, resulting in a recovery percentage of 99.43% which was significantly higher than the conventional isolation method of 3.6%.

The synthesized modified silica was characterized using Scanning Electron Microscope (SEM) and Fourier Transform Infrared (FTIR) techniques. The results showed that it can be utilized as an adsorbent for Solid Phase Extraction (SPE).

Methods

Tools

The study involved the use of several tools, including an analytical balance (Ohaus®), a set of reflux and distillation apparatus, an orbital shaker (IKA® KS 130 Basic), an ultrasonicator (Elmasonic® S 30H), a centrifuge (Hettich® EBA 20), a UV-Visible spectrophotometer (Shimadzu® UV 1800), an SEM (Hitachi SU 3500®), an FTIR (Thermo® Scientific), a micropipette (FISHER Elite®), and glassware commonly used in the Chemistry Laboratory.

Materials

The materials used in this study were silica gel 60 (70-230 mesh ASTM) for column chromatography (MERCK®), andrographolide (Tokyo Chemical Industry®), (3-aminopropyl) triethoxysilane (APTES) (Tokyo Chemical Industry®), tetraethoxysilane (TEOS) (MERCK®), methanol pro-HPLC (MERCK®), glacial acetic acid (Fulltime®), and demineralized water (Amidis®), all the reagents used in this research were analytical grade.

MIP-Modified Silica Gel Synthesis

Before application, silica gel was activated by refluxing it for 12 hours using a 3 M HCl solution with a volume of 200 mL. Subsequently, it was filtered and washed with demineralized water to neutralize silica. The final product was then dried in an oven at 110˚C for 12 hours and stored in a closed container, as described by Yang, *et.al.*[10]

3-aminopropyl triethoxysilane (APTES) as a functional monomer and andrographolide solution as a template molecules in methanol were mixed using a magnetic stirrer at room temperature for 30 minutes. Furthermore, 1 g of activated silica gel was added to the mixture, along with tetraethoxysilane (TEOS) as a crosslinker and glacial acetic acid. The mixing process was continued for 24 hours. Subsequently, modified silica was filtered and the template was removed using methanol: acetic acid solvent at 9:1 through sonication for 7 hours. The sonication was continued until the absorption wavelength of andrographolide in extracting solvent was no longer detected by UV-Vis spectrophotometry. Finally, the synthesized modified silica was dried in an oven at 100°C for 8 hours, following the method described by Yang *et.al.* [10] and Winingsih *et.al.*[11]

The composition of the components constituting the modified MIP-silica gel can be seen in the Table 1.

Characterization of MIP -Modified Silica Gel using SEM

The surface morphology of modified silica was observed using SEM. For the analysis, the sample was placed on a double-sided metal plate, and subsequently, it was gold-plated under vacuum conditions. The scanning process was conducted using a current of 60 mA and an electric power of 15 V.

Characterization of MIP - Modified Silica using FTIR

To analyse the functional groups in modified silica, FTIR spectroscopy was employed. Approximately 50 mg of the sample was placed in ZnSe ATR and IR radiation was performed at wave numbers that ranged from 400 to 4000 cm^{-1}.

Imprinting Factor Determination

Imprinting factor was determined using equations 1 and 2:

$$KD = \frac{(Ci - Cf)V}{Cf\ W} \quad (1)$$

$$IF = \frac{KDMS}{KDUMS} \quad (2)$$

KD is the distribution coefficient, Ci and Cf (mg/L) are andrographolide concentration before and after the adsorption experiment, V (L) is the volume of andrographolide solution, and W (g) is the polymer weight. IF stands for imprinting factor, KD Ms is the distribution coefficient of modified silica, and KD UMS is the distribution coefficient of modified silica without the addition of template compounds.[12-16]

Application of modified silica for andrographolide purification

A total of 5 grams of modified silica was mixed with 100 mL of the *Andrographis paniculata* (Burm.f.) Nees methanol extract solution using a shaker. The mixing process lasted for 1 hour at a speed of 240 rpm. Upon completion, the mixture was separated into filtrate and modified silica was separated. Furthermore, the solvent was evaporated, and the purity of the purified isolate was determined using HPLC.[11]

Results and Discussion

Modified Silica Gel synthesis

The synthesis process began by reacting andrographolide with APTES as the functional monomer in methanol as porogen solvent, with the expected occurrence of hydrogen bonding between the amine group of APTES and the hydroxyl group of andrographolide. Next, TEOS was added as the cross-linking agent, silica gel as the solid support, and acetic acid as the catalyst to the mixture of andrographolide and APTES, resulting in a reaction as shown in Figure 1. After the reaction took place, it was expected that Molecularly Imprinted Polymers (MIP) will be formed on the surface of the silica gel (MIP - modified silica gel before template removal). Upon template removal, specific MIP cavities for andrographolide will be created on the surface of the silica gel (MIP - modified silica gel).

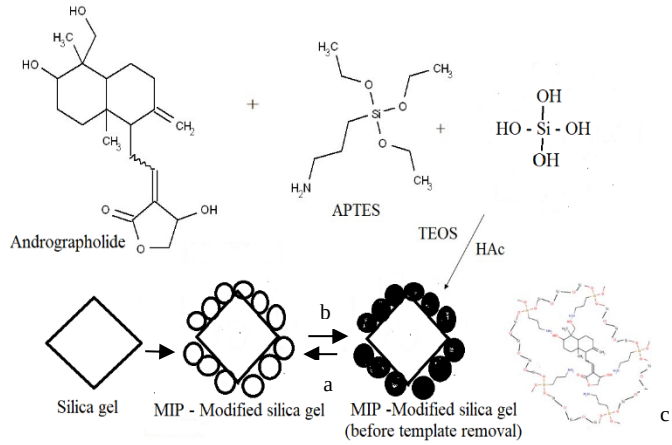

Figure 1: Ilustration of Modified silica gel synthesis. a: template removal process; b : rebinding of target molecules to the MIP cavity ; c : illustration of interaction between andrographolide and functional monomer in polymers matrix of MIP

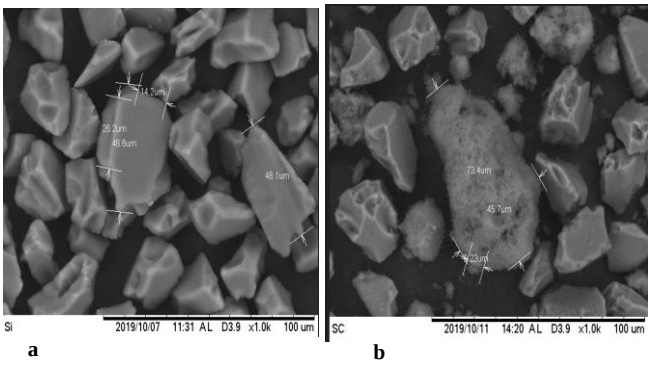

Figure 2: Characterization results using SEM, a: silica gel b: MIP - modified silica gel

Characterization of Modified Silica using FTIR and SEM

Characterization Results Using SEM

The SEM characterization results provided information about the surface morphology of modified silica gel. Figure 2 shows the difference in the surface morphology between silica gel and modified silica gel. Modified silica gel exhibited a rougher texture. Additionally, a change in particle size was observed in silica gel before and after modification. This occurred due to the formation of MIP on its surface.

Characterization Results using FTIR

The objective of FTIR characterization of modified silica was to observe the formation of functional groups, following the synthesis process. The surface modification of silica gel involved the hydrolysis and conjugation of TEOS and APTES, leading to the elimination of alkyl groups in both compounds. This was indicated by the absence of absorption at wavenumbers 2980 and 2670 cm^{-1} as well as 2980 and 2850 cm^{-1}. Furthermore, the presence of absorption within the wavenumber range of 1000-1100 cm^{-1} showed the successful formation of MIP on the silica gel surface[17], attributed to the presence of Si-O-Si groups.[17]

FTIR characterization results of modified silica did not provide a clear overview of the interactions between andrographolide and the polymer. However, they could indicate the success of synthesis process through the absence of absorption at wavenumbers 2980 and 2670 cm^{-1} as well as 2980 and 2850 cm^{-1}, as shown in Figure 3. The differences in the spectrum shape between modified silica and its constituent materials (silica gel, TEOS, and APTES) suggested the occurrence of a polymerization process.[17]

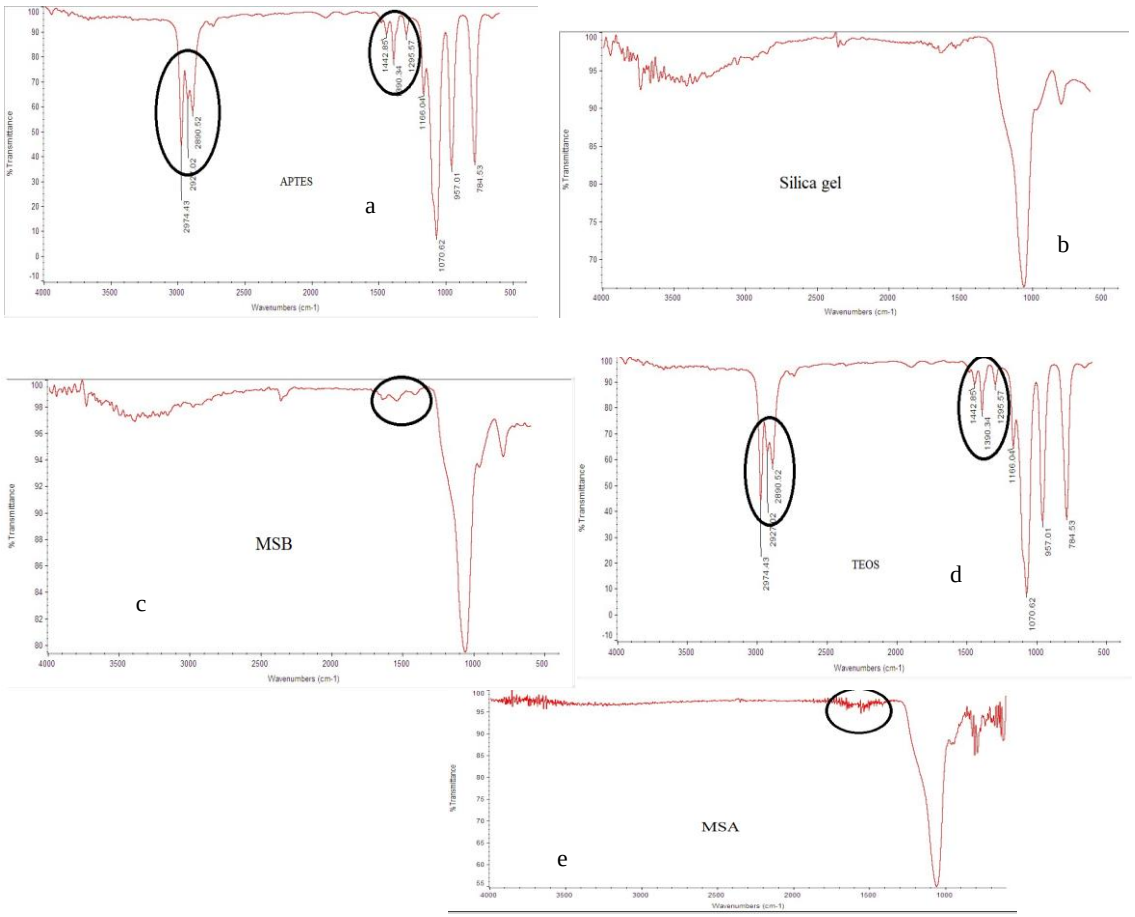

Figure 3: Characterization results using FTIR. a:APTES; b: silica gel; c: MSB d : TEOS ; e : MSA

Imprinting Factor determination

Imprinting factor is a parameter that shows the performance of MIP on modified silica gel, the greater the imprinting factor, the better the MIP of modified silica gel performance. MIP – modified silica gel showed good performance since it has an IF value more than 1.[11,12]

The composition of a molecularly imprinted polymer (MIP) coating on silica gel significantly influences the imprinting factor. Consequently, variations in the imprinting factor values are observed between MIP-silica gel fabricated using a functional monomer ratio of 1:4 (MSA) and those prepared with a template: functional monomer ratio of 1:5 (MSB).[18] The results of the imprinting factor test can be observed in Table 2.

Modified Silica Gel Application for Andrographolide Purification

The purification method using MIP (Molecularly Imprinted Polymer) for andrographolide through direct mixing has been previously conducted in the study by Winingsih et al[5,10] with a stirring time of 2 hours. However, when using MIP-modified silica gel, the equilibrium time is significantly reduced to 1 hour. This indicates that purification using MIP-modified silica gel provides better time efficiency.The purification process involved mixing the *Andrographis paniculata* (Burm.F) Nees methanol extract with a certain amount of MIP modified silica gel for 1 hour. The modified MIP-silica gel used for purification was the MIP-silica gel modification with the best imprinting factor (MSA). The purified extract showed an increase in purity from 1.46% to 65.97% (retention time: 3.520). This was higher compared to purification with unmodified silica gel from 1.46 % to 20.57%. (RT: 3.352). The chromatograms of methanol extract before and after purification are shown in Figure 4.

Conclusion

Based on characterization of modified silica gel using SEM, FTIR, and imprinting factor test, MIP was formed on the surface of silica gel, which is selective for andrographolide. MIP - modified silica gel with a molecular template: functional monomer ratio : of 1:4 (MSA) exhibits a better imprinting factor compared to a ratio of 1:5 (MSB). The increased selectivity, achieved through modification with MIP, was further evidenced by the higher purity obtained in the isolate purified using modified silica gel. Therefore, MIP-modified silica gel proved to be a valuable tool for purification process of andrographolide from the *Andrographis paniculata* (Burm.f) Nees methanol extract. Therefore, MIP-modified silica gel can be employed as an adsorbent for column chromatography and solid-phase extraction.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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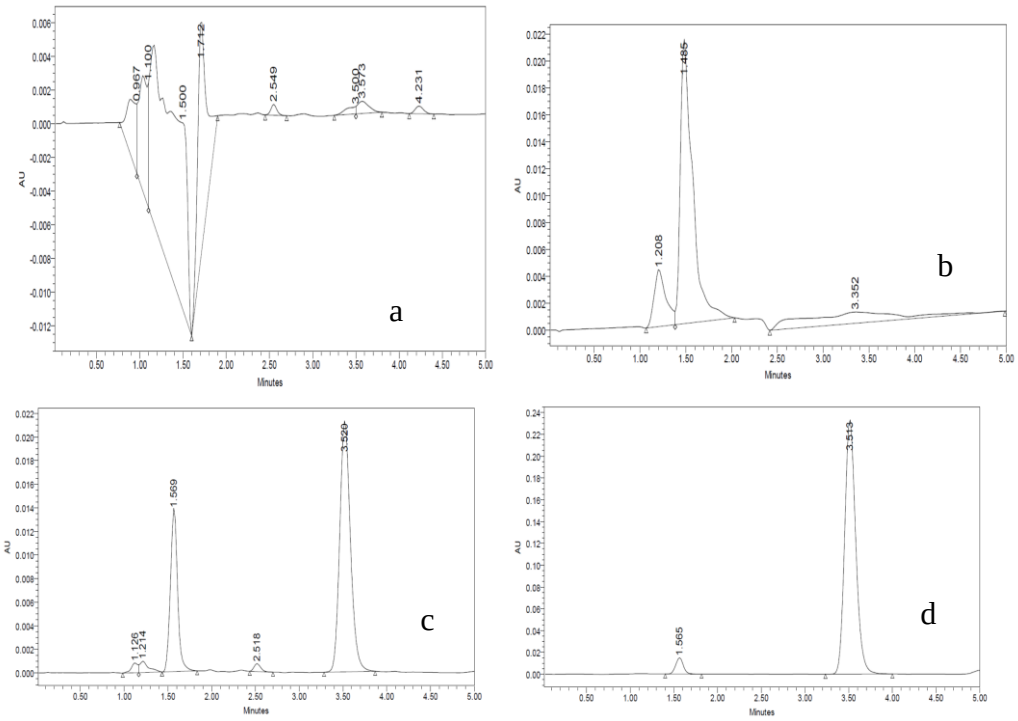

Figure 4: The chromatogram of the purity test results of the methanol extract of *Andrographis paniculata* (Burm.f.) Nees before purification (a), after purification using silica gel (b), and after purification using modified MIP-silica gel (c), were compared with the standard (d).

Table 1: Composition of modified MIP-silica gel

Adsorbent	Materials	Total
Modified Silica A (MSA)	andrographolide	280 mg
	APTES	0.94 mL
	TEOS	18 mL
	Acetic acid glacial	1 mL
	silica gel	1 g
	Methanol	30 mL
MSA without template addition (NSA)	andrographolide	0
	APTES	0.94 mL
	TEOS	18 mL
	Acetic acid glacial	1 mL
	silica gel	1 g
	Methanol	30 mL
Modified Silica B (MSB)	andrographolide	280 mg
	APTES	1.175 mL
	TEOS	4.43 mL
	Acetic acid glacial	1 mL
	silica gel	1 g
	Methanol	30 mL
MSB without template addition	andrographolide	0
	APTES	0.24 mL

(NSB)	TEOS	4.43 mL
	Acetic acid glacial	1 mL
	silica gel	1g
	Methanol	30 mL

Note : MSA and MSB had different comparison of template molecule and functional monomer. The comparison of template molecules: functional monomer of MSA 1:4. While the comparison in MSB was 1:5.

Table 2: The result of Imprinting Factor determination

Adsorbent	KD	IF
MSA	0.104075	1.22
NSA	0.085477	
MSB	0.084338	0.99
NSB	0.085477	

Note : KD is Distribution coefficient of the sample, IF is imprinting factor of the sample

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