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Original Article

BORON-SILICA BASED MESOPOROUS FOR CURCUMINOID ISOLATION FROM TURMERIC EXTRACT

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ABSTRACT

Objective: The purpose of this study was to develop the isolation method for curcuminoid from turmeric extract using boron-silica based mesoporous as an adsorbent.

Methods: The formation of mesoporous materials were conducted using the sol-gel technique. The characterization of mesoporous materials was analyzed using a scanning electron microscope (SEM), transmission electron microscope (TEM), and Fourier transforms infrared spectrometry (FTIR). The extraction of turmeric was done by solvent extraction using ethanol 96 %. The isolation of curcuminoid was achieved by the adsorption method using mesoporous materials, both for silica-based mesoporous (MCM) and boron-silica based mesoporous (BMCM). The elution of curcuminoid-loaded mesoporous was conducted using various solvents. The concentration of total curcuminoid and its compounds was measured by visible spectrometry and high-performance liquid chromatography (HPLC).

Results: Morphology of MCM and BMCM shows the homogenous regular spherical shape, but having a different size. MCM has a smaller diameter particle size (500-600 nm) compared to BMCM (700-900 nm). On the other hand, BMCM has a smaller pore size (1-5 nm) compared to MCM (5-20 nm). The FTIR spectra of BMCM shows the additional vibration at 1400-1600 cm for B-O-H bond. Visible spectrometry measurement shows that the highest concentration of curcuminoid eluted from BMCM is 65.411±0.056 ppm by using ethyl acetate as a solvent, while the concentration of curcuminoid eluted from MCM is 11.503±0.054 ppm by using the same solvent. The results of curcuminoid adsorption and elution, indicating that ethyl acetate is the best solvent to elute curcuminoid due to its 98.83 % purity using HPLC analysis.

Conclusion: It was concluded that boron-silica based mesoporous showed stronger curcuminoid adsorption than silica-based mesoporous therefore found to be a potential adsorbent for curcuminoid isolation from turmeric extract.

Keywords: Boron-silica based mesoporous, Curcuminoid, Isolation, Turmeric extract

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INTRODUCTION

Turmeric (*Curcuma longa* L.) is a herbal plant of the *Zingiberaceae* family. It has been widely used for the treatment of a variety of diseases due to its pharmacological properties, including anti-inflammatory, antimicrobial, antioxidant, and anticancer [1-3]. Its pharmacological activities have been attributed mainly to the three different curcuminoids (fig. 1), namely curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) [4]. Effectivity of curcuminoid extraction and fractionation is usually limited to curcuminoid crude. Several methods have been developed to obtain purer curcuminoid or even pure compound of three different curcuminoids, including low-pressure solvent extraction and supercritical fluid extraction (SFE) [5]. Other studies have been reported for the separation and isolation of curcuminoid, including thin-layer chromatography (TLC), column chromatography (CC), high-performance liquid chromatography (HPLC), pressurized liquid extraction, and microwave-assisted extraction [6-11]. Another method that can be applied for curcuminoid isolation is using mesoporous material.

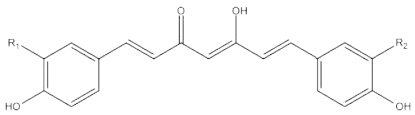

Fig. 1: Chemical structure of curcuminoid curcumin (R_1 = OCH_3, R_2 = OCH_3), demethoxycurcumin (R_1 = OCH_3, R_2 = H), and bisdemethoxycurcumin (R_1 = H, R_2 = H)

Mesoporous material is a material with a large number of nanometer-sized pores. Mesoporous material has a high surface area, so it can adsorb and interact with atoms, ions, or molecules on the outer surface [12]. One type of mesoporous material is silica-based mesoporous. Since silica-based mesoporous can be used to adsorb natural compounds, it is possible to use silica-based mesoporous as a functional material for adsorbing curcuminoid. Silica-based mesoporous has been used for carbon dioxide adsorption [13]. Other studies have been reported to use silica-based mesoporous as a suitable carrier for drugs, including curcumin [14-17].

Silica-based mesoporous can be used as a carrier system due to its unique properties, including high surface area, facile modification of surface area, stability, and nontoxic structure [18]. Moreover, silica-based mesoporous also has low toxicity, so it can be used as a drug carrier [19]. Our earlier study of silica-based mesoporous and boron-silica based mesoporous adsorption for curcuminoid shows promising results and the possibility of using these materials as an adsorbent for curcuminoid isolation from turmeric [20]. This study aims to develop boron-silica based mesoporous as an adsorbent for curcuminoid isolation from turmeric extract.

MATERIALS AND METHODS

Chemicals and reagents

Turmeric, curcuminoid standard (Business Department of Sekolah Tinggi Farmasi Indonesia, Indonesia), cetyltrimethylammonium bromide/CTAB (Amresco, Ohio), distilled water, ethanol (Merck, Germany), ammonia (Merck, Germany), boric acid (Merck, Germany), tetraethylorthosilicate/TEOS (Merck, Germany), acetonitrile (Fulltime, China), and phosphoric acid (Merck, Germany).

Instrument

The characterization of mesoporous materials was done using JEOL JSM Scanning Electron Microscope (SEM), HITACHI HT7700 Transmission Electron Microscope (TEM), and Thermo Fisher® Nicolet iS5 FTIR

spectrometer with the holder of ZnSe iD3 ATR (Attenuated Total Reflectance). Total curcuminoid content in the turmeric extract was analyzed using Shimadzu UV-1800 Spectrophotometer. The chromatographic technique for curcuminoid separation was performed on Waters 1525 Binary HPLC Pump with Waters 486 Tunable Absorbance Detector, reversed-phase Agilent ZORBAX Eclipse plus C18 (4.6 mm x 150 mm) and Empower 2 software.

Formation of boron-silica based mesoporous

The formation method of mesoporous materials in this work was adopted from Günaydin and Yilmaz and modified from Trong on *et al.* [21, 22]. Boron-silica based mesoporous (BMCM) was prepared using the sol-gel technique. Cetyltrimethylammonium bromide (CTAB) as a template, dissolved in 200 ml mixture of H_2O and 96 % ethanol in the ratio of 1:1 under vigorous stirring at 70 °C. pH was adjusted to 10-11 using 25 % ammonia, then 300 mg boric acid was added to the mixture. Tetraethylorthosilicate (TEOS) 20 ml was added dropwise, and the mixture was stirred vigorously for four hours. The mixture was heated at 100 °C for 24 h. The solid product was washed with distilled water until a neutral pH. The removal of the organic template was performed by the calcination process at 600 °C for eight hours. Silica-based mesoporous (MCM) was prepared using the same procedure but without the addition of boric acid.

Characterization of mesoporous

The mesoporous materials formed were characterized by scanning electron microscope (SEM), transmission electron microscope (TEM), and Fourier transforms infrared (FTIR). Particle morphology was analyzed by SEM using JEOL JSM at a voltage of 15 kV. The sample powder was coated with gold to form a conductive layer on a carbon stub to evaluate the surface properties. TEM image was recorded with HITACHI HT7700 at an accelerating voltage of 120 kV to assess the size and shape of the pores of mesoporous. FTIR spectra were taken in Nicolet iS5 Thermo Fisher Spectrometer using the resolution of 4 cm in 4000–400 cm range.

Extraction of turmeric

Turmeric extract was prepared by soaking turmeric in ethanol 96 % in the ratio of 1:5. The mixture then stands for 24 h. These steps were repeated three times. All mixtures were collected and evaporated to obtain a viscous extract.

Adsorption and elution of curcuminoid

Adsorption was conducted by mixing 200 mg mesoporous materials with 50 ml turmeric extract. The mixture then stands for 18-24 h. The mixture was filtered to obtain the residue, followed by washing with n-hexane and 25 % ethanol. 25 mg of dried residue was eluted with 5 ml of various solvents. Eluates were evaporated and diluted with methanol. The solutions were measured by Visible Spectrometer at 425 nm for the quantitative analysis of curcuminoid. The solutions were also separated by high-performance liquid chromatography. The chromatographic separation was conducted on the Agilent ZORBAX Eclipse Plus C18 column (4.6 mm x 150 mm) using a mobile phase mixture of phosphoric acid and acetonitrile (50:50), which was filtered and degassed before use. The flow rate was 1.0 ml/min and ultraviolet/ visible detection at 425 nm.

RESULTS AND DISCUSSION

Formation and characterization of mesoporous

From the results of mesoporous formation, the percent yield for silica-based mesoporous (MCM) formation was 12.76 % in ratio to TEOS as a source of silica, while the percent yield for BMCM was 20.48 %. Both mesoporous materials have a white color, while the MCM was relatively more voluminous than the BMCM. It is necessary to ensure that there is no residual boron in the synthesis product because it can affect the determination of curcuminoid concentration in the adsorption and elution process. Therefore, the washing process using water not only be checked by the pH value but qualitative analysis of boron using visible spectrometry to ensure the wash filtrate was free of boron.

The mesoporous formed was characterized by SEM, TEM, and FTIR. SEM was used to observe the morphology of mesoporous, including shape, size, and size distribution. Fig. 2 shows SEM and TEM images of mesoporous materials. Fig. 2B shows a homogenous regular spherical shape of BMCM particle compared to MCM (fig. 2A). Moreover, MCM particle still shows agglomeration. BMCM has a larger diameter particle size (700-900 nm) compared to MCM (500-600 nm). Compared to another study on silica mesoporous synthesis, the result indeed showed that grafted MCM tends to have larger particles than MCM [23]. Fig. 2C and 2D show TEM image of silica-based mesoporous and boron-silica based mesoporous materials. TEM was used to observe the pore shape and size of mesoporous. The images indicating the irregular shape of the pore structure of BMCM compared to MCM. MCM has a well-ordered hexagonal-shaped pore structure. However, the hexagonal shape pore structure still can be observed in BMCM. BMCM has a smaller pore size (1-5 nm) compared to MCM (5-20 nm). This result is relevant to other studies that grafted MCM has smaller pores and tends to have irregular pore shape [22, 24].

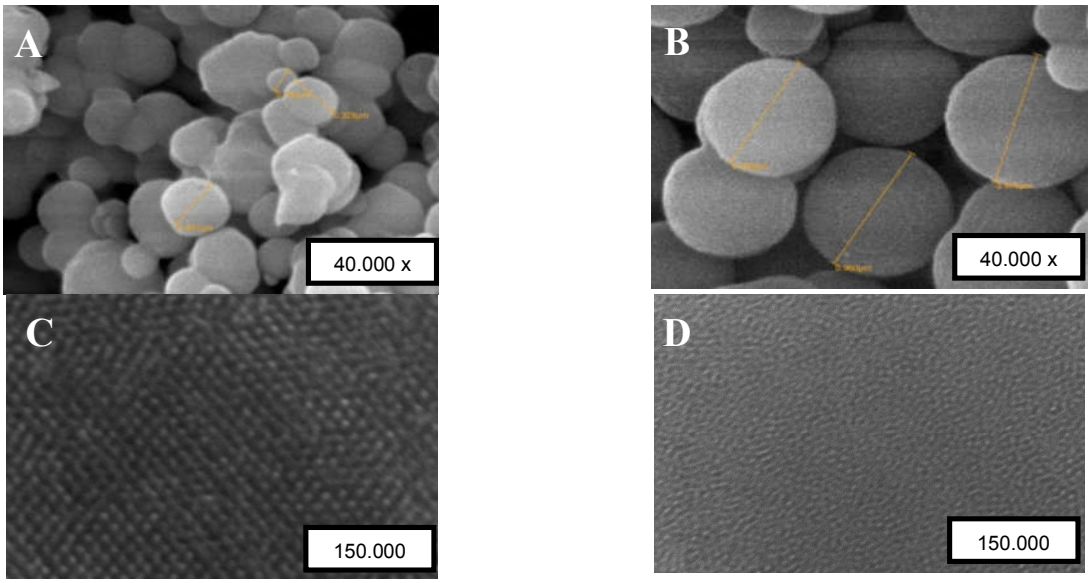

Fig. 2: SEM image of (A) silica-based mesoporous MCM and (B) boron-silica based mesoporous BMCM and TEM image of (C) silica-based mesoporous MCM and (D) boron-silica based mesoporous BMCM

Fig. 3A shows the FTIR spectra of MCM, while fig. 3B shows the FTIR spectra of BMCM. The spectra of MCM in fig. 3A shows stretch vibration bands of Si-O-Si at 1100-1200 cm, bend vibration bands of Si-OH at 950 cm, and several silanol bands at 3400-3600 cm. The spectra of BMCM in fig. 3B has similarities with fig. 3A, with the addition of vibration bands of B-O-H bond at 1400-1600 cm. The results of the mesoporous materials characterization by FTIR analysis in this study are in line with previous studies, where the mesoporous silica material shows Si-O-Si vibration band at 1000-1200 cm and silanol group Si-O-H band at 950 cm [25].

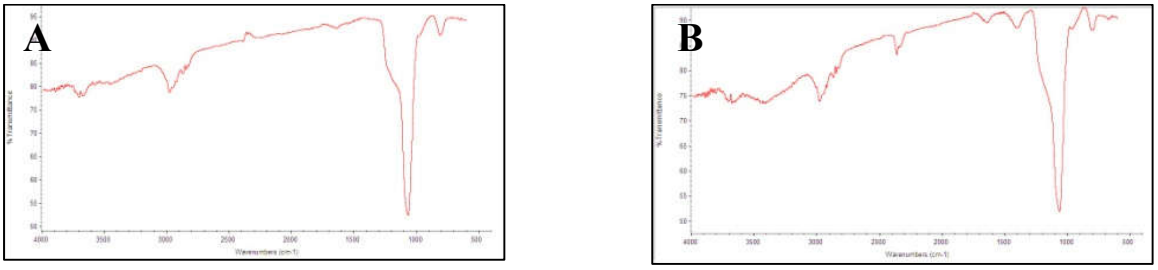

Fig. 3: FTIR spectra of (A) silica-based mesoporous MCM and (B) boron-silica based mesoporous BMCM

Adsorption and elution of curcuminoid

Adsorption and elution process was conducted to observe the adsorption strength of mesoporous materials. This process was divided into two purposes; the first purpose was to determine total curcuminoid concentration, while the second purpose was to determine the concentration of each curcuminoid content. Total curcuminoid concentration was determined by a visible spectrometer at 425 nm. The results of this stage were presented in table 1.

Table 1: Total Curcuminoid concentration eluted from mesoporous materials with various solvents

Solvents	Average of total curcuminoid Concentration (ppm)	
	MCM	BMCM
Cold ethanol	11.703±0.075	36.152±0.047
Ethanol 50 %	3.287±0.012	14.910±0.056
Methanol	12.906±0.064	43.768±0.054
Acetone	20.321±0.078	46.774±0.038
Ethyl acetate	11.503±0.054	65.411±0.056
Chloroform	20.922±0.070	49.178±0.087

Note: n = 3

From the results in table 1, it was concluded that the adsorption strength of BMCM is stronger than MCM due to the high concentration of total curcuminoid eluted from BMCM. The main purpose of the addition or dopping of boron to the silica-based mesoporous is based on the properties of curcuminoid (curcumin, demethoxycurcumin, bisdemethoxycurcumin) as a specific target compound. Curcuminoid can bind to boron by coordinate covalent bonds so the mesoporous materials are expected to have a higher affinity for curcuminoid. In the curcuminoid adsorption process on boron-silica based mesoporous, two boron bonds are used to bind oxygen atoms on the silanol group, while the other two boron bonds are bound to curcuminoid. Thus, each mole of boron binds to one mole of curcuminoid. Silica structure, hypothesis structure of the bond of boron and silica, and hypothesis structure of the bond of boron-silica and curcuminoid are shown in Fig. 4.

A

B

C

Fig. 4: Molecular structure of (A) silica, (B) hypothesis structure of the bond of boron-silica, and (C) hypothesis structure of the bond of boron-silica and curcuminoid

In this case, it means that the curcuminoid adsorbed to the MCM mesoporous only through the physisorption process. On the other hand, curcuminoid adsorbed to the BMCM mesoporous not only through physisorption but also partly chemisorption by coordinate covalent bonds, which are supposed to be much stronger than physisorption. This is proven by the results of curcuminoid concentration in BMCM, which are relatively higher than in MCM. The results were also indicating that ethyl acetate is the best solvent to elute total curcuminoid from BMCM.

The concentration of three different curcuminoid, namely curcumin, demethoxycurcumin, and bisdemethoxycurcumin, was determined by HPLC with ultraviolet/visible detection at 425 nm. The results of this stage were presented in table 2 for MCM and table 3 for BMCM.

Table 2: Three different curcuminoid purity eluted from silica-based mesoporous MCM with various solvents

Curcuminoid	Average of compound purity (%)					
	Cold ethanol	Ethanol 50 %	Methanol	Acetone	Ethyl acetate	Chloroform
Curcumin	57.10±2.45	46.68±1.97	55.17±2.38	53.58±2.11	53.60±2.06	66.89±2.24
DMC	21.70±0.76	24.45±1.01	21.74±0.78	20.62±0.70	22.78±0.83	20.41±0.78
BDMC	17.57±0.56	23.73±0.98	18.42±0.72	16.94±0.66	20.44±0.76	9.26±0.09
Total of curcuminoid	96.37	94.86	95.33	91.14	96.82	96.56

Note: n = 3

Table 3: Three different curcuminoid purity eluted from boron-silica based mesoporous BMCM with various solvents

Curcuminoid	Average of compound purity (%)					
	Cold ethanol	Ethanol 50 %	Methanol	Acetone	Ethyl Acetate	Chloroform
Curcumin	57.64±2.32	39.02±1.36	56.66±2.01	55.84±1.93	55.58±1.27	73.91±1.22
DMC	21.16±0.85	20.14±0.71	20.59±0.73	21.45±0.34	22.51±0.52	15.04±0.21
BDMC	17.69±0.63	22.61±0.80	18.64±0.71	20.92±0.40	20.74±0.44	3.82±0.06
Total of curcuminoid	96.49	81.77	95.89	98.21	98.83	92.77

Note: n = 3

The results in table 2 and table 3 indicating that ethyl acetate is the best solvent to elute curcuminoid compounds, both from MCM and BMCM. These results are in line with the results from visible spectrometry measurement. The uses of various solvents to elute curcuminoid from the mesoporous materials aim to determine strategies for obtaining specific compounds of curcuminoid. As we can see from the results, if the purpose is to obtain a high purity of total curcuminoid, we can use BMCM and the elution using ethyl acetate to obtain total curcuminoid purity up to 98.83 %. If the purpose is to obtain curcuminoid with a high purity of curcumin, then we can use BMCM, and the elution using chloroform to obtain the curcumin concentration reaches 73.91 %, while BMCM also can be used to obtain curcuminoid with a high purity of BDMC by the elution using ethanol 50 %. Based on the results, the purity of curcuminoid compounds eluted from BMCM is higher than from MCM by using cold ethanol, methanol, acetone, or ethyl acetate as a solvent. The curcuminoid isolation method used in this study giving curcuminoid with greater purity compared to the method in previous studies, such as microwave-assisted extraction method giving 75% purity and hydrotropic extraction method giving maximum purity of 95% [26, 27].

CONCLUSION

In this research work, boron-silica based mesoporous (BMCM) was successfully prepared and characterized by SEM, TEM, and FTIR analysis. Morphological analysis by SEM showed a large particle size of BMCM. Pores' shape and size analysis by TEM showed small irregular pores of BMCM. FTIR spectra of BMCM showed additional vibration bands of B-O-H at 1400-1600 cm. Adsorption and elution test using BMCM revealed strong adsorption of curcuminoid, while ethyl acetate is the best solvent to elute curcuminoid from BMCM. These results showed the potential use of BMCM as an adsorbent for curcuminoid isolation from turmeric extract. This method also could be considered as a new method for curcuminoid isolation.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declared no conflict of interests.

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