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Synthesis, Characterization and Application of ZS/HMS Catalyst in the Esterification of Gossypol

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ABSTRACT

A solid acid catalyst of zirconium sulfate (ZS) on a pure hexagonal mesoporous silica (HMS) sieve was prepared and characterized by small angle X-ray diffraction, NH_3-temperature programmed desorption, and thermogravimetric analysis. The obtained ZS/HMS catalyst displayed a typical mesoporous structure, ZS was well dispersed on the HMS support, and the acidity increased with the amount of ZS loading. Gossypol was extracted from cottonseed cake with acetone as solvent, and then the gossypol solution was esterified with ZS/HMS as catalyst to yield products of acetic acid gossypol. Under the optimal conditions, the conversion efficiency of gossypol was as high as 96.7%.

Keywords: Gossypol; Zirconium Sulphate; Hexagonal Mesoporous Silica; Esterification

1. Introduction

Gossypol is a polyphenolic compound derived from the yellow pigment glands of cotton plants that plays an important role in pest resistance [1,2]. Acetylated gossypol is the esterification product of gossypol and acetic acid. Acetylated gossypol has been studied in a wide range of biological and medicinal fields due to its purported antitumor and antifertility activities [3-5]. Gossypol is usually extracted from cottonseed kernels using aniline as intermediates [5,6]. The toxic residue in the cottonseed cake is harmful to both humans and animals. Therefore, development of a non-toxic extraction method for acetylated gossypol from cottonseed cake is of great importance.

Solid acid catalysts are widely used in esterification reactions because of their low acute toxicity. Furthermore, there is no evidence of carcinogenicity to humans. Several recent studies reported that zirconium sulfate (ZS) showed high activity and selectivity as a solid acid catalyst for the esterification of fatty acids [7]; however, ZS is easily soluble in water and difficult to remove from the reaction mixture. To circumvent this problem, many recent studies have investigated the synthesis of immobilized ZS supported on various supports such as activated carbon [8], *γ*-alumina [9], silica [10], carbon nanotubes [11], or MCM-41 [12] as a solid catalysts.

Acetylated gossypol is the esterification product of gossypol and solid acid catalyst may be able to enhance the conversion ratio of this reaction. In this paper, we describe the preparation of immobilized ZS on hexagonal mesoporous silica (HMS) sieve. The ZS/HMS was characterized and applied as a solid acid catalyst for the esterification of gossypol. The loading of ZS on HMS, the amount of catalyst, the reaction time, and reaction temperature were investigated to optimize the reaction conditions for gossypol esterification.

2. Experimental

2.1. Catalyst Preparation

Pure siliceous HMS material was synthesized according to a procedure described previously [13]. Briefly, dodecylamine (DDA, 4.9 mmol) was dissolved in 5 mL of ethanol, and 45 mL of distilled H_2O was then added to afford a 90:10 (V/V) H_2O/EtOH solution of the surfacetant. At room temperature, tetraethyl orthosilicate (TEOS 19.6 mmol) was added to the solution to yield a reaction mixture. Into this reaction mixture, we added 7 mL ethanol during the mixing process to compensate for the volatilization. The reaction flask was sealed with cling film and shaken at 220 rpm in a heated water bath at room temperature for 20 h. The reaction product was then filtered, washed, and dried at room temperature for 24 h. The obtained samples were calcined in a muffle furnace at about 600˚C for 4 h.

The ZS/HMS materials were synthesized following procedures similar to those of Joon and his co-works [14]. The ZS-impregnated HMS catalyst was prepared by first dispersing the HMS in a water solution and then adding

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ZS. The mixture was stirred overnight and then dried at 120˚C for 10 h. The samples obtained after drying were denoted X% ZS/HMS (X% refer to loading of the ZS). Before use in the reaction, the ZS/HMS catalyst was stored in desiccators.

2.2. Characterization

The powder X-ray diffraction (XRD) data was collected on a Brunker D8 Advance X-ray diffractometer using Cu-Kα irradiation ($\lambda = 1.5406$ Å) as source at 40 kV and 40 mA. The samples were recorded from 1 to 20˚ (2θ) with a step scan of 0.01˚/s.

Brunauer-Emmett-Teller (BET) surface area analysis was performed from the nitrogen adsorption isotherms at 77 K using a Micromeritics Model ASAP 2020 instrument. All samples were degassed at 110˚C under vacuum for 6 h. Average pore diameter (d) and pore volume were calculated based on the Barret-Joyner-Halenda (BJH) method.

The NH_3-temperature programmed desorption (NH_3-TPD) data were collected using a ChemBet 3000 analyzer. About 200 mg samples were activated under flowing heat (500˚C) for 2 h, and cooled to 120˚C under continuous evacuation. The sample was equilibrated with gaseous NH_3 at 0.04 kPa and the temperature increased by 10˚C/min under flowing heat 20 cm^3/min.

Thermogravimetric analysis (TGA) was performed on a Netzsch STA-449F3 (Jupiter OR, Germany) analyzer under an oxygen atmosphere. Heating rates were typically 10˚C/min under an oxygen atmosphere. The gas flow through the system was 20 mL/min.

2.3. Extraction of Gossypol

A sample of cottonseed cake raw material was weighed. After grinding, cottonseed cake was put into a grind port flask. An appropriate amount of acetone was added to the flask. The cottonseed cake extracting solution was removed from the residue by vacuum filtration. The remaining residue was washed three times with 50 mL of distilled water to remove residual solvents. The liquid product was filtered through a 0.45 μm organic membrane and 20 μl of the filtrate was analyzed by high performance liquid chromatography. The chromatographic conditions, an C_{18} reversed-phase column was used. The particle size was 5 μm, and the column dimensions were 4.6 mm × 150 mm, the mobile phase were methanol and 2% phosphoric acid solution (90:10), the column temperature was 40˚C, the flow rate was 1 mL/min and the samples were detected by Waters a 2487 UV detector at 235 nm. The extraction yield of gossypol from cottonseed cake was calculated by the following formula:

$$R(\%) = (W_1/W_2) \times 100\% \qquad (1)$$

The formula: R—extraction yield of gossypol;
W_1—extracting amount of gossypol;
W_2—the total content of gossypol in cottonseed cake.

2.4. Esterification of Gossypol with Acetic Acid

Esterification was performed at atmospheric pressure in a grinding glass bottle. A typical esterification reaction consists of acetic acid (1 mL), 0.6 mg/mL of gossypol (5 mL), distilled water (3.2 mL), and fresh ZS/HMS solid acid catalyst (0.1 g). After acetic acid and gossypol were mixed and stirred for 15 minutes, distilled water was added drop by drop. The reaction mixture was kept on stir for esterification. At the beginning of the reaction, the catalyst was handed by ultrasonic dispersion. Speed of agitation had only a small effect on the reaction rate. All subsequent experiments were conducted at a stirrer speed of 200 rpm to ensure that there was no external mass transfer resistance. After standing for 10 minutes, the produced acetic acid gossypol was precipitated due to its low solubility in water, and then the upper supernatant was taken using HPLC to detect the remaining amount of gossypol and computed the conversion of gossypol. The yellow precipitation was dissolved in acetone with a certain amount. After stirring for 10 minutes, the sample was filtered in order to obtain a pure gossypol acetic acid.

3. Results and Discussion

Figure 1 shows the typical XRD pattern for the ZS/HMS reaction product with different ZS loading. The patterns all contain an intense diffraction peak $2\theta = 2.3˚$ corresponding to the diffraction of (100) plane, indicating that these samples had the typical characteristic peaks of a mesoporous molecular sieve in agreement with previous reports [15].

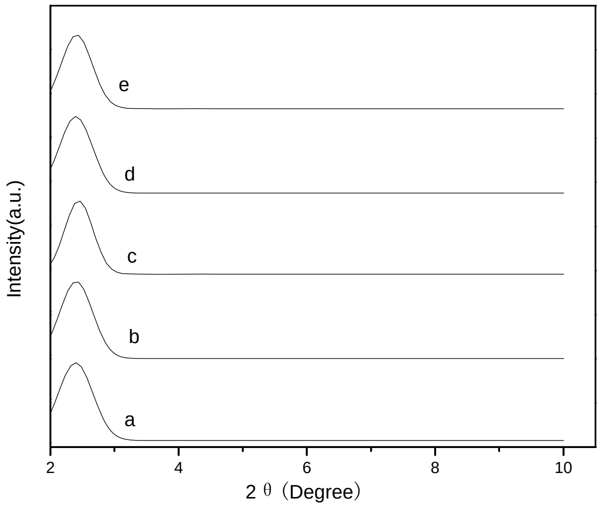

Figure 1. XRD patterns of ZS/HMS material for different ZS/HMS ratios: a: HMS; b: 30% ZS/HMS; c: 40% ZS/HMS; d: 50% ZS/HMS; and e: 60% ZS/HMS.

The textural properties of samples were examined by surface area and pore size distribution (**Table 1**). The average surface area of the HMS was 637 m^2/g. Surface area and pore volume decreased with increasing ZS loads, while the average pore size of ZS/HMS expanded from 3.2 to 4.1 nm with increasing ZS. At higher ZS loads, ZS particles will enter into the surface the microporous structure and block superficial pores, reducing surface area and surface pore volume. The pore volume reduced from 0.60 cm^3/g to 0.20 cm^3/g. Pore size distributions are shown in **Figure 2** and measured by N2 adsorption technique. The pore sizes of catalyst decrease with increment of ZS loading. As these surface microporous are blocked, the remaining pores of the support must be larger, so the average pore size underestimates the average pore diameters within the ZS/HMS solid acid catalyst.

The nitrogen adsorption isotherms for HMS and ZS/HMS samples with different ZS loading are shown in **Figure 3**. All samples exhibited the shape of II isotherms according to the IUPAC classification for nitrogen adsorption-desorption isotherms. The adsorption and desorption branches were not parallel and hysteresis loops emerge between 0.9 and 1. The hysteresis loops of ZS/HMS were similar to those of parent of HMS, indicating that ZS had good loading dispersion, consistent with the XRD and results.

Table 1. The structure of various ZS/HMS samples.

Sample	S_{BET} (m^2/g)	d (nm)	Pore volume (cm^3/g)
HMS	637	3.2	0.60
20% ZS/HMS	571	3.4	0.48
30% ZS/HMS	315	3.7	0.29
40% ZS/HMS	253	4.0	0.26
50% ZS/HMS	213	4.1	0.22
60% ZS/HMS	193	4.1	0.20

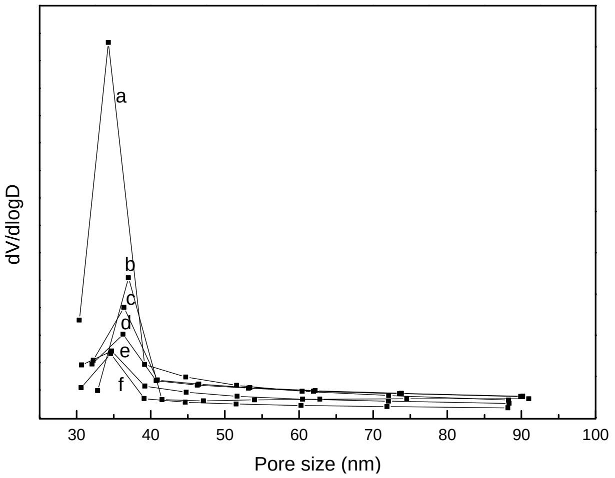

Figure 2. Pore size distributions for a: HMS; b: 20% ZS/HMS; c: 30% ZS/HMS; d: 40% ZS/HMS; e: 50% ZS/HMS; and f: 60% HMS.

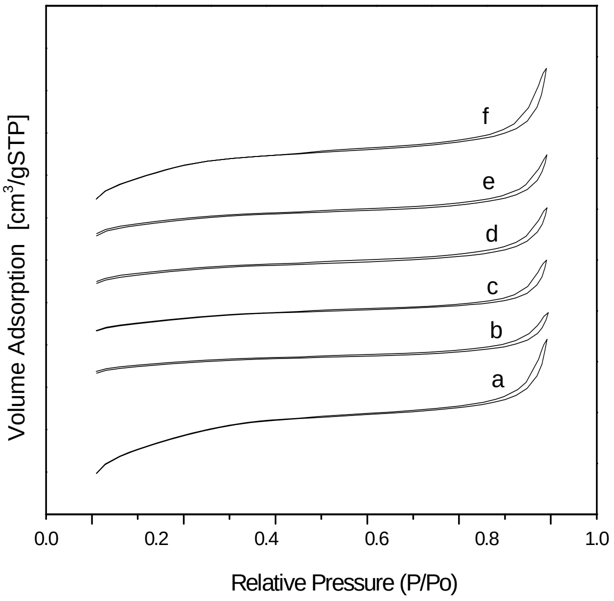

Figure 3. Nitrogen adsorption isotherms for a: HMS; b: 20% ZS/HMS; c: 30% ZS/HMS; d: 40% ZS/HMS; e: 50% ZS/HMS, and f: 60% HMS.

There are many methods for acidity measurements [16, 17]. Ammonia TPD is widely used to determine the acidity of solid acid. The NH_3-TPD profiles of HMS and ZS/-HMS are shown in **Figure 4**. Gannapati *et al.* [18] reported that desorption temperature could be divided into three categories: 1) intermediate or medium (100˚C - 200˚C); 2) strong (200˚C - 400˚C); 3) very strong acid (>400˚C). The HMS exhibited one peak at 125˚C, reflecting the physical adsorption of NH_3 on the HMS surface. When zirconium was loaded onto the HMS, peaks of different intensities (heights) at 242˚C will appear that indicate strong acid strength. As the zirconium content increased, the peak intensity also increased. The peak height was equivalent at 50% zirconium and 60% zirconium loads, indicating that these two load levels deposited equal amounts of ZS on the HMS.

Thermogravimetric analysis was conducted under atmospheric conditions to test the thermal stability of bulk ZS and HMS-supported ZS. The thermograms of HMS and ZS/HMS materials with various ZS loadings are shown in **Figure 5**. The first change in TGA curve between room temperature and 100˚C is ascribed to removal of physiccally adsorbed and structural water. At higher temperatures, TGA curves yield the decomposition pattern of ZS/HMS. The profiles indicated that the unmodified HMS showed little loss; however, TGA curves of ZS/HMS revealed degradation between 650˚C and 750˚C, reflecting breakdown of ZS groups on the silica surface, in agreement with earlier reports [19].

The orthogonal test results for optimal extraction of gossypol from cottonseed cake are listed in **Tables 2** and

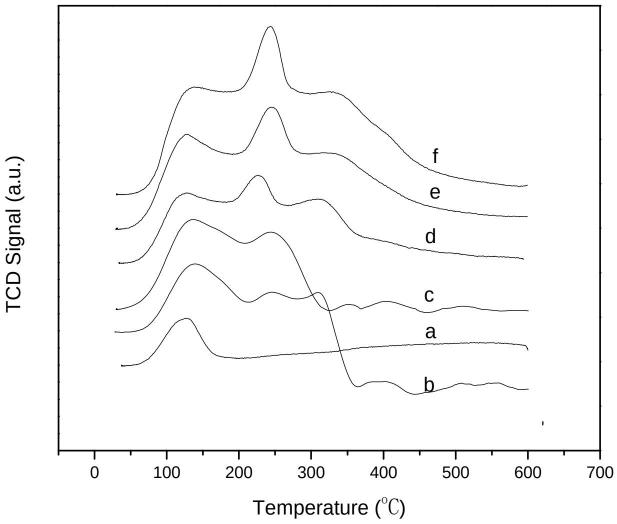

Figure 4. NH_3-TPD curves for a: HMS; b: 20% ZS/HMS; c: 30% ZS/HMS; d: 40% ZS/HMS; e: 50% ZS/HMS; and f: 60% HMS.

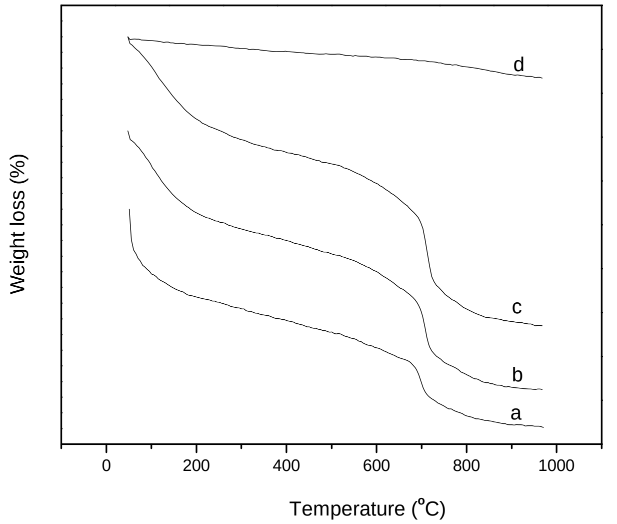

Figure 5. TGA curves for a: 40 wt% ZS/HMS; b: 50 wt% ZS/HMS; c: 60 wt% ZS/HMS; and d: HMS.

Table 2. Variables and levels for orthogonal test.

Level	A (time, h)	B (Phosphoric acid, mol/L)	C (Acetone, %)	D (solvent, mL)
1	8	1.0	60	25
2	16	1.4	70	30
3	24	1.8	80	35

3. **Table 2** is variables and levels for orthogonal test; **Table 3** is three factors three levels ($L_9(3^4)$) orthogonal experiment and through 9 sets of experiments to determine the optimal conditions for gossypol extraction from cottonseed cake using acetone. The influence of each variable on the extraction yield of gossypol was determined for extraction time (A), phosphoric acid concentration (B), acetone concentration (C), and the amount of solvent (D). From **Table 3**, it can be seen that the rank order of influence was A > C > D > B. Extracting time had the strongest influence on gossypol extraction, while the phosphoric acid concentration was the least influential variable. The results of range analysis revealed that the optimal combination of these four factors was $A_3B_1C_3D_1$. The optimum conditions for gossypol extraction from cottonseed cake using acetone were an extraction time of 24 h, phosphate concentration of 1.0 mol/L, 80% acetone, and solvent dosage of 25 mL. At these optimal values, the average removal rate of gossypol reached 63.75%.

The effects of the ZS/HMS catalyst on gossypol esterification are presented in **Figure 6**. It is obvious that the conversion of gossypol to acetylated gossypol increased with increasing ZS loading on the catalyst and was as high as 97% at 50 wt% ZS loading on HMS. No further increase was observed at high ZS loading on HMS. Again, this high efficiency indicated that the loaded ZS was well dispersed on the HMS surface, thus maximizing catalytic surface area. At ZS loading below 50%, the weaker catalytic activity was due to lower dispersion of ZS on HMS.

The amount of ZS/HMS catalyst used in the esterification process also affected the acetylated gossypol conversion efficiency. The effect of the catalyst dose on the conversion efficiency is shown in **Figure 7**. The catalyst amount was varied from 10 - 70 mg in 10 mg increments. The conversion efficiency of acetylated gossypol increa-

Table 3. The $L_9(3^4)$ orthogonal experiment for determining the optimal conditions for gossypol extraction from cottonseed cake using acetone.

Experiment no.	A	B	C	D	Extraction of gossypol (mg/g)
1	8	1.0	60%	25	0.123
2	8	1.4	70%	30	0.184
3	8	1.8	80%	35	0.388
4	16	1.0	70%	35	0.159
5	16	1.4	80%	25	0.646
6	16	1.8	60%	30	0.146
7	24	1.0	80%	30	1.173
8	24	1.4	60%	35	0.327
9	24	1.8	70%	25	0.831
K1	0.232	0.486	0.199	0.533	
K2	0.317	0.386	0.391	0.501	
K3	0.777	0.455	0.736	0.291	
R	0.545	0.099	0.537	0.242	

K1: Average extraction of gossypol for the four factors in level 1; K2: Average extraction of gossypol for the four factors in level 2; K3: Average extraction of gossypol for the four factors in level 3; R: mean range.

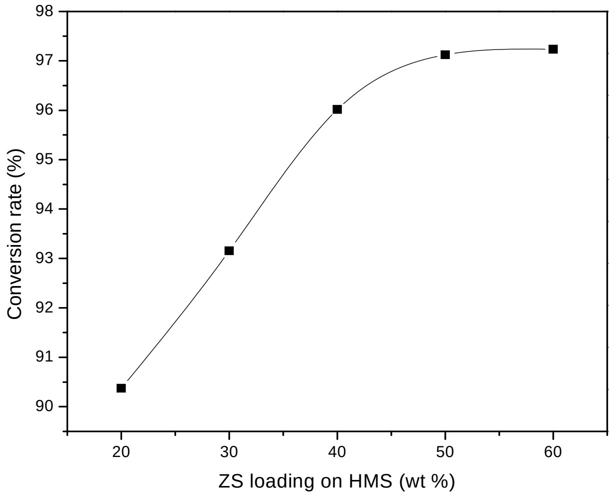

Figure 6. Catalytic activities of solid acid catalysts with different ZS loading on HMS for esterification of gossypol.

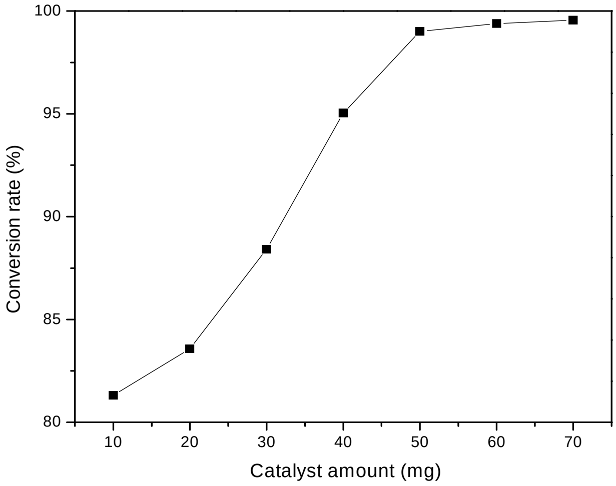

Figure 7. Effect of catalyst amount on the esterification of gossypol.

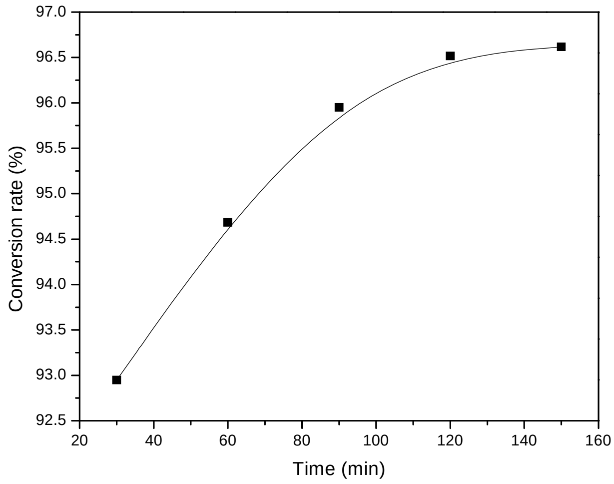

Figure 8. Effect of reaction time on the esterification of gossypol.

sed to reach the maximum conversion efficiency at 50 mg ZS/HMS, while addition of additional catalyst did not significantly increase gossypol esterification efficiency. These results confirmed that the average size of catalyst particle (60 - 80 mesh) was small enough to limit the internal mass transfer inside the catalyst pores.

The reaction was carried out at room temperature with 50 mg ZS/HMS (50 wt%) and 2.5 mL gossypol (0.6 mg/mL). The mixture was stirred at 200 rpm for 15 min. Then 1.6 mL distilled H_2O was added to the solution and it was left standing. Samples were removed every 30 min to determine gossypol ester production (**Figure 8**). The conversion rate increased with reaction time and gradually peaked at 120 min. At 120 min, the conversion rate of acetylated gossypol reached 96.5%.

4. Conclusion

The synthesized ZS/HMS shows good dispersion by TG, XRD and BET characterizations. The acid activity of the catalysts increases with the increment of the ZS loading. When ZS loading on HMS was 50%, the optimal amount of catalyst was 50 mg catalyst for an esterification reaction of 120 min, the yield of acetylated gossypol reached up to 96.7%.

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