GREEN SYNTHESIS OF ZnO NANOPARTICLES USING *BACILLUS SUBTILIS* AND THEIR CATALYTIC PERFORMANCE IN THE ONE-POT SYNTHESIS OF STEROIDAL THIOPHENES

Shamsuzzaman,[a]* Abad Ali,[a] Mohd Asif,[a] Ashraf Mashrai[a] and Hena Khanam[a]

Keywords: ZnO nanoparticles; *Bacillus subtilis;* steroidal thiophenes.

In the present work, we describe a low-cost, unreported and simple procedure for biosynthesis of zinc oxide nanoparticles (ZnO NPs) using *Bacillus subtilis* as eco-friendly reducing and capping agent. The synthesized ZnO NPs were characterized by UV-Vis spectroscopy, FTIR, XRD, SEM, TEM, PL and TGA techniques. The biosynthesis methods were carried out for its intrinsic advantages, as it is simple, cost-effective, environment-friendly and can be easily scaled up for large scale synthesis. The prepared nano-particles were used as catalyst for the fast and efficient synthesis of steroidal thiophenes. The one pot three-component mixture of steroidal ketones (**1**-**3**), malononitrile/ethyl cyanoacetate and elemental sulfur were converted into the corresponding steroidal thiophenes (**4**-**9**) in moderate to high yields with excellent selectivity.

Corresponding Authors
Tel: +919411003465
E-Mail: shamsuzzaman9@gmail.com
[a] Steroid Research Laboratory, Department of Chemistry, Aligarh Muslim University, Aligarh 202 002, India

Introduction

Green chemistry is an innovative research field, that reduce or eliminate the use and generation of hazardous substances[1,2] and offering a research-based approach in synthesizing chemicals such as pharmaceuticals, perfumes, flavorants, etc., with reduced needles environmental impact which benefit the environment and contribute to environmental sustainability[3] and there is no need to use high temperature, pressure, energy and hazardous chemicals. Therefore, green chemistry has advantages over chemical and physical methods.[4] Nanotechnology is emerging interdisciplinary science for synthesis of nanoparticles at the nanoscale level.[5,6] The field of nanotechnology is one of the most active areas of research in modern material science.[7] Nanoparticles can be synthesized by different physical, chemical and biological methods.[8] The biosynthesized nanoparticles have received broad attention due to their non-toxic and eco-friendly nature.[9,10] The biosynthesized nanoparticles are different in shape and size in comparison with those produced by other organism and has led to the development of new biocidal agents.[11] The biosynthesized nanoparticles have a wide variety of applications such as drug carriers for targeted delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science and magnetic resonance imaging (MRI).[12] The synthesized ZnO nanoparticles possess high specific surface area, high catalytic activity and high adsorption capacity. ZnO nanoparticles has gained considerable interest due to their vast applications in various areas.[13-15] The Gewald reaction of sulfur, an active nitrile, and oxo-component provide 2-aminothiophene derivatives via a three-component one pot reaction (G-3CR). It has found diverse applications in combinatorial and medicinal chemistry. Chemistry of 2-aminothiophenes is one of the most extensive and dynamic field of present-day research. Since 1961 when first report on the Gewald reaction was published it became a universal method for synthesis of substituted 2-aminothiophenes and has gained prominence in recent times.[16] Thiophenes possess considerable attention for organic synthetic and medicinal chemists because of their unique applications in pharmaceuticals, organic semi-conductors, conducting polymers, organic light-emitting diodes (OLEDs) and lasers.[17-20] Encouraged by the above facts and in continuation of our previous work in green chemistry[21] and heterosteroids synthesis.[22] We here report a green methodology for the synthesis of highly substituted thiophenes *via* biosynthesized ZnO NPs. UV-vis spectroscopy, FTIR, XRD, SEM, TEM, PL and TGA were performed to ascertain the formation and characterization of biosynthesized ZnO NPs. The structures of newly synthesized compounds have been established on the basis of their elemental analysis and spectral data.

Experimental

All the chemicals used were purchased from Sigma Aldrich and Merck as analytical grade. The solvents were purified prior to use. The optical absorbance spectra were taken by using UV-vis double beam Perkin-Elmer LAMBDA 35 spectrophotometer at room temperature in the wavelength range of 250-800 nm. X-ray diffraction (XRD) patterns of the samples were obtained at room temperature, with a step of 0.02°, using Bruker D8 ADVANCE X-ray diffractometer with Cu K_α radiation (λ = 1.54178 Å) in the range of $0° < 2\theta < 100°$ at 40 kV. SEM images were obtained using a field emission scanning electron microscope (JSM-7600F, JEOL, Tokyo Japan) at an accelerating voltage of 15 kV and TEM images were obtained with ultra-high resolution FETEM (JEOL, JEM-

2100F) at an accelerating voltage of 200 kV. Photoluminescence (PL) spectra were measured using a Cary Eclipse EL06063917 fluorescence spectrophotometer with a xenon arc lamp as the light source. The thermal studies were carried out using TGA instrument (SHIMADZU) at a heating rate of 20 ^{o}C min^{-1}. Melting points were recorded on Riechert Thermover instrument and are uncorrected. IR spectra (KBr disks) were recorded on Perkin Elmer FT-IR Spectrometer spectrum two and its values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra in dilute $CDCl_3$ solutions at 303 K were run on a Bruker Avance II 400 NMR spectrometer equipped with a 5 mm diameter broad band inverse probehead working at 400 MHz for ^{1}H and at 100 MHz for ^{13}C, respectively. ^{1}H chemical shifts were referenced to the trace signal of $CHCl_3$ (7.26 ppm from int. TMS). Following abbreviations were used to indicate the peak multiplicity s -singlet, d -doublet, m -multiplet and values are given in parts per million (ppm) (δ) and coupling constants (J) are given in Hertz and ^{13}C chemical shifts to the center peak of the solvent signal (77.00 ppm from int. TMS). Mass spectra were recorded on a JEOL D-300 mass spectrometer.

Elemental analyses of all the new compounds were recorded on Perkin Elmer 2400 CHN Elemental Analyzer within ± 0.4 % of the theoretical values. The progress of all reactions was monitored by thin layer chromatography (TLC) plates with 0.5 mm layer of silica gel G, light petroleum ether refers to a fraction of b.p. 60-80 ^{o}C, and exposed to iodine vapors to check the purity as well as the progress of reaction. Sodium sulfate (anhydrous) was used as a drying agent.

Synthesis of ZnO nanoparticles

Bacillus subtilis cells were allowed to grow as a suspension culture in sterile distilled water containing nutrient broth media for 24 h. 25 mL of culture was taken and diluted four times by adding 75 mL of sterile distilled water containing nutrients. This diluted culture solution was again allowed to grow for 24 h. 50 mM zinc acetate dihydrate aqueous solution was injected into the bacterial suspension at a rate of 10 mL min^{-1} through a buret. After the mixture was stirred for 30 min, 200 mL of a 50 mM $NaBH_4$ aqueous solution as the reducing agent, was slowly poured into the mixture at a flow rate of 10 mL/min through the buret. Then the final mixture was robustly stirred for 24 h at room temperature. After the mixture was fully reacted, the mixture was centrifuged and subsequently the precipitate was collected.[23]

General procedure for synthesis of steroidal thiophene derivatives (4-9)

A mixture of 5α-cholestan-6-one **1-3** (1 mmol), malononitrile/ ethyl cyanoacetate (1 mmol), S_8 (1 mmol) and ZnO nanoparticles catalyst (0.003 g), in ethanol (15 mL) was refluxed for 12-18 h. The progress of the reaction was followed by TLC. After completion of the reaction, the mixture was filtered to remove the catalyst and the filtrate was taken in diethyl ether, washed with water and dried over anhydrous sodium sulfate. Removal of solvent gave the crude product which was recrystallized from methanol to obtain the pure compounds (**4-9**).

3β-Acetoxy-5α-cholest-6-eno-[6,7-d]-2´-amino-3´-cyanothiophene (4)

Brown powder, yield 70 %, m.p. 167-169 °C; IR (KBr, ν_{max}/cm^{-1}): 3344 (NH_2), 2210 (CN), 1713 ($OCOCH_3$), 1623, 1621 (C=C), 1068 (C-O), 714 (S-C); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.21 (2H, brs, NH_2, exchangeable with D_2O), 4.7 (1H, m, C_3α-H, $W½$ = 14 Hz), 2.3 (dd, 1H, J = 7.55, 4.52 Hz, C_5-H), 2.05 (3H, s, $OCOCH_3$), 1.2 (3H, s, C_{13}-CH_3), 1.14 (3H, s, C_{10}-CH_3), 1.04 and 1.02 (other methyl protons); ^{13}C NMR ($CDCl_3$, δ, ppm): 172.5, 151.1, 145.2, 137.7, 120.4, 85.1, 75.5, 58.6, 50.1, 46.9, 45.2, 43.4, 40.6, 37.3, 35.3, 34.2, 33.9, 32.1, 31.0, 29.5, 27.5, 26.9, 25.2, 24.1, 23.9, 22.7, 21.2, 20.9, 19.5, 17.6, 15.3, 13.1; Anal. Calcd for $C_{32}H_{48}N_2O_2S$ %: C, 73.24, H, 9.22, N, 5.34. Found: C, 73.29, H, 9.24, N, 5.37; MS: m/z 524 [$M^{+\cdot}$].

3β-Chloro-5α-cholest-6-eno-[6,7-d]-2´-amino-3´-cyanothiophene (5)

Green brown powder, yield 72 %, m.p. 154-156 °C; IR (KBr, ν_{max}/ cm^{-1}): 3346 (NH_2), 2215 (CN), 1620, 1624 (C=C), 742 (C-Cl), 716 (S-C); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.24 (2H, brs, NH_2, exchangeable with D_2O), 3.8 (1H, m, C_3α-H, $W½$ = 17 Hz), 2.2 (dd, 1H, J = 7.53, 4.56 Hz, C_5-H), 1.2 (3H, s, C_{13}-CH_3), 1.14 (3H, s, C_{10}-CH_3), 1.04 and 1.02 (other methyl protons); ^{13}C NMR ($CDCl_3$, δ, ppm): 152.7, 148.3, 137.1, 120.4, 87.4, 66.3, 59.9, 55.7, 51.7, 48.5, 45.9, 41.6, 40.2, 39.4, 38.9, 37.4, 36.2, 34.9, 32.7, 31.5, 30.7, 28.6, 27.4, 26.3, 25.4, 24.9, 23.7, 20.6, 16.8, 14.8; Anal. Calcd. for $C_{30}H_{45}ClN_2S$ %: C, 71.89, H, 9.05, N, 5.59. Found: 71.87, H, 9.09, N, 5.57; MS: m/z 500/502 [$M^{+\cdot}$].

5α-Cholest-6-eno-[6,7-d]-2´-amino-3´-cyanothiophene (6)

Brown powder, yield 71%, m.p. 149-151 °C; IR (KBr, ν_{max}/ cm^{-1}): 3343 (NH_2), 2217 (CN), 1630, 1617 (C=C), 718 (S-C); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.26 (2H, brs, NH_2, exchangeable with D_2O), 2.7 (dd, 1H, J = 7.51, 4.53 Hz, C_5-H), 1.2 (3H, s, C_{13}-CH_3), 1.14 (3H, s, C_{10}-CH_3), 1.04 and 1.02 (other methyl protons); ^{13}C NMR ($CDCl_3$, δ, ppm): 150.3, 147.5, 139.1, 119.7, 85.1, 61.4, 59.7, 55.1, 49.4, 46.1, 43.5, 42.7, 41.5, 40.2, 38.7, 37.4, 35.2, 32.6, 31.5, 30.4, 29.3, 27.1, 26.3, 25.6, 24.1, 23.7, 20.5, 17.2, 14.3, 12.9; Anal. Calcd. for $C_{30}H_{46}N_2S$ %: C, 77.20, H, 9.93, N, 6.00. Found: C, 77.24, H, 9.97, N, 6.02; MS: m/z 460 [$M^{+\cdot}$].

3β-Acetoxy-5α-cholest-6-eno-[6,7-d]-2´-amino-3´-carboethoxythiophene (7)

Brown powder, yield 74 %, m.p. 175-177 °C; IR (KBr, ν_{max}/cm^{-1}): 3340 (NH_2), 1710 ($OCOCH_3$), 1669 (OCOC), 1620, 1618 (C=C), 1246, 1136 (C-O), 718 (S-C); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.22 (2H, brs, NH_2, exchangeable with D_2O), 4.34 (2H, q, J = 7.02 ,CH_2),4.7 (1H, m, C_3α-H, $W½$ = 14 Hz), 2.6 (dd, 1H, J = 7.52, 4.51 Hz, C_5-H), 2.05 (3H, s, $OCOCH_3$), 1.2 (3H, s, C_{13}-CH_3), 1.14 (3H, s, C_{10}-CH_3), 1.04 and 1.02 (other methyl protons); ^{13}C NMR ($CDCl_3$, δ, ppm): 175.5, 168.3, 151.5, 142.1, 139.5, 118.7, 77.8, 65.3, 59.7, 56.9, 52.1, 45.7, 43.2, 42.1, 40.5, 39.5, 37.4, 36.2, 35.6, 33.1, 30.6, 29.9, 28.1, 26.6, 24.1, 23.9, 22.8, 21.0, 20.9, 19.9, 16.1, 15.6, 13.9, 12.4; Anal. Calcd. for $C_{34}H_{53}NO_4S$ %: C, 71.41, H, 9.34, N, 2.45. Found: C, 71.45, H, 9.38, N, 2.49; MS: m/z 571 [$M^{+\cdot}$].

3β-Chloro-5α-cholest-6-eno-[6,7-d]-2´-amino-3´-carboethoxy-thiophene (8)

Green brown powder, yield 72 %, m.p. 176-178 °C; IR (KBr, ν_{max}/cm^{-1}): 3339 (NH_2), 1665 (OCOC), 1623, 1615 (C=C), 1140 (C-O), 740 (C-Cl), 720 (S-C); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.27 (2H, brs, NH_2, exchangeable with D_2O), 4.35 (2H, q, J = 7.01, CH_2), 3.8 (1H, m, C_3α-H, $W½$ = 16 Hz), 2.7 (dd, 1H, J = 7.55, 4.53 Hz,C_5-H), 1.2 (3H, s, C_{13}-CH_3), 1.14 (3H, s, C_{10}-CH_3), 1.04 and 1.02 (other methyl protons); ^{13}C NMR ($CDCl_3$, δ, ppm): 169.5, 152.2, 143.6, 140.1, 114.6, 67.1, 64.2, 59.9, 56.1, 53.7, 49.4, 45.4, 41.5, 40.2, 39.1, 38.3, 37.2, 36.5, 34.7, 32.9, 30.5, 29.3, 28.9, 26.3, 25.2, 24.5, 23.9, 21.7, 20.6, 17.2, 15.7, 13.1; Anal. Calcd. for $C_{32}H_{50}ClNO_2S$ %: C, 70.10, H, 9.19, N, 2.55. Found: C, 70.13, H, 9.16, N, 2.52; MS: m/z 547/549 [$M^{+\cdot}$].

5α-Cholest-6-eno-[6,7-d]-2´-amino-3´-carboethoxythiophene (9)

Brown powder, yield 71 %, m.p. 175-177 °C; IR (KBr, ν_{max}/cm^{-1}): 3335 (NH_2), 1668 (OCOC), 1623, 1621 (C=C), 1138 (C-O), 716 (S-C); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.25 (2H, brs, NH_2, exchangeable with D_2O), 4.32 (2H, q, J = 7.02, CH_2), 2.7 (dd, 1H, J = 7.53, 4.54 Hz, C_5-H), 1.2 (3H, s, C_{13}-CH_3), 1.14 (3H, s, C_{10}-CH_3), 1.04 and 1.02 (other methyl protons); ^{13}C NMR ($CDCl_3$, δ, ppm): 171.4, 152.3, 145.7, 138.2, 118.1, 65.7, 59.6, 57.7, 53.1, 46.2, 44.5, 43.2, 42.9, 41.7, 40.5, 39.7, 37.3, 35.1, 32.1, 31.7, 29.1, 28.3, 27.2, 25.9, 24.1, 23.4, 22.9, 21.5, 20.9, 17.3, 15.6, 13.9; Anal. Calcd. for $C_{32}H_{51}NO_2S$ %: C, 74.80, H, 10.00, N, 2.73. Found: C, 74.83, H, 10.02, N, 2.70; MS: m/z 513 [$M^{+\cdot}$].

Result and discussion

Characterization of ZnO nanoparticles

UV spectrophotometry study

The UV-vis absorption spectrum findings demonstrate a novel technique for the preparation of ZnO nanoparticles (**Figure 1**), by dispersing ZnO nanoparticles in distilled water and using distilled water as the reference. An absorption peak focused at 375 nm. The electronic band gap (E_g) of ZnO nanoparticles was determined by employing the following relationship:

$$E_g = \frac{hc}{\lambda}$$

whereas

h is Planck's constant,

c is speed of light and

λ is the cut off wavelength value of ZnO nanoparticles.

The E_g value was determined to be 3.31 eV which is in good agreement with the previous work.[24]

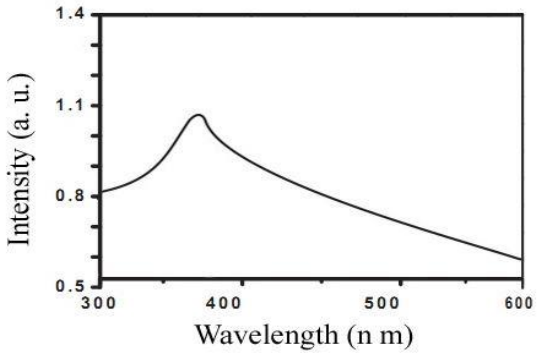


Figure 1. UV-vis spectrum of ZnO nanoparticles.

Fourier transform infrared spectroscopy

The synthesized zinc oxide nanoparticles have peaks at 3414.57 cm^{-1} for NH_2 stretching in adenine, cytosine, quinine and H-bonded OH groups while 2934.14 cm^{-1} for C-H stretching in aliphatics of cell walls (fatty acids, carbohydrates). 1625.64 cm^{-1} for NH_2 bending, C=O, C=N stretching (amide I band). The peak at 1385.69 cm^{-1} for amide III band and 1052.22 cm^{-1} for C-O-C asymmetric stretching in aliphatic esters.[25] Zn-O-Zn stretching modes at 609.33 cm^{-1} were well supported by available literature.[26] These peaks are the combined characteristics of zinc oxide and bacterial strain

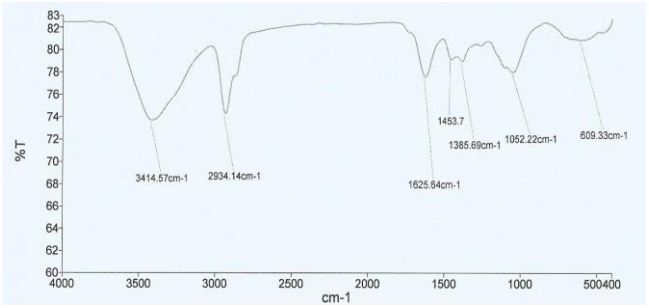


Figure 2. FTIR spectrum of ZnO nanoparticles.

XRD analysis

X-ray diffraction is taken in order to further confirm ZnO phase of the nanoparticles. The XRD patterns of the obtained ZnO nanoparticles are shown in **Figure 3**. Powder XRD of the product was carried out with Cu K_α radiation (λ = 1.54056 Å), employing a scanning rate of 0.02° s^{-1} and 2θ ranges from 20^o to 80^o for ZnO. The observed peaks correspond to the Bragg angle for the (100), (002), (101), (102), (110) and (200) planes of the crystalline ZnO, which are consistent with standard JCPDS No. 89-7102 and no indication of a secondary phase. All the peaks of XRD are very well matched with the hexagonal phase (wurtzite structure). The strong and narrow diffraction peaks indicate that the product has good crystalline structure. The crystallite size of the nanoparticles was calculated using Debye Scherrer formula

$$D = K\frac{\lambda}{\beta\cos\theta}$$

where,

K is constant,

λ is the wavelength of employed X-rays (1.54056 Å),

β is corrected full width at half maximum and

θ is Bragg's angle.

The 2θ value from the equation comes out to be at 35.81 and therefore the calculated crystallite size of the powder particles is about 24 nm.

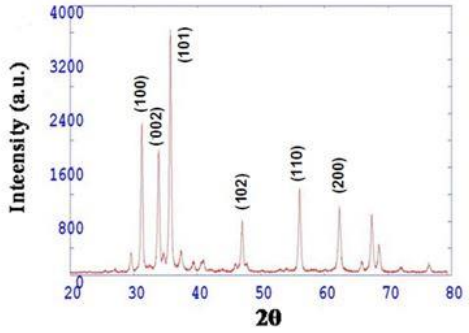


Figure 3. XRD analysis of ZnO nanoparticles.

SEM and TEM analysis

The conformation of the nanostructure morphology of ZnO particles comes from the analysis of SEM and TEM micrographs. SEM micrograph (**Figure 4**) showed the average size of nanoparticles between 20 and 30 nm.

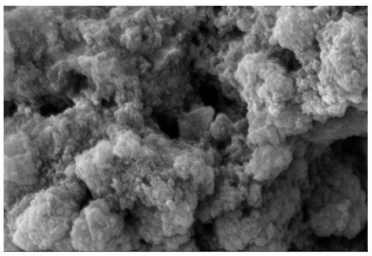


Figure 4. SEM image of the ZnO nanoparticles

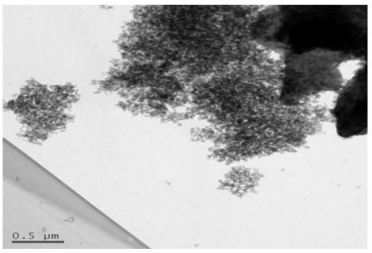


Figure 5. TEM image of the ZnO nanoparticles.

The size and morphology of ZnO particles analyzed by TEM is represented in **Figure 5**. This image reveals that most of the ZnO nanoparticles are quasi-spherical and their diameter is about ~25 nm. This result is in agreement with the value calculated from the X-ray diffraction.

Photoluminescence analysis

The Photoluminescence spectrum of ZnO nanoparticles consists of two emission peaks (**Figure 6**). A weak deep-level emission at 2.25 eV in the visible range is caused by a structural defect.[27] The other peak in the UV range at 3.1 eV which can be explained by the direct combination of excitons through an exciton-exciton collision process and the lower energy peak in the asymmetric UV emission is associated with band-to-acceptor transitions due to the large binding energy of ZnO.[28]

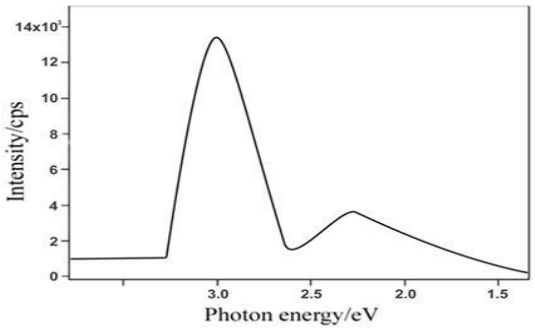


Figure 6. The Photoluminescence spectrum of ZnO nanoparticles.

Thermal stability

The thermo gravimetric analysis (TGA) has been performed on the biosynthesis of ZnO nanoparticles. TGA curve in **Figure 7** indicates that the weight loss starts at 200 ^{o}C because of the evaporation of water, the major weight loss occurs between 320 and 430 ^{o}C, which is around 40 % of the original weight due to the removal and decomposition of organic groups present during the biosynthesis. No decomposition or reaction occurs at temperatures above 500 °C.

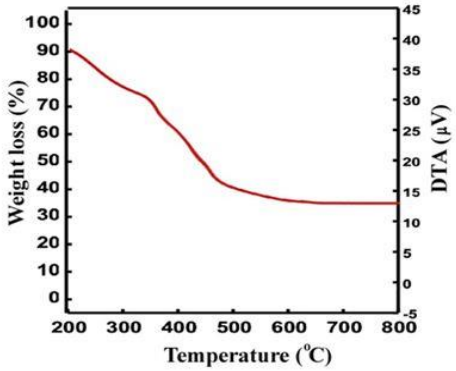


Figure 7. TGA curve of ZnO nanoparticles

 DOI: 10.17628/ECB.2014.3.939

The catalytic performance of ZnO in the synthesis of steroidal thiophenes

The catalytic system is influenced by various parameters, such as amount of the catalyst employed, effect of catalyst and solvent system. 3β-Acetoxy-cholestan-6-one, malononitrile and sulfur powder in DMF were selected as model substrates for carrying out the optimization studies for the synthesis of steroidal thiophenes. Initially, the model reaction was performed in the absence of ZnO nanoparticles and the reaction did not proceeded even with very long reaction time. When the model reaction was examined with ZnO nanoparticles the reaction was accelerated.

Catalytic loading

It was observed that yield was increased with enhancing catalyst concentration. The yield was increased from 38 to 70 % by enhancing the catalyst amount from 0.5 to 2.5 mol% (**Table 1**). Further increase in the catalyst concentration from 2.5 to 3.5 mol %, did not show any profound effect on the reaction rate as well as the yield this may be attributed to the coagulation of ZnO nanoparticles which decreased the effective surface area of the catalyst.[29]

Table 1. Effect of catalyst loading on the model reaction**.**

Entry	Catalysts, mol %	Time, h	Yield, %[a]
1	0.5	19	38
2	1	18	44
3	1.5	14	53
4	2.5	12	70
5	3	12	70
6	3.5	12	70

[a]Yield are related to isolated pure products

Effect of solvent

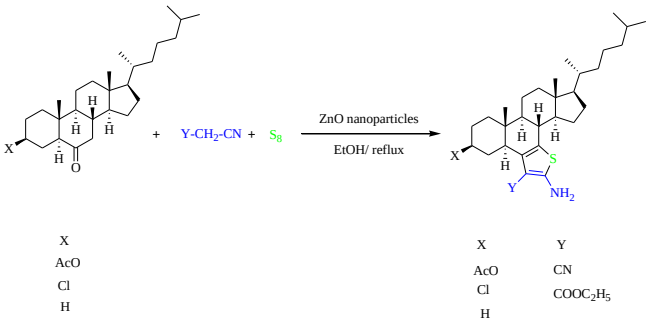
We then tried to screen the reaction in various organic solvents in order to optimize the reaction conditions using ZnO nanoparticles as catalyst (**Table 2**).

Table 2. Solvent screening for the model reaction.

Entry	Solvent	Yield, %[a]
1	DMF	70
2	Methanol	60
3	Ethanol	62
4	1,4-Dioxane	45
5	Benzen	30

[a]Yield are related to isolated pure products

The solvent screening experiments revealed that the reaction yield is dependent on the polarity and the coordinating ability of the solvents. The polar solvents afforded better yield than the nonpolar ones and the best result was obtained in ethanol in which ZnO nanoparticle catalyst worked most efficiently by phasing out of the desired product. In order to investigate the scope of this reaction, a variety of different steroidal compounds were subjected to this reaction (**Scheme 1**).



Scheme 1. Synthesis of steroidal thiophene derivatives using biosynthesized ZnO nanoparticles

All the reactions proceeded smoothly and the reaction was completed within 12 to 18 h to afford the products (**4-9**) in excellent yields (70-74 %).

Recyclability of catalyst

After completion of the model reaction in specified time, the catalyst was recovered by filtration, washed with dichloromethane and methanol and dried at 150 ºC for 4 h and used for the subsequent cycle (**Figure 8**).[30] The results revealed that the catalyst exhibited good catalytic activity up to five cycles.

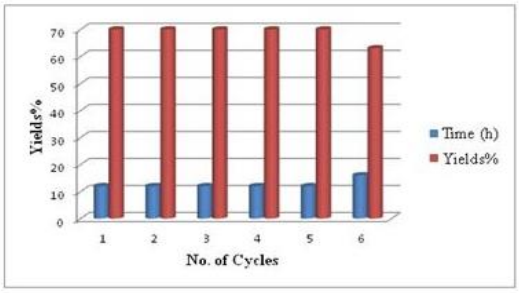


Figure 8. Recyclability of ZnO nanoparticles.

Studying the superiority of ZnO nanoparticles over some other catalyst

Various catalysts were employed to evaluate the capability and efficiency of the catalyst (**Table 3**). The model reaction was examined with imidazole, morpholine, diethyl amine and ZnO (bulk) using 2.5 mol% of each catalyst separately the reaction took longer time period for completion with lower yield of the product.

Table 3. The superiority of ZnO nanoparticles over other reagents.

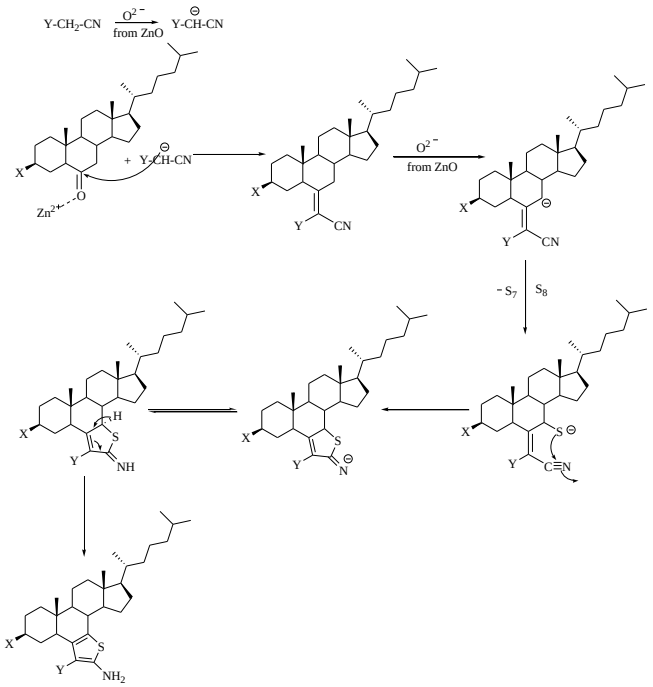
Entry	Catalysts	Time, h	Yield, %[a]
1	-	-	-
2	Imidazole	22	58
3	Morpholine	20	45
4	Diethyl amine	26	52
5	ZnO (bulk)	18	53
6	ZnO	12	70

[a]Yield are related to isolated pure products

With ZnO nanoparticles the reaction was accelerated and yield of the desired product was maximum.

Proposed reaction pathway for the synthesis of steroidal thiophenes

A mechanistic route for the synthesis of steroidal thiophenes using nano-ZnO as catalyst is presented in **Scheme 2**. The mechanism involves a three-step process and nano-ZnO has dual characters of Lewis acidic (Zn^{2+}) in one hand and Lewis basic sites (O^{2-}) on the other.[31] In the first step, Lewis acid sites of ZnO (Zn^{2+}) coordinates to the oxygen of the carbonyl group, hence reactivity of carbonyl group increases. Moreover, the Lewis basic sites of nano-ZnO (O^{2-}) deprotonated the malononitrile/ cyano ethylacetate and then a nucleophilic attack to the activated carbonyl group proceeds the reaction forward. The ring closure step is the most crucial step which is performed as an intra-molecular nucleophilic attack of the sulfur anion to triple bond of the cyano group.[32]



Scheme 2. Plausible reaction mechanism for the synthesis of steroidal thiophene derivatives (**4-9**).

Conclusion

In conclusion, we have synthesized zinc oxide nanoparticles (ZnO NPs) using *Bacillus subtilis* as eco-friendly reducing and capping agent. ZnO nanoparticles were used as green catalyst for one-pot three-component preparation of steroidal thiophene derivatives. The attractive features of this protocol are simple procedure, cleaner reaction and use of reusable nano catalyst. Satisfactory yields of reaction, as well as a simple experimental, isolation and purification of the products make it a useful protocol for the green synthesis.

Acknowledgments

The authors would like to thank the Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh for providing necessary research facilities. Facilities provided by SAP (DRS-I) for their generous research support are also gratefully acknowledged.

References

[1]Kirchhoff, M. M., *J. Chem. Educ.*, **2001**, *78*, 1577.

[2]Anastas, P. T., *Crit. Rev. Anal. Chem*., **1999**, *29*, 167-175.

[3]Banitaba, S. H., Safari, J., Khalili, S. D., *Ultrason. Sonochem*., **2013**, *20*, 401-407.

[4]Jayaseelan, C., Ramkumar, R., Rahuman, A., Perumal, P., *Ind. Crops Prod*., **2013**, *45*, 423-429.

[5]Jr, L. A. P., *J. Chem. Educ*., **2007**, *84*, 259-264.

[6]Sadik, O. A., Zhou, A. L., Kikandi, S., Du, N., Wang, Q., Varner, K., *J. Environ. Monit*., **2009**, *11*, 1782-1800.

[7]Schmitt, S. W., Schechtel, F., Amkreutz, D., Bashouti, M., Srivastava, S. K., Hossmann, B., Dieker, C., Spiecker, E., Rech, B., Christiansen, S. H., *Nano Lett*., **2012**, *12*, 4050-4054.

[8]Mohanpuria, P., Rana, N. K., Yadav, S. K., *J. Nanopart. Res*., **2008**, *10*, 507-517.

[9]El-Rafie, H. M., El-Rafie, M. H., Zahran, M. K., *Carbohydr. Polym*., **2013**, *96*, 403-410.

[10]Shankar, S. S., Rai, A., Ahmad, A., Sastry, M., *J. Colloid Interface Sci*., **2004**, *275*, 496-502.

[11]Patel, V. K., Bhattacharya, S., *Appl. Mater. Interfaces*, **2013**, *5*, 13364-13374.

[12]Sapsford, K. E., Algar, W. R., Berti, L., Gemmill, K. B., Casey, B. J., Oh, E., Stewart, M. H., Medintz, I. L., *Chem. Rev*., **2013**, *113*, 1904-2074.

[13]Wang, Z. L., *Annu. Rev. Phys. Chem*., **2004**, *55*, 159-196.

[14]Kuo, C. L., Kuo, T. J., Huang, M. H., *J. Phys. Chem.* **2005 B**, *109*, 20115-20121.

[15]Wang, H. H., Xie, C., *J. Cryst. Growth*, **2006**, *291*, 187-195.

[16]Puterová, Z., Krutošíková, A., Végh, D., *Arkivoc* **2010 (i)** , 209-246.

[17](a)Holmes, J. M., Lee, G. C. M., Wijono, M., Weinkam, R., Wheeler, L. A., Garst, M. E., *J. Med. Chem*., **1994**, *37*, 1646-1651; (b) Luker, T. J., Beaton, H. G., Whiting, M., Mete, A., Cheshire, D. R., *Tetrahedron Lett*., **2000**, *41*, 7731-7735; (c) Pinto, I. L., Jarvest, R. L., Serafinowska, H. T., *Tetrahedron Lett*., **2000**, *41*, 1597-1600.

[18](a) Mishra, A., Ma, C. Q., Bauerle, P., *Chem. Rev*., **2009**, *109*, 1141-1276; (b) Katz, H. E., Bao, Z., Gilat, S. L., *Acc. Chem. Res*., **2001**, *34*, 359-369.

[19](a) Noda, T., Imae, I., Noma, N., Shirota, Y., *Adv. Mater*., **1997**, *9*, 239-241; (b) Noda, T., Ogawa, H., Noma, N., Shirota, Y., *J. Mater. Chem*., **1999**, *9*, 2177-2181.

[20]Pisignano, D., Anni, M., Gigli, G., Cingolani, R., Zavelani-Rossi, M., Lanzani, G., Barbarella, G., Favaretto, L., *Appl. Phys. Lett*., **2002**, *81*, 3534.

[21]Shamsuzzaman, Mashrai, A., Khanam, H., Aljawfi. R. N., *Arab. J. Chem.*, http://dx.doi.org/10.1016/j.arabjc.2013.05.004

[22](a) Shamsuzzaman, Khan, M. S., Alam, M., Tabassum, Z., Ahmad, A., Khan, A. U., *Eur. J. Med. Chem*., **2010**, *45*, 1094-1097; (b) Shamsuzzaman, Khanam, H., Mashrai, A., Sherwani, A., Owais, M., Siddiqui, N., *Steroids*, **2013**, *78*, 1263-1272.

[23]Shim, H. W., Jin, Y. H., Seo, S. D., Lee, S. H., Kim, D. W., *ACS Nano*, **2011**, *5*,443-449.

[24]Ni, Y. H., Wei, X. W., Hong, J. M., Ye, Y., *Mater. Sci. Eng. B*., **2005**, *121*, 42-47.

[25]Filip, Z., Herrmann, S., Kubat, J., *Microbiol. Res*., **2004**, *159*, 257-262.

[26]Dutta, R. K., Sharma, P. K., Pandey, A. C., *J. Nanopart. Res*., **2010**, *12*, 1211-1219.

[27]Soares, J. W., Whitten, J. E., Oblas, D. W., Steeves, D. M., *Langmuir*, **2008**, *24*,371-374.

[28]Ha, B., Ham, H., Lee, C. J., *J. Phys. Chem. Solids*, **2000**, *869*, 2453-2456.

[29]Bhattacharyya, P., Pradhan, K., Paul, S., Das, A. R., *Tetrahedron Lett*., **2012**, *53*, 4687-4691.

[30]Ghomi, J. S., Ghasemzadeh, M. A,. *Acta Chim. Slov*., **2012**, *59*, 697-702.

[31]Ghosh, P. P., Das, A. R., *J. Org. Chem*., **2013**, *78*, 6170-6181.

[32]Tayebee, R., Javadi, F., Argi, G., *J. Mol. Catal. A: Chem*., **2013**, *368/369*, 16-23.

Received: 22.08.2014.
Accepted: 25.09.2014.