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Antibacterial and catalytic properties of silver nanoparticles loaded zeolite: green method for synthesis of silver nanoparticles using lemon juice as reducing agent

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Zeolite Y is a cage-like alumina silicate which is widely used as solid support to immobilize metal and metal sulfide nanoclusters. We have attempted to synthesis silver nanoparticle-loaded zeolite Y by an ion exchange method followed by a biogenic reduction method using lemon juice as a reducing agent. The antimicrobial activity of the silver ion, silver nanoparticles and silver chloride-modified zeolite was investigated against various Gram negative and Gram positive microorganisms. The silver nanoparticle-loaded zeolite was further functionalized with amoxicillin antibiotic which exhibited a strong antimicrobial action to kill drug resistant microorganisms. The catalytic behavior of silver nanoparticles was investigated to reduce 4-Nitrophenol in presence of $NaBH_4$. The catalytic reaction is found to be pseudo-first order, resulting in a rate constant that was comparable with previously-reported results.

Keywords: zeolite-Y, silver nanoparticles, amoxicillin, catalytic reduction of 4-aminophenol, antibacterial studies.

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1. Introduction

Zeolites are an important group of crystalline aluminosilicates currently available for various fields of applications. These minerals are widely used as sorbents, ion exchangers, catalysts and biosensors [1–4]. The catalytic nature, reactivity and other properties of zeolite can be greatly improved by cation exchange methods [5]. These materials are negatively charged with a high density of active acid sites, high thermal stability, high size selectivity and unique porous properties, which impart their ability act as catalysts for various industrial catalytic studies at elevated temperature with wider pH ranges. Because of void space and swelling behavior which can be used in petrochemical cracking, ion-exchange, gas and solvent separation, and removal of pollutants [6]. Metal ion, charged species, redox and photoactive molecules can be immobilized within the pores of zeolite system and these composites can be used in sensors and catalytic applications [7–10].

Silver (Ag) is a metallic element that has been widely used in various excellent fields [11]. Silver ions (Ag^+) and silver nanoparticles are effective in inhibiting bacterial growth and may damage the DNA of both Gram-positive and Gram-negative bacteria [12–15]. Silver modified zeolite has been used in various fields such as catalyst, biosensors, water purification, antifungal and antimicrobial activity [16, 17].

The aim of this study is to prepare AgNPs/Zeo-Y nanocomposites using lemon juice as reducing agent by green synthesis method and study of its applications in the catalytic reduction of 4-Nitrophenol and the antibacterial activity of the system.

2. Experimental Section

2.1. Chemicals

Zeolite-Y and silver nitrate were purchased from Sigma Aldrich, USA. 4-Nitrophenol and liquid ammonia (30% v/v) were received from Fisher Scientific Pvt. Ltd., India. Sodium borohydrate, sodium chloride and amoxicillin were received from Merck, India. Hydrochloric acid and sulphuric acid were purchased from SRL Pvt. Ltd., India. All reagents and chemicals were used received from commercial source with an analytical grade and without further purification.

2.2. Preparation of AgNPs/Zeo-Y

To prepare AgNPs/Zeo-Y nanocomposite, 0.1 M $AgNO_3$ was dissolved in 20 mL of distilled water and then 1 % solution of NH_4OH was added dropwise to the aq. solution of $AgNO_3$ [18]. The color of the solution changed from greenish gray to colorless. 1 g of natural zeolite-Y was added to above mixture and stirred overnight. The product was collected and washed with distilled water and then dried at 100 °C for 2 h. Finally, the AgNPs/Zeo-Y nanocomposite was collected for further studies.

2.3. Synthesis of AgCl/ Zeo-Y

The Ag^+ ion loaded Zeo-Y was placed in a sealed 100 ml round bottom flask and then purged with HCl gas which was generated from NaCl in presence of conc.H_2SO_4 continuously to obtain AgCl immobilized Zeo-Y for a period of 2 h.

2.4. Preparation of AgNPs/Zeo-Y using lemon juice as reducing agent

To 3 mL of DD water was added a fresh 1 mL of lemon juice and the pH of the medium was adjusted to 11 followed by 0.01 M of silver nitrate solution was added. Finally, the yellowish green colored solution was changed to reddish brown color which indicated the formation of silver nanoparticle immobilized zeolites.

2.5. Preparation of Amoxicillin modified AgNPs/Zeo-Y

A known amount of AgNPs/Zeo-Y was dispersed an aqueous solution of 0.01M amoxicillin and then allowed stand for 1 h to obtain amoxicillin protected AgNPs/Zeo-Y. The final product was obtained by centrifugation.

2.6. Instrumentation

UV-Visible spectral studies were carried out using Shimadzu UV-Visible Spectrophotometer, Japan (Model UV-1800). The XRD patterns with diffraction intensity versus 2 θ were recorded in a JSO Debye Flex 2002 Seifert diffractometer using Cu Kα radiation (λ=1.5406 Å) from 10 to 80° at a scanning speed of 1° min^{-1}. X-ray tube voltage and current were set at 40 kV and 40 mA, respectively. Morphological and structural investigations were carried out using field emission scanning electron microscopy (FE-SEM, SU6600, Hitachi, Japan).

2.7. Antibacterial test

To evaluate the antibacterial properties of AgNPs/Zeo-Y and amoxicillin modified AgNPs/Zeo-Y, *Bacillus subtilis* and *Salmonella typhi* were selected as Gram-negative and Gram-positive bacteria, respectively. One has the ability to measure the effectiveness of an antibacterial agent by determining a zone of inhibition. A standard inoculum of the test organism with 1×10^7 colony forming units (CFU)/mL was swabbed onto the surface of a LB agar plate, AgNPs/Zeo-Y and Amoxicillin/AgNPs/Zeo-Y antibacterial agents were placed on the surface of agar. The plates were incubated overnight at 37°C, and the clear zones around the antibacterial agents were then measured. The above experiments were repeated thrice and the average values were taken.

3. Results and discussion

3.1. Characterization of AgNPs loaded Zeolite Y

The UV-Visible spectra of a) AgNPs/Zeo-Y, b) lemon juice, c) amoxicillin and d) AgNPs/Zeo-Y with amoxicillin are shown in Fig. 1. The silver nanoparticles loaded zeolite Y nanocomposite shows a silver nanoparticle peak at 420 nm due to surface plasmon resonance (SPR). The lemon juice not showing any peak and the amoxicillin peak was obtained at 270 nm. After the incorporation of amoxicillin drug into the silver nanoparticles loaded zeolite Y nanocomposite two major peaks were observed one at 360 nm and another one at 250 nm. Thus, these results confirm that amoxicillin drug was effectively attached to the silver nanoparticles loaded zeolite Y nanocomposite through ionic interaction.

The X-ray diffraction pattern of a) zeolite-Y and b) AgNps/Zeo-Y nanocomposite are shown in Fig. 2. As shown in Zeo-Y, the peaks at 31.04, 32.74, 33.24, 34.34, 35.94, 36.64, 37.76, 40.24, 41.74, 41.92, 52.80, 54.48, 56.54, 57.58, 58.74, 65.22, 66.54, 69.34, 71.10, 72.92, 75.96, and 77.82° are assigned for the presence of zeolite (JCPDS: 01-072-2344) [19]. In AgNPs/Zeo-Y, the characteristics peaks are due to the presence of silver chloride are attributed at 27.6, 32.05, 46.05, 54.6, 57.3, 67.4, 74.5 and 76.6° with plane of 111, 200, 220, 311, 222, 400, 311 and 420 reflections owing to the AgCl phase. These results confirm that the silver nanoparticles loaded within zeolite Y matrix.

The structural and morphology were confirms by FE-SEM images. The mesoporous material of zeolite has octahedral shaped particles with various sizes around 1 μm (Fig. 3(a)) [20]. From AgNPs loaded zeolite Y, the

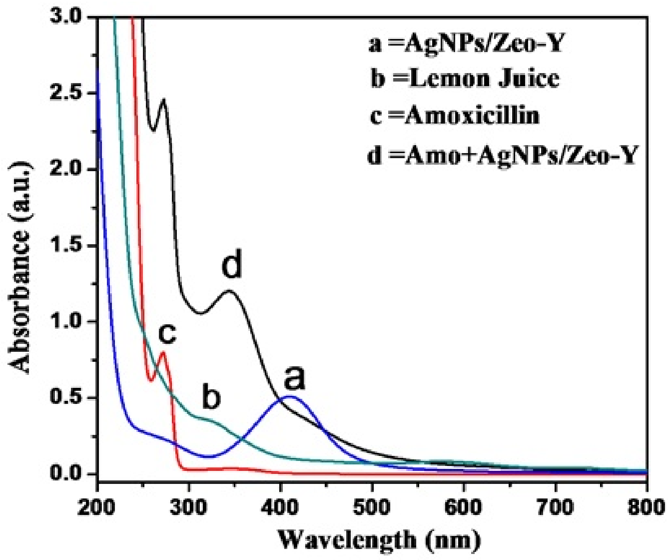

FIG. 1. UV-Visible spectrum of a) AgNPs/Zeo-Y, b) lemon, c) amoxicillin and d) AgNPs/Zeo-Y with amoxicillin

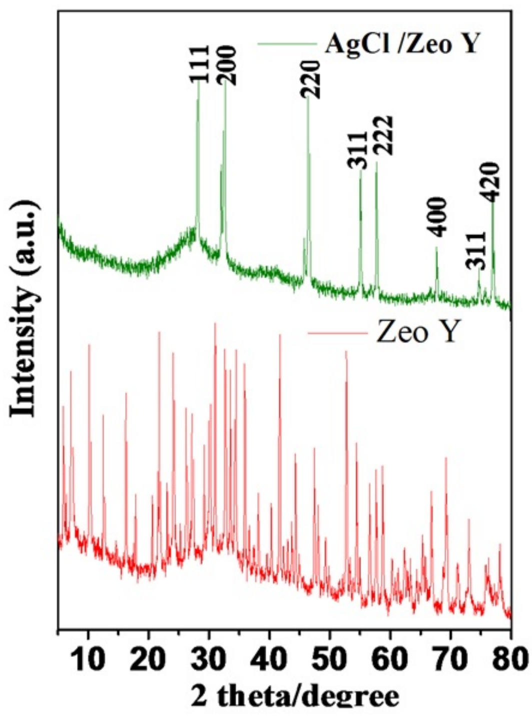

FIG. 2. XRD analysis a) zeolite Y and b) AgNPs loaded Zeo-Y

silver nanoparticles loaded on surface of mesoporous zeolite and also regular shaped silver nanoparticles and the size of the silver nanoparticles are roughly 0.1 μm as shown in Fig. 3(b). Therefore, the present method can be exploited for the effective loading of silver nanoparticles on zeolite surface.

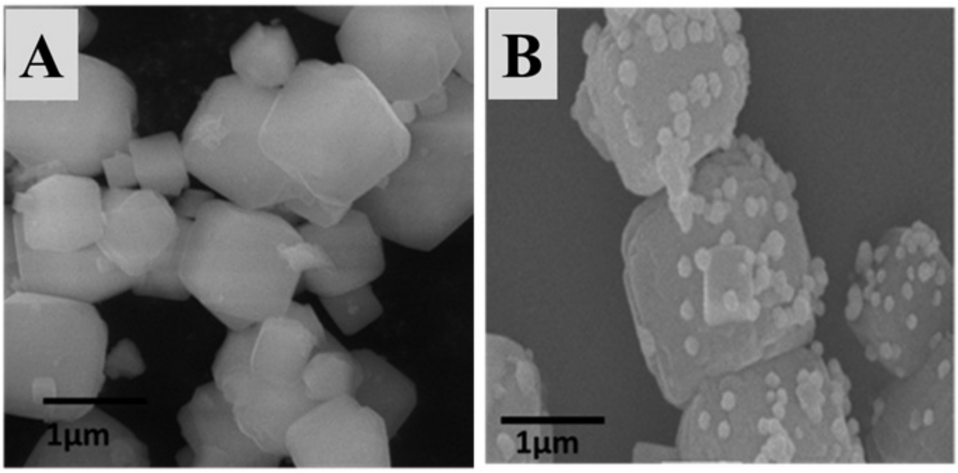

FIG. 3. FE-SEM images for Zeolite-Y (A) and AgNPs/Zeo-Y (B)

3.2. Catalytic activity of Zeolite Y loaded AgNPs

The catalytic activity of AgNPs/Zeo-Y was tested against the catalytic reduction of 4-Nitrophenol (4-NP) in the presence of $NaBH_4$. Upon the addition of $NaBH_4$ (Fig. 4(a)), the absorption peak of 4-Nitrophenol undergoes an immediate red-shift from 317 nm to 400 nm indicating the formation of 4-Nitrophenolate ions, evidenced by the visible color changes from light yellow to yellow green in color. After the addition of AgNPs/Zeo-Y using lemon juice nanocomposite, the absorption peak at 400 nm gradually decreased in intensity along with increase in absorption of a new peak at 300 nm, indicating the formation of 4-Aminophenol (4-AP). Under these reaction conditions, the reduction was completed within 10 and 5 mins for the addition catalytic amount 5 mg and 10 mg respectively (Fig. 4(b) and Fig. 4(c)).

The rate of reaction forb the reduction of 4-NP in presence of the AgNPs/Zeo-Y nanocomposite is due to decrease in absorbance values at 400 nm. Therefore, the rate constant of the reaction can be calculated using the following formula:

$$\ln C/C_0 = -kt, \quad (1)$$

where k is the constant, t is the reaction time; C and C_0 are the concentration of 4-NP at time t and 0, respectively. The concentration or borohydride used in the entire study was 0.1 M, which is large excess when compared to the concentration of 4-NP, thus the reduction reaction is considered to be pseudo first order so that the rate of the reaction depends primarily on the concentration of 4-NP. The rate of the reaction (k) obtained from the slope of the straight line was found to be 0.083 min^{-1} and 0.32 min^{-1} for 5 and 10 mg of catalyst used for the catalytic studies (Fig. 4(d)). The rate of the reaction increases with increasing of the catalyst concentration which is due to the increase of the catalytic activities.

3.3. Antibacterial activity

It is well established that silver nanoparticles modified substrates have shown an enhanced antimicrobial activity [12, 13]. In the present study, we studied the antimicrobial activity of silver nanoparticles, AgNPs/Zeo-Y and amoxicillin incorporated AgNPs/Zeo-Y against various Gram negative and Gram positive bacteria. Obviously, amoxicillin modified AgNPs/Zeo-Y showed a good antimicrobial activity against *Bacillus subtilis* and *Salmonella typhi.* The antimicrobial activity of AgNPs/Zeo-Y and Amoxicillin/AgNPs/Zeo-Y against various Gram positive and Gram negative bacteria as shown in Table 1.

4. Conclusion

In summary, the biogenic method was established to synthesize silver nanoparticle-loaded zeolite Y through an ion exchange approach followed green chemical reduction using lemon juice. This method is a fast and easy way to synthesize the AgNPs/Zeo-Y in large scale. The activity of AgNPs/Zeo-Y was studied, displaying excellent

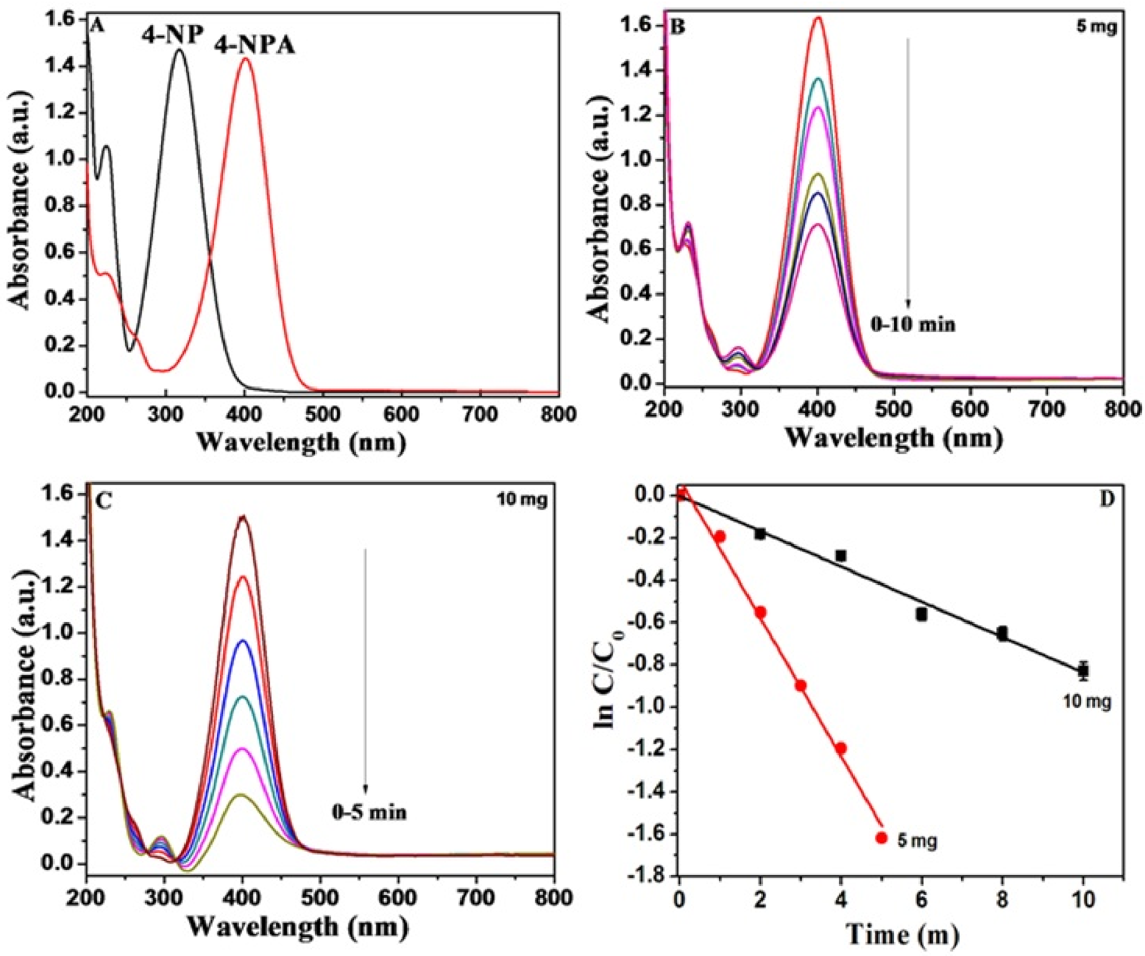

FIG. 4. UV-Visible spectra capturing the conversion of 4-NP to 4-AP upon reaction with $NaBH_4$(A), in presence of 5 mg and 10 mg of AgNPs/Zeo-Y (B), (C) and kinetic plot. Error bars designates the standard deviation for five independent measurements (D)

TABLE 1. Antibacterial activity of AgNPs/Zeo-Y and Amoxicillin/AgNPs/Zeo-Y against *Bacillus subtilis* and *Salmonella typhi*

Sample (mg/mL)	Gram Positive Bacteria					Gram Negative Bacteria				
	Bacillus subtilis					*Salmonella typhi*				
	a	b	c	d	e	a	b	c	d	e
AgNPs/Zeo-Y	2.2	6.5	7.3	16.4	-	3.3	6.5	8.4	20.5	-
Amoxicillin AgNPs/Zeo-Y	-	3.1	3.5	7.5	-	-	-	-	9.4	-

a = (25 μg/ml), b = (50 μg/ml), c = (75 μg/ml), d = (25 μg/ml (Standard) chloramphenicol), e = 50 μg/ml 0.1 % DMSO catalytic activity for the conversion of 4-NP to 4-AP. The amoxicillin-protected AgNPs/Zeo-Y system can be exhibited an enhanced antimicrobial activity as compared to AgNPs/Zeo-Y.

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