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Antibacterial Activities and Biofilm Inhibition of CdS Nanoparticles Prepared from Tri Substituted Imidazolium Ionic Liquids

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Abstract: ***In this paper, cadmium sulphide nanoparticles were synthesized by a simple and low temperature chemical method using cadmium acetate and thioacetamide precursor in a green solvent, the ionic liquid([EBMIM] BF_4, [EBMIM]PF_6) in a very short time scale. Highly substituted imidazolium based ionic liquids were synthesized and the structures were confirmed by spectral studies such as FT-IR,^{1}H-NMR and ^{13}C-NMR. The synthesized nanoparticles were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX),have been employed for the characterization of size, structure and morphology of the synthesized CdS nanoparticles. Energy dispersive X-ray spectroscopy (EDX) investigations reveal that the products are very pure and nearly stoichiometric. In addition, the antibacterial activity and bio film inhibition of the as synthesized CdS nanoparticles were also tested against S. aureus and P.aeruginosa pathogens by broth dilution method. The results indicate that anionic part of ionic liquids plays an important role in the formation, size and shape of nanoparticles. Nanoparticles synthesized in ionic liquids with PF_6^- are the most effective products against the tested bacterial strains compared with nanoparticles prepared in ionic liquids with BF_4^- anion. Compared with two different microorganism's gram negative bacteria is more effective than grampositive bacteria.***

Keywords: Ionic liquids, Cadmium Sulphide nanoparticles, Chemical method, Antimicrobial activity, Biofilm inhibition

1. Introduction

Metallic nanoparticles exhibit novel dimension-dependent properties leading to attractive applications in catalysis, optoelectronics and environmental remediation. Metallic nanoparticles of specific sizes and morphologies can be readily synthesized using various chemical and physical methods. Room temperature ionic liquids (RTILs) have been widely studied as a reaction media due to their unique physicochemical properties [1]–[3].CdS is one of the most important II-IV group semiconductors; the band gap is ~2.4 eV, having vital applications in many technical fields including mechanical and optoelectronic fields, and use in solar cells, and the photo degradation of water pollutants [4]. Especially, they have been extensively exploited to be used in biological systems, living organisms and drugs [5]. The preparation of CdS nanoparticles has become a very popular research area in recent years [6].Various methods are devoted for the synthesis of CdS nanoparticles namely a solgel template [7], microwave - solvothermal route [8], hydrothermal reaction [9], laser ablation [10], chemical bath deposition [11] and chemical method [12], [13]. Chemical method is a simple, less time consuming and inexpensive technique to obtain CdS nanoparticles. Our research focuses on to investigate the role of the different counter anion and same countercation of imidazolium ionic liquids in the formation, stabilization and morphology of CdS nanoparticles.

2. Experimental Section

2.1 Materials and Method

All chemicals were of AR grade. They were purchased from Merk, SD Fine Chemicals Limited and used without further purification. All the solvents and reagents were used as received and all reactions were run in oven-dried glassware. The homogeneity of the products was checked on TLC plates coated with silica gel-G and visualized by exposure to iodine vapors. 1-ethyl-3-methylimidazolium bromide ([EMIM] Br), 1-ethyl-2-butyl-3-methylimidazoliumbromide ([EBMIM] Br), 1-ethyl-2-butyl-3-methylimidazolium tetrafluoroborate ([EBMIM] BF_4), 1-ethyl-2-butyl-3-methylimidazolium hexafluorophosphate ([EBMIM] PF_6) was synthesized according to previous report [14]-[16]. ILs was characterized by FT-IR, ^{1}H - NMR and ^{13}C - NMR spectral studies. Nanoparticles were well characterized by powder X-Ray Diffraction (powder XRD), Scanning Electron Microscope (SEM) and Energy Dispersive X-ray spectroscopy (EDX).

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$and DMSO-d_6 on a Jeol JNN ECX 400P spectrometer. The IR spectra were obtained on a Varian 800 FT-IR as thin films or for solid samples. The X-ray diffraction (XRD) patterns were recorded on a Philips X-pert X-ray diffractometer with Cu Kα radiation (λ = 0.15406 nm) employing a scan rate of 1o/min in the 2θ range from 20 ^{o} to 80^o. Surface morphology and the distribution of particles were characterized by a LEO 1430VP scanning electron microscopy (SEM) using an accelerating voltage of 15 kV.EDX observations were prepared by transferring the particles, which were first dispersed in ethanol, to a glass substrate attached to the SEM stage. After the evaporation of ethanol from the substrate, the particles on the stage were coated with a thin layer of gold and palladium.

2.2 Synthesis of Cadmium sulphide Nanoparticles

In addition, 1.50 g of thioacetamide (TAA) was dissolved in 12.5 ml of distilled water, and 12.5 ml of IL. Then, the TAA solution was slowly added into the solution of cadmium acetate under magnetic stirring. The solution was refluxed approximately at 95°C for 60 minutes [2]. The formed

yellow color suspension was centrifuged to get the precipitate which was then washed three times with double distilled water and ethanol, respectively to remove the unreacted reagents and dried in an oven at 50°C for 24 h.

2.3Antimicrobial activity

Minimum inhibitory concentrations (MICs)
The minimum inhibitory concentrations of the CdS nanoparticle were determined by broth dilution method [17]. The strains were grown in Mueller Hinton broth to exponential phase with an *A*560 of 0.8, representing 3×10^8 CFU/ml. Different concentration (5, 10, 15 and 20 µg/ml) of the synthesized particles (1mg of particles in 1 ml of deionized water) were added on to separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 10^8 CFU/ml. Each MIC was determined from five independent experiments performed in triplicates. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% methanol were used as bacterial control, 4.5 ml of inoculated Mueller Hinton broth and 0.5 ml 7 % methanol served as a blank. The tubes were incubated at 37°C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at *A*560 nm.

2.4Biofilm formation inhibition of pathogenic bacteria

Effect of CdS nanoparticle on biofilm formation a modified microtitre plate assay [18] was performed to see the effect alkaloid extract on pathogenic bacteria biofilm formation. In brief, sterile, polystyrene microtitre plate wells were inoculated with 100 µl nutrient broth containing 10^7 CFU/ml and loaded with different concentrations of alkaloid extract (5-20 µg/ml). These plates were incubated in static condition at 37 ºC for 24 h. Controls wells were maintained with medium containing bacterial suspension. Then, medium in the wells was removed and washed with sterile phosphate buffer saline to remove loosely attached bacteria. Wells were stained with 150 µl of 0.25% crystal violet and incubated for 30 min. Further these wells were washed, air dried, bound stain was solubilized in 150 µl of 95% ethanol and the absorbance at 595 nm was recorded using the plate reader (Tecan Infinite M200, Switzerland).

3. Results and Discussion

3.1 Spectral studies of Ionic liquids

3.1.1 Synthesized 1-ethyl-3-methylimidazolium bromide [EMIM] Br
FT-IR (neat): 3,155, 3,105, 2,927, 2,857, 1,572, 1,460, 1,169, 837, 753, 620 cm^{-1}; **^{1}H-NMR (400 MHz, CDCl3):** δ 9.66 (s, 1H), 7.42 (t, 1H), 7.18 (t, 1H), 4.11 (t, 2H), 3.79 (s, 3H), and 1.27 (t, 3H). **^{13}C -NMR** (75 MHz) δ = 136.24, 123.59, 121.94, 44.91, 36.42, 15.46.

3.1.2 Synthesized 1-ethyl-2-butyl-3-methylimidazolium bromide [EBMIM] Br
FTIR (neat): 3,149, 2,960, 2,869, 1,658, 1,571, 1,168, 1,033 cm^{-1}; **^{1}H -NMR (400 MHz, CDCl3):** δ = 7.61 (d, 1H, J = 2.07 Hz), 7.60 (d, 1H, J = 2.04 Hz), 4.35 (t, 2H), 4.03 (s, 3H), 3.25 (t, 2H), 1.95-1.86 (m, 2H), 1.47-1.42 (m, 2H), 1.39-1.34 (m, 4H), 0.98-0.93 (m, 6H); **^{13}C -NMR (75 MHz)** δ = 136.85, 123.78, 121.22, 49.66, 35.90, 32.054, 29.16, 23.45, 22.33, 19.54, 13.51, 13.37.

3.1.3 Synthesized 1-ethyl-2-butyl-3-methylimidazolium tetrafluoroborate [EBMIM] BF_4 [IL-1]
FT-IR (neat): 3139, 2921, 2855, 1653, 1529, 1454, 1378, 1282, 1232, 1166, 1042, 724, 620, 517 cm^{-1}; **^{1}H NMR:** δ 7.567(d, 1H), 7.406(d, 1H), 4.226(q, 2H), 3.960(s,3H), 3.279(t,2H), 3.098-2.792(m,2H), 1.523-1.130(m,2H), 0.962-0.913(m,6H); **^{13}C NMR:** δ 148.04, 122.33, 123.80, 47.64, 39.83, 30.94, 19.31, 15.19, 13.71, 10.81

3.1.4 Synthesized 1-ethyl-2-butyl-3-methylimidazolium hexafluorophosphate [EBMIM] PF_6 [IL-2]
FT-IR (neat): 3660, 3399, 3170, 3124, 2967, 2877, 1574,1520,1464, 1386,1340,1288,1235, 1169,1112, 1084,1032, 875, 749, 656, 623, 559cm^{-1}; **^{1}H NMR:** δ 7.771(d,1H), 7.641(d,1H), 4.192(q, 2H), 4.125(s, 3H), 3.856(t, 2H), 3.071-3.033(m, 2H), 1.329-1.153(m, 2H), 0.943-0.906(m, 6H); **^{13}C NMR:** δ 136.85, 121.22, 123.78, 49.66, 35.90, 32.05, 29.16, 23.45, 19.54, 13.51

3.2 XRD Analysis

The XRD pattern of the as prepared CdS nanoparticle in [EBMIM] BF_4 and [EBMIM] PF_6 (figure 1) shows a hexagonal crystal structure. The major strong characteristic peaks of CdS particles are obtained at 2θ = 26.60, 36.06, 43.60, 47.76, 51.53 and 70.8, which are corresponding to crystal faces (100), (102), (110), (103), (112) and (211) of CdS respectively. All the reflection n peaks could be indexed to hexagonal, primitive, (JCPDS NO 41-1049). In addition to identification of the crystalline phases, XRD data were used to estimate size of the constituent crystallites by Scherrer equation [19].

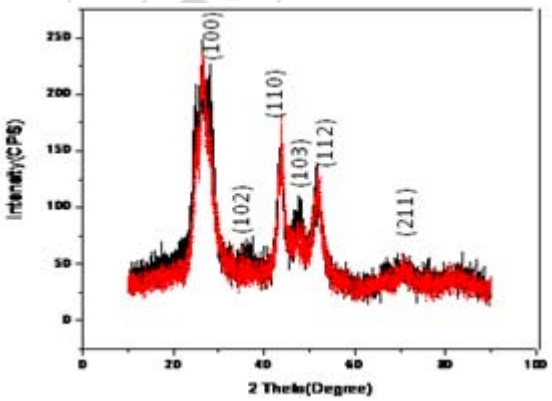

Figure 1: XRD patteren of CdS nanoparticle in [EBMIM] BF_4 and [EBMIM] PF_6

The average particle size, D, was determined using D = K λ /β Cosθ Where, λ is the wavelength of X-ray radiation (0.15406), K, the Scherrer's constant (K=0.9), θ the characteristic X-ray radiation (2θ = 44.30) and β is the full-width-half-maximum of the (220) plane (in radians). According to the full width at half maximum of the diffraction peaks, the average size of the particles could be estimated from the Scherer equation to be about 7.8 and 5.2 nm for CdS nanoparticle prepared in [EBMIM] BF_4 and

[EBMIM] PF_6respectively. Same cation ([EBMIM]) with different anionic part ($BF_4^-$and $PF_6^{-)}$ of the ionic liquids does not change the crystallite nature of the nanoparticles.

3.3 SEM analysis

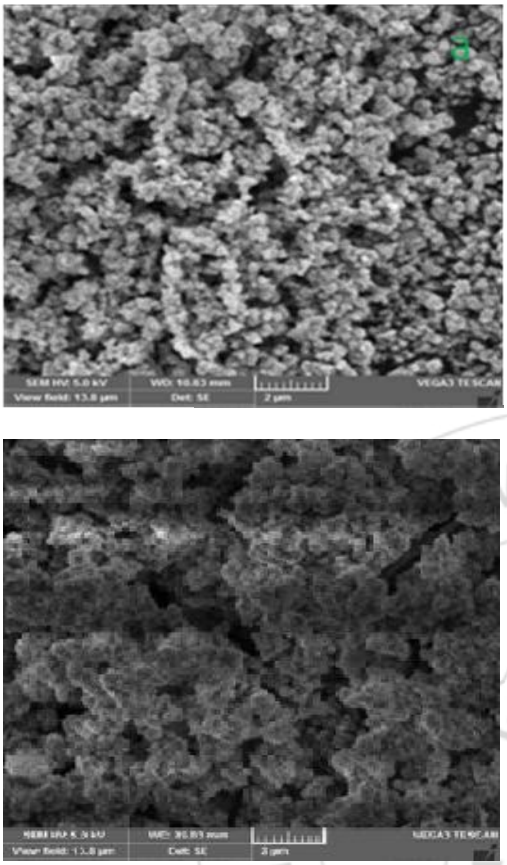

Figure 2: SEM image of as prepared CdS nanoparticles in (a) [EBMIM] BF_4 (b) [EBMIM] PF_6

Morphology of the as synthesized CdS nanoparticles in two different ILs was investigated by scanning electron microscope (SEM). Figure 2a and 2b shows the SEM image of CdS nanoparticle prepared in [EBMIM] BF_4 and [EBMIM] PF_6 respectively. Morphologies of nanoparticles can be explained by the assistance of IL. The already formed CdS get coated by IL because of electrostatic phenomena between the cations of IL and nuclei of CdS. It is evident from the figure 2a that the synthesized CdS nanoparticles in [EBMIM] BF_4 exhibit the morphology of spherical shaped particles. When the anion PF_6^- is introduced instead of BF_4^-, the morphology and size of CdS also tuned as flakesshaped particles.

3.4 EDX Analysis

The purity and composition of the products (CdS nanoparticles in [EBMIM] BF_4 and [EBMIM] PF_6) were studied by energy dispersive X-ray spectroscopy (EDX). The results are displayed in figure 3a-b. The other peaks in the figure corresponded to gold, palladium, and silicate which were due to sputter coating of the glass substrate on the EDX stage, and these were not considered in the elemental analysis of CdS nanoparticles. It is clear that the CdS nanoparticles prepared were sufficiently pure.

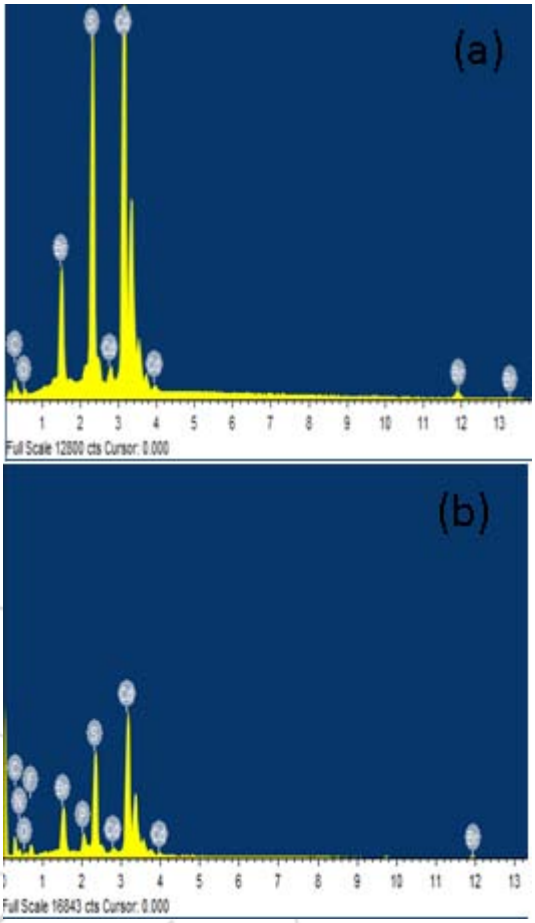

Figure 3: EDX pattern of as prepared CdS nanoparticles in (a) [EBMIM] BF_4 (b) [EBMIM] PF_6

3.5 Antimicrobial activity of CdS nanoparticles

The antibacterial activities of as synthesized CdS nanoparticles in two different ILs were screened with one gram positive (*Staphylococcusaureus*) and one gram negative bacteria (*Pseudomonasaeruginosa*) using broth dilution technique. Figure 4 shows the comparative account of the anti-bacterial activity of CdS nanoparticles synthesized in two different ILs ([EBMIM] BF_4 and [EBMIM] PF_6) with different concentrations.

In view of the results, it appeared that in both the pathogens antimicrobial activity increases with increase in concentrations. Nanoparticles synthesized in ionic liquids with $PF_6^-$are the most effective products against the tested bacterial strains compared with nanoparticles prepared in ionic liquids with BF_4^- anion. Compared with two different microorganism's gram negative bacteria is more effective than gram positive bacteria.

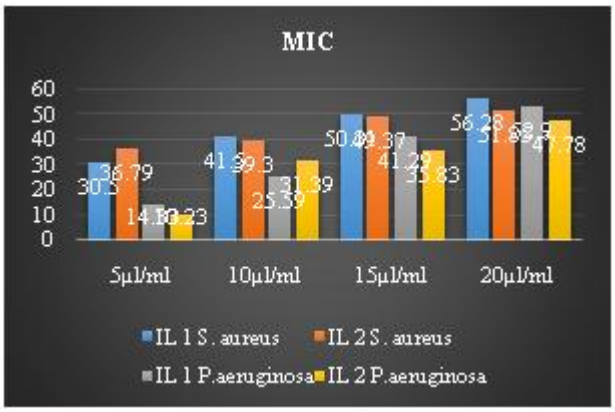

Figure 4: Comparison of MIC values of synthesized nanoparticles

3.6 Effect synthesized particles on inhibition of biofilm formation on pathogenic bacteria

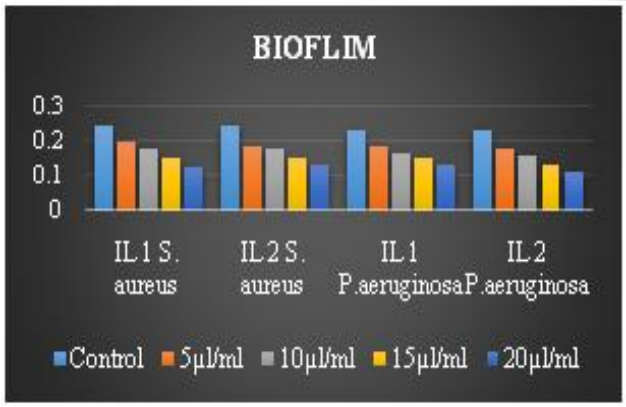

Figure 4: Comparison of Biofilm formation of synthesized nanoparticles

Static biofilm quantification assay was performed to evaluate the effect of CdS nanoparticles in [EBMIM] BF_4 and [EBMIM] PF_6on *S. aureus*and *P.aeruginosa*biofilm formation. Figure 5 showsthe comparative account inbiofilm formation between the control (i.e., no particlesaddition) and the culture containing particles (5, 10, 15 and 20µg/ml). The microtiter plates showed that biofilm formation for the experimental group (i.e. cultures with synthesized particles) was 39–56% less than the amount of formation in the control. The results suggested that the biofilm formation of both the bacteria were inhibited by the addition of synthesized CdS in[EBMIM] BF_4 and [EBMIM] PF_6 particles in microtiter plates.

4. Conclusions

Among the different ionic liquids, imidazolium based room temperature ionic liquids have attracted considerable attention due to their extended hydrogen bond network, highly structured and the ability of ions to form classical ion aggregates. Nowadays, these advantages would encourage the ionic liquids to act as template or capping agent in the preparation of well-defined and extended ordering of nanostructures. Therefore, in the present work, an attempt was made to synthesize two imidazolium based ionic liquids such as [EBMIM] BF_4 and [EBMIM] PF_6. Under the given optimal conditions, synthesis of two ionic liquids gave excellent yield and the products were identified and purified by column chromatography methods and ` by FT-IR, 1H-NMR and 13C-NMR studies.

Towards the synthesis of nanostructured CdS nanoparticles, template solvents, imidazolium based ionic liquid assisted chemical method was explored. The main goal of this work was to investigate the role of the different counter anion of imidazolium based ionic liquids in the formation, stabilization and morphology of these nanoparticles. The XRD pattern of CdS nanoparticles showed that the materials to be at the nanometeric size regime with hexagonal primitive crystal structure and no other phases were noticed.

SEM observations of CdS nanoparticles exhibit the different structures of spherical and flakes like shaped morphologies for [EBMIM] BF_4 and [EBMIM] PF_6respectively. To screen the bacteriostatic effects of the nanoparticles were conducted on the bacteria of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The result shows CdS in [EBMIM] BF_4 and [EBMIM] PF_6 particles inhibited growth of gram-negative bacteria (Staphylococcus aureus) than gram-positive bacteria (pseudomonas aeruginosa). The results also, suggested that the biofilm formation of all bacteria were inhibited by the addition of synthesizedCdS nanoparticles in IL-1 and IL2 in microtiter plates.

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