Synthesis of Copper–Polystyrene Nanocomposite Particles Using Water in Supercritical Carbon Dioxide Medium and Its Antimicrobial Activity

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ABSTRACT: Copper-encapsulated polystyrene nanocomposite particles were prepared through *ex situ* dispersion of Cu nanoparticles into monomer droplets and subsequent polymerization using water in supercritical carbon dioxide (water-in-sc-CO_2) at 70°C. First, colloidal dispersion of copper nanoparticles was synthesized by chemical reduction of copper chloride ($CuCl_2$) using sodium borohydrate ($NaBH_4$) as reducing agent. Colloidal dispersion of copper nanoparticles was added slowly during the polymerization of styrene using water-in-sc-CO_2 medium at 70°C and 20.68 MPa. Cu nanoparticle encapsulated polymer particles were characterized by UV, X-ray diffraction, thermogravimetric analysis, SEM, and TEM. Cu nanoparticles were uniformly distributed inside the polymer matrix during the polymerization process. This work represents a simple way to prepare a variety of metal nanoparticles encapsulated polymer particles using water-in-sc-CO_2 medium. The Cu/polystyrene nanocomposite particles exhibit antimicrobial activity against a number of bacteria. The current work represents a simple, cheap and universal way to prepare a variety of metal–polymer nanocomposite materials. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 1027–1033, 2011

Key words: supercritical carbon dioxide; nanocomposite; antimicrobial activity

INTRODUCTION

Polymer/metal nanocomposites synthesis is an ongoing challenge in materials research. A number of techniques are being used currently, but till now, no general, effective, and green preparative method is available. Polymer/inorganic materials nanocomposites have generated extensive interest in view of their novel properties such as enhanced conductivity, mechanical toughness, optical, and catalytic activity.[1–4] Different approaches have been used to synthesize polymer/metal nanocomposites. The *in situ* synthesis of metal nanoparticles in polymer matrices had been widely used.[5]

Supercritical carbon dioxide (sc-CO_2) has attracted extensive interest as a polymerization and processing medium, primarily driven by the need to replace conventional solvents with more environmentally benign and economically viable systems.[6] It is an attractive substitute for the organic non polar solvents for a variety of chemical reactions.[7–10] It has many advantages over the conventional organic solvents because of its nontoxic, nonflammable, inexpensive, high diffusivity, and compressibility nature.[11] Moreover, the pure product can easily be separated by depressurizing the carbon dioxide gas used. Although many reagents/chemicals are insoluble in CO_2, but they can easily be emulsified in sc-CO_2 by selecting proper fluorinated or siloxane-based surfactants/stabilizers.[12,13] DeSimone et al.[14] pioneered the dispersion polymerization of methyl methacrylate in sc-CO_2. They used a CO_2-soluble fluorinated homopolymer [poly(dihydroperfluorooctyl acrylate)] as the stabilizer for the polymerization system.

It is interesting and challenging to prepare polymer/metal nanocomposites in which the smaller nanosized metal particles are dispersed in polymer nanospheres. Zhang et al.[15] synthesized polystyrene nanospheres immobilized with Copper nanoparticles by using compressed CO_2. This method describes the advantages that the polymer chains collapse and entangle in the presence of compressed CO_2, which acts as antisolvent. The metal nanoparticles and polymers can be precipitated simultaneously from the micellar solutions by easy controlling of CO_2 pressure. Yang et al.[6] reported a novel strategy to synthesize hybrid metal–polymer nanocomposites based on the *in situ* free radical suspension and bulk polymerizations. An organometallic precursor complex was dissolved in a liquid monomer phase before polymerization, where upon the precursor

molecules are immobilized inside the polymer matrix during its formation.

Horsch et al.[16] reported the preparation of clay/polymer nanocomposites in sc-CO_2. Polydimethylsiloxane (PDMS) was used as a stabilizer for the dispersion of the nanoclays. The nanoclays can be delaminated by the sc-CO_2 process, and the extent of dispersion is dependent on the CO_2-philicity of the nanoclay. Wong et al.[17] prepared gold nanoparticle composites supported on silica using sc-CO_2. Gold nanoparticles can easily be incorporated into polyamide, polypropylene, and poly(tetrafluoroethylene) in sc-CO_2.

Synthesis of nanoparticles has been investigated widely because of their effective application in catalysis and antimicrobial activity. The antimicrobial potential of nanoparticles (Ag, Cu, etc.) is a subject of great interest to chemists, biologists in the recent years. Numerous methods for the preparation of metallic nanoparticles have been reported. The synthesized metal nanoparticles cannot be directly used for the antimicrobial purpose without any support as the amount of metal nanoparticle is very small for a particular application. Their application varies widely on their physical properties such as apparent density, surface area, and morphology, which are strongly related to the preparation method and the synthesis medium.

Authors herein wish to report one simple, effective, and green preparation method for the synthesis of copper/polystyrene nanocomposite particles using water in sc-CO_2 medium. The antimicrobial activity of these nanoparticles encapsulated polystyrene particles that were tested against a number of bacterial strains.

EXPERIMENTAL

Materials

Copper chloride ($CuCl_2$) (Aldrich), sodiumborohydrate ($NaBH_4$) (Merck), and PDMS (Fluka) were used as received. Styrene (Merk) was washed with 10% NaOH solution and then double distilled water to make inhibitor free. Azobisisobutyronitrile (AIBN) (Allied Industries, Bombay) was recrystallized twice from methanol. Carbon dioxide (SCF/SFE Grade) (99.99%pure) (Rass Chryogenics) was used as received.

Apparatus

All experiments were conducted in a 60-mL high-pressure stainless steel reactor (SCF-System, Reaction Eng, Korea). The schematic diagram of the apparatus is shown in Figure 1. The SCF reactor is connected with a high-pressure CO_2 cylinder, one high-pressure metering pump, an efficient cooler, stirrer, and thermocouple with temperature controller. There is a provision for continuously addition of
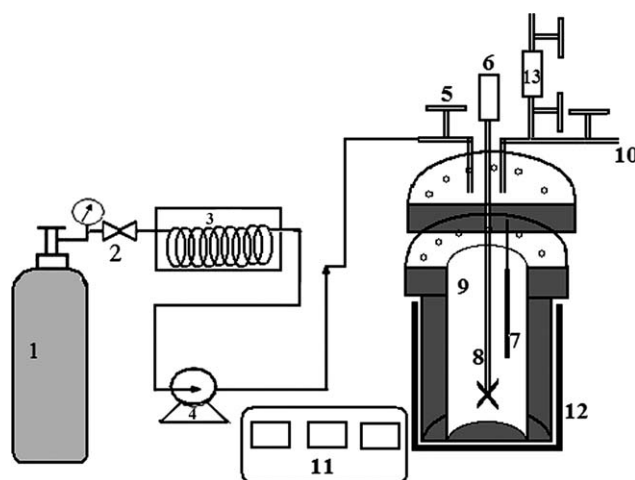

Figure 1 Schematic diagram of the apparatus. (1) Carbon dioxide cylinder; (2) back pressure valve; (3) refrigeration unit; (4) high-pressure liquid pump; (5) pressure valve; (6) motor for mechanical stirrer; (7) heating probe; (8) mechanical stirrer; (9) SFE vessel; (10) vent; (11) digital display unit; (12) heating jacket; (13) specially designed dropper.

reactants to the reactor during the reaction at high pressure (13 in Fig. 1). The pressure inside the reactor can be raised up to 42 MPa. The pressure inside the reactor is measured with a pressure transducer.

Procedure

Synthesis of Cu nanoparticles in supercritical carbon dioxide

In a typical reaction, 25 mL of $CuCl_2$ solution with a concentration of 0.03 mol/L and PDMS (2 mL) were mixed thoroughly in a round-bottomed flask. The whole mixture was then purged into the SCF reactor. The pressure inside the reactor was raised up to 20.68 MPa, and the temperature was set at 45°C. When the desired temperature and pressure is attained, 8 mL sodium borohydrate ($NaBH_4$) solution of concentration 0.03 mL/L was added slowly with a specially designed feeder (13 in Fig. 1) into the $CuCl_2$ solution. The reaction was continued for 4 h at a rotational speed of 900 rpm and at 20.68 MPa. After depressurizing the CO_2, the brown colloid containing copper nanoparticle was collected. The reaction was repeated by varying the reaction pressure up to 23.44 MPa.

Synthesis of Cu/polystyrene nanocomposite particles

In a typical reaction, PDMS and AIBN were mixed thoroughly in double-distilled water in a round bottom flask. To this mixture, styrene monomer was added with vigorous stirring for the preparation of pre emulsion. The pre-emulsion was then purged into 60 mL stainless steel reactor. The reactor was filled with CO_2, and the pressure inside the reactor

was raised up to 20.68 MPa. The temperature inside the reactor was set at 75°C and stirred at a rotation speed of 900 rpm. After half an hour, the stable dispersion of Cu nanoparticles (0.5% V/V of dispersed Cu nanoparticle solution with respect to monomer) was added drop wise to the reaction mixture. The concentration of Cu nanoparticles in the nanocomposite was varied from 0.5 to 1.5% (V/V of the stable dispersion with respect to monomer). The reaction was continued for 8 h. After polymerization, the reactor was depressurized, and the milky white emulsion was separated from the reactor. The emulsion was precipitated out in methanol and washed thoroughly with water and ethanol for several times. The whole reaction was performed at various pressures up to 23.44 MPa. Each reaction was repeated twice to obtain the reproducible data.

Characterization

The UV–vis absorption spectra of the dispersed nanoparticles in water were recorded in the range 300–900 nm using Hitachi U-2001 UV–vis spectrophotometer. Powder X-ray diffraction (XRD) data were collected on a Rigaku Miniflex X-ray diffractometer with a scanning rate of 0.05 degree/s in 2θ ranges from 10 degree to 90 degree. The morphology of copper nanoparticles and nanocomposite were determined by using JEOL, JEMCXII transmission electron microscopy (TEM) at an operating voltage of 80 kV and a Jeol-Jsm-6390LV scanning electron microscope (SEM). For SEM analysis, samples were mounted on an aluminum stub using an adhesive carbon tab and were coated with platinum to a thickness of 200 Å. For TEM analysis, two to three drops of the emulsion were transferred onto a 3 mm diameter carbon coated copper grid. The solvent was allowed to evaporate at room temperature before loading the sample in the microscope. Conventional bright field imaging was used to observe the particle morphology with diffraction patterns. Molecular weight of the polymer was determined by a modular Waters 600 gel permeation chromatograph (GPC) with ultrastyragel columns of linear, 10^3 and 10^4 Å porosities, and a 410 differential refractometer. Tetrahydrofuran (THF) was used as a mobile phase with a flow rate of 1.0 mL/min. Thermal analysis was carried out using (Shimadzu TGA-50) thermogravimetric analyzer. A preweighted amount of polymer (mass 10 mg) was loaded in a pan, and the heating speed was maintained at 10°C min^{-1} under nitrogen atmosphere.

Antimicrobial activity test

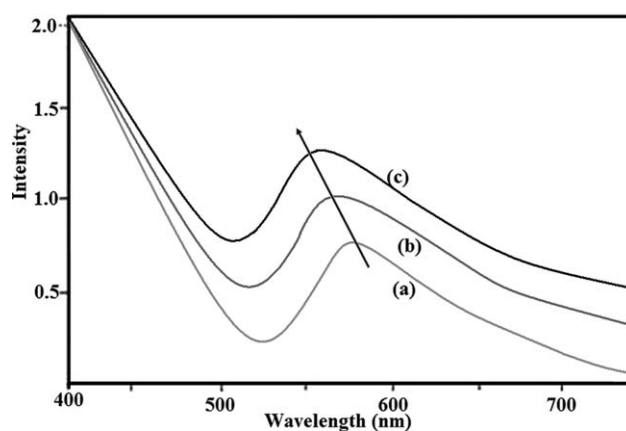Four kinds of bacterial strains, that is, *Pseudomonas fluorescens* BS3, *Bacillus circulens* BP2, *Eschericia coli*

Figure 2 UV–vis spectra of Cu nanoparticles at (a) 23.44 MPa, (b) 22.06 MPa, and (c) 20.68 MPa.

DHSα, and *staphylococcus aureus* strain were used as test organisms for the experiment. The test organisms were grown on nutrient agar plates by evenly spreading over the entire surface of the agar plates. After the agar surface has dried, 50 μL of the test samples was placed on each bacterial growth plates. Triple parallel experiments were carried out for each sample. Antimicrobial activity was determined by measuring the diameters of the zone of inhibition to the nearest millilmeter of the well on the plate. The wider the zone surrounding the well, the susceptibility of the test organism is more.

RESULTS AND DISCUSSION

Synthesis of copper nanoparticles

It is well known that weak intermolecular forces, such as van der Waal attraction and π–π interaction etc. contributes to the aggregation of nanoparticles.[18] Water-in-sc-CO_2 offers an excellent medium to synthesize nanoparticles. In our experiment, Cu-nanoparticles were formed by the reduction of $CuCl_2$ with $NaBH_4$ in the water-in-sc-CO_2 medium. Water-in-sc-CO_2 acts as a protective agent, and PDMS acts as stabilizer. After the formation of nanoparticles, the CO_2 was depressurized, and finally a stable dispersion of Cu-nanoparticles in water was obtained. The dispersion was kept at room temperature for observing stability of the dispersion and found to be stable up to 60 days. UV–vis spectra (Fig. 2), WAXD diffractogram (Fig. 3), and the TEM micrograph (Fig. 5) confirmed the formation and dispersion of Cu nanoparticles.

Synthesis of copper/polystyrene nanocomposite

Nanoparticles have very high-chemical activity due to its high-active surface area. Polymer molecules are very good stabilizer for the stabilization of the
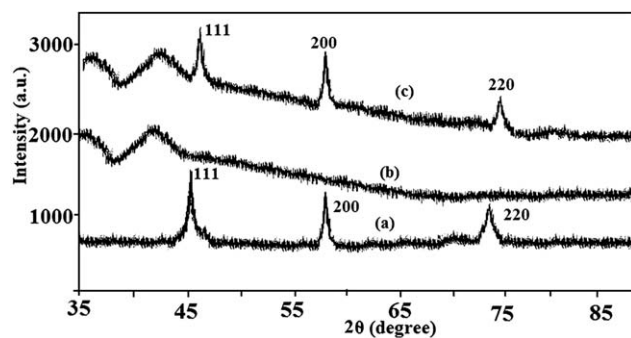
Figure 3 XRD pattern of the samples. (a) Cu-nanocomposite, (b) pristine polystyrene, and (c) Cu/polystyrene nanocomposite.

metal nanoparticles.[19] By incorporating nanoparticles into polymer matrix, it is possible to create reliable protection against corrosion and other undesirable factors.[20]

The Cu nanoparticles are well dispersed into the microemulsion of the monomers in the water-in-sc-CO_2 medium in the presence of PDMS stabilizer. The monomers interact with the Cu nanoparticles forming some active monomer droplets. The initiator added to the mixture undergoes decomposition at 75°C and generate the free radical on the active monomer droplets. These active monomer droplets contain the Cu nanoparticles at the center and undergo free radical polymerization on the surface of the nanoparticles. Thus, the nanoparticles are encapsulated into the polymer particles [Fig. 5(b)]. Stabilization of nanoparticles in the polymer matrix is affected by the polymer nature and its functional groups. The excess fragments of polystyrene molecules aggregate the nanoparticles to enhance the stability of the nanoparticles. More is the electron donor properties of the polymer functional groups, the stronger is their adhesion to dispersed phase particles.[19] In case of polystyrene, the aromatic π-electrons interact with the atoms of the metal surface layer, which increase the stability of the nanoparticles in the Cu/polystyrene nanocomposite.

UV–vis spectral analysis

The analysis of the various samples showed the absorption peak in a range of 580–600 nm, which confirms the formation of Copper nanoparticles.[21] A characteristic "blue-shift" was observed from the analysis with the decrease in the pressure of CO_2 (23.44–20.68 MPa) in the reaction (Fig. 2). This particular "blue-shift" implies that, with the decrease in pressure, the particle size of Cu nanoparticle decreases. At higher pressure, the concentration of the fluid (sc-CO_2) increases, which leads to the formation of larger particle size.

XRD analysis

The X-ray diffraction (XRD) analysis of the synthesized Cu nanoparticles shows a cubic crystal structure. The major strong characteristic peaks of Cu nanoparticles are at $2\theta = 46.16°$, $57.28°$, and $73.74°$, which were in consistent with crystal faces of (111), (200), and (220) of Cu. All these reflection peaks could be indexed to face-centered cubic copper. Cu nanoparticles prepared using water-in-scCO_2 did not change the crystalline structure of neat Cu nanoparticles. The lattice constant is calculated to be 3.614 Å, which is in good agreement of pure copper crystals. The WAXD patterns of pure Cu nanoparticle (a), pristine polymer (b), and polymer/Cu nanocomposite (c) are shown in Figure 3. The XRD pattern confirms the proper incorporation of Cu nanoparticles into the polymer microparticles, which can be further confirmed in TEM images.

The crystallite size distribution is determined from the analysis of line broadening method using Schrrer equation.[20] According to the full width at half-maximum of the diffraction peaks, the average size of the particles could be estimated from the Scherrer equation to be about 6.3 nm [reaction conditions: $t = 40°C$, $c(CuCl_2) = 0.033$ mol/L]. These results are consistent with the TEM images.

SEM analysis

The SEM images of the pristine polymer (a) and the copper–polystyrene nanocomposite particles (b) are as shown in Figure 4. The morphology of the pristine polymer particle and Cu/polymer nanocomposite particles is different. In the pristine polymer, the polymer particles are interlinked like foam structures. It is observed that the particle formation in the nanoparticle encapsulated polymer particle is clearer than the pristine polymer. Cu nanoparticles inside the monomer droplets enhance the particle formation for which discrete particle formation is observed in the Cu/polystyrene nanocomposite particles. This is possibly due to the electronic interaction between Cu and unsaturated styrene monomers.
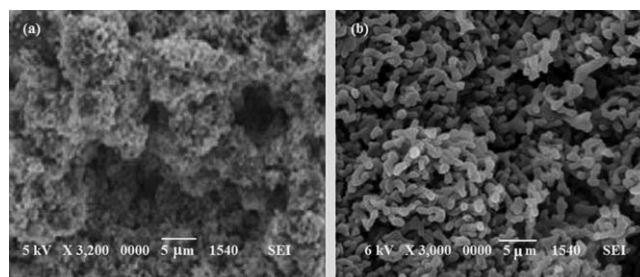

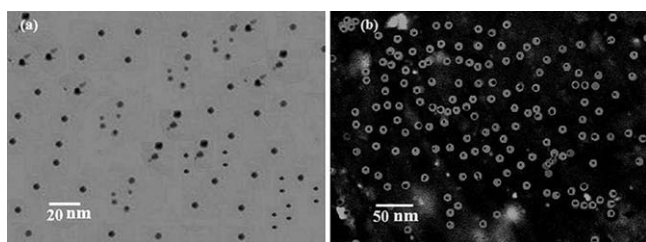**Figure 4** SEM images of (a) pristine polymer and (b) Cu/polystyrene nanocomposite.

Figure 5 TEM images of (a) Cu nanoparticles and (b) Cu-polystyrene nanocomposite particles.

TEM analysis

TEM image of the prepared Copper nanoparticles is shown in Figure 5. The representative TEM images demonstrate the homogeneous dispersion of Copper nanoparticles in the medium. Cu nano particles are spherical in shape with a smooth surface morphology. The TEM image of the Copper nanoparticle yielded an average size of 7 nm. This value is in good agreement with that of the XRD analysis.

TEM image also shows that the produced nanoparticles are more or less uniform in size and shape. The Copper nanoparticles are encapsulated into the polymer particles, which can be clearly observed in the Figure 5(b). No bare Cu nanoparticles left out within the polymer matrix. The average size of the Cu/polymer nanocomposite particle is 20 nm.

GPC analysis

The molecular-weight distribution and polydispersity indices of the polymer samples were obtained from gel permeation chromatographic (GPC) data. All the data are summarized in Table I. The copper nanoparticles present in the composites were separated by making a solution in THF and centrifuging at 5,000 rpm for 1 h. The nanoparticles settled down, and the clear solution was collected for polymer molecular weight determination. Number average molecular weight (M_n) of the polymer was found to be in the range of (43,000–45,000 g/mol). The polydispersity indices of the polymers are observed in the range of (1.26–1.34). From these GPC data, it can be confirmed that the molecular weight and the polydispersity indices of the polymer nanocomposite are independent of the percentage of nanoparticles pres-
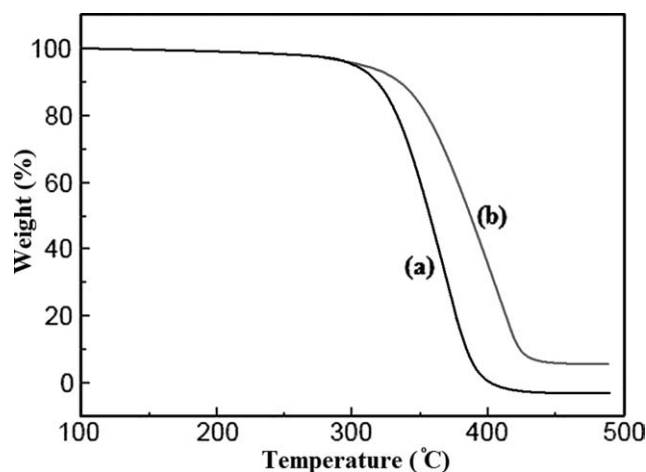

Figure 6 TGA thermogram of the samples: (a) pristine polymer and (b) Cu/polystyrene nanocomposites.

ent in the composite. In all the cases, the yield of the polymer was found to be above 80%.

TGA analysis

The thermal stability of the polymer was studied by thermogravimetric analysis (TGA). TGA curves (residual weight percentage versus temperature) for pristine polymer (a) and for the metal polymer nanocomposite (b) are shown in the Figure 6. The decomposition temperature of the pristine polystyrene is 320°C, and the Cu/polystyrene nanocomposite is 350°C. Evidently, the decomposition onset for Cu/Polystyrene nanocomposite shifts to higher temperature compared to pristine polystyrene. This shows that the thermal stability of the polymer/metal nanocomposite is higher than the pristine polymer.

Antimicrobial activities

The bacteriostatic effects of the Cu/polymer nanocomposite particles were studied against four kinds of bacterial strains, that is, *Pseudomonas fluorescens* BS3, *Bacillus circulens* BP2, *Eschericia coli* DHSα, and *staphylococcus aureus* strain. It is clear from Figure 7 and Table II that the test samples with a Cu nanoparticles concentration 1% Cu nanoparticles (c) and 1.5% Cu nanoparticles (d) show inhibitory effect against all the four bacterial strains. The sample with 1.5% Cu nanoparticles concentration show the highest degree of inhibition against all the four kinds of bacterial strains for which the diameter of zone of inhibition is more. The sample with no Cu nanoparticles (a) did not show any antimicrobial activity toward the bacterial strains, samples with 0.5% Cu nanoparticles show a very little affect against the test organism, and therefore the inhibitory zone of this sample is very narrow. The test samples (a) and (b) show no antimicrobial activity against the test
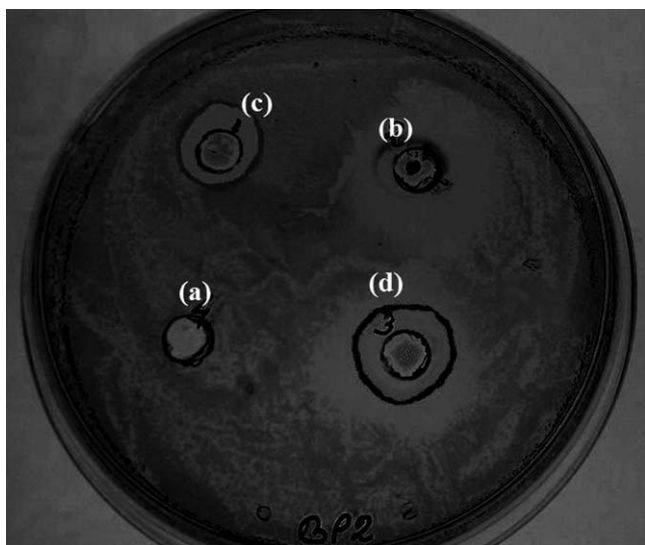
TABLE I
Molecular Weight of the Cu/Polymer Nanocomposites

SI no	Polymer	Mol. weight (g/mol) (M_n)	Polydispersity index	Yield (%)
1	Pure polystyrene	44,000	1.31	80
2	0.5% Cu/polystyrene	43,000	1.26	81
3	1% Cu/polystyrene	44,000	1.34	85
4	1.5% Cu/polystyrene	45,000	1.30	82

Figure 7 Antimicrobial test against the bacterial strain *Bacillus circulens* BP2. (a) Pure polystyrene particle, (b) Cu-polystyrene nanocomposite (0.5% Cu), (c) Cu-polystyrene nanocomposite (1% Cu), and (d) Cu-polystyrene nanocomposite (1.5% Cu).

organism. Thus, the test samples (c) and (d) exhibit good bacteriostatic effect on the tested bacteria.

It has been proposed that the bacterial DNA loses its replication ability once the bacteria have been treated with copper nanoparticles.[22] Other studies have shown evidence of structural changes in the cell membrane as well as the formation of small electron-dense granules formed by silver. Copper nanoparticles when come in contact with the bacteria, it destroys the cell walls of the bacteria showing its antimicrobial activity.[23]

The bar diagram (Fig. 8) shows the antimicrobial activity toward the four different kinds of bacterial strains. The inhibitory effect of the samples with 1% Cu nanoparticles (c) and 1.5% Cu nanoparticles (d) is shown in the diagram. It is clear from the above set of tests that *Bacillus circulens* BP2 bacteria is very sensitive toward the Cu-encapsulated polystyrene nanocomposite particles, and *Eschericia coli* DHSα shows the lowest degree of inhibition.
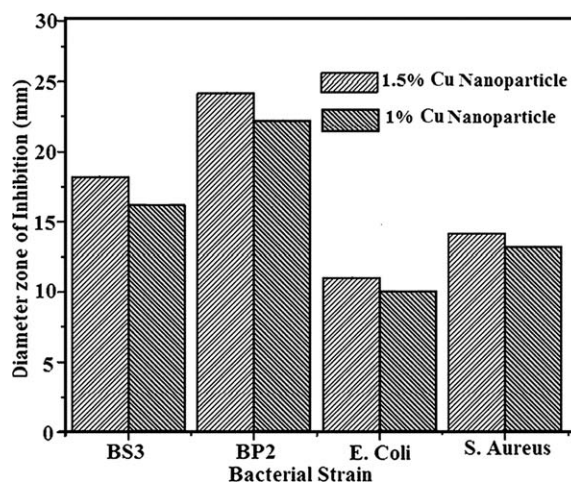

Figure 8 Bar diagram for the antimicrobial activity.

CONCLUSIONS

In summary, this work describes an easy method to synthesize the copper/polystyrene nanocomposites by *ex situ* addition of Cu nanoparticles during the polymerization. The size of the nanoparticles can be easily controlled by adjusting the pressure inside the reactor. The average size of the Cu nanoparticles was found to be 7 nm. The metal nanoparticles were uniformly distributed inside the polymer particles, and the inclusions of the metal precursor have no significant influence on the polymerization process. Moreover, this method has some potential advantages for applications as it is simple, timesaving, and the solutions can be recycled. The polymer nanocomposite particles show thermal stability upto 350°C, whereas the pristine polymer shows stability up to 320°C. Molecular weight of the polymer particles is found to be in the range of (43,000–45,000 g/mol). The antimicrobial activity of the copper/polystyrene nanoparticles was tested against four different bacterial strains, *Pseudomonas fluorescens* BS3, *Bacillus circulens* BP2, *Eschericia coli* DHSα, and *staphylococcus aureus*. Among all the bacterial strains, *Bacillus circulens* BP2 is very sensitive toward the Cu-encapsulated polystyrene nanocomposite particles.

TABLE II
The Antimicrobial Activity of the Cu-Polystyrene Nanocomposite Against Bacteria

		*Average diameter zone of inhibition (mm)			
SI. No	Micro organisms	0% Cu-(pure polystyrene)	0.5% Cu-polystyrene	1% Cu-polystyrene	1.5% Cu-polystyrene
1	*Pseudomonas fluorescens*	0[b] (0)[a]	9[b] (0.1)[a]	13[b] (0.2)[a]	19[b] (0.2)[a]
2	*Bacillus circulens*	0 (0)	11 (0.2)	16 (0.2)	24 (0.2)
3	*Eschericia coli*	0 (0)	7 (0.1)	8 (0.1)	10 (0.1)
4	*staphylococcus aureus*	0 (0)	4 (0.2)	10 (0.2)	0.1)

* Average diameter zone is reported from the average of three experiments, and the value in the bracket shows standard deviation.
[a] Standard deviation.
[b] Average diameter.

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