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Silver Nanoparticles in Polyacrylamide and Hyperbranched Polyamine Matrix

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Silver nanoparticles have been prepared in a polyacrylamide (PA) matrix, as well as in the presence of a hyperbranched polyamine/polyacrylamide combined system (HB-PA) by using a reductive technique. The stability of colloidal solution of silver nanoparticles is higher (5 months) in combined matrix compared to PA alone (4 months). The prepared silver nanoparticles were characterized by different spectroscopic and analytical techniques such as FTIR, UV-visible, X-ray diffraction, TEM etc. TEM and XRD studies confirmed the formation of well-dispersed nanoparticles with an average size of 9.91 nm and 8.5 nm for PA and HB-PA matrices, respectively. The antibacterial activity of silver nanoparticles in both the matrices was tested against *Bacillus Subtilis* bacteria by using the diffusion disc technique. The result shows that the antibacterial activity of the active agent, Ag(0) is a little higher in the case of HB-PA system. The dielectric constant of the matrices decreases with an increase in frequency, but the values increase with an increase of concentration of silver nanoparticles in PA matrix.

Keywords: silver nanoparticle; hyperbranched polyamine; polyacrylamide; s-triazine; dielectric property; antibacterial activity

1 Introduction

Nanotechnology is the manipulation or self-assembly of individual atoms, molecules, or molecular clusters into structures to create materials and devices with new or vastly different properties. The word nanotechnology is generally used when referring to materials with the size of 0.1 to 100 nm. However, it is also inherent that these materials should display different properties from bulk (μm and larger size) materials as a result of their size differences. These differences include physical strength, chemical reactivity, electrical conductance, magnetism, optical effects, etc. Nanoparticles were used as dye materials in ceramics by ancient people (1).

Silver is one of the most widely used noble metals that find their applications in its colloidal or nanocrystalline form in many applications. These include as a dopant in a dielectric medium for the enhancement of optical properties (2), in biological applications where the use of colloidal silver for medicinal purposes is an age-old practice (3–5). Silver metal particles in nanometer size range have attracted considerable interest in recent years, as they have tremendous applications in the area of biomedical, catalysis, optoelectronics, etc. (6) due to their unique size dependent properties. Thus, effort can be made to prepare stable nanoparticles of this metal with control size in a suitable matrix.

Polymer coated functionalized noble metal nanoparticles have recently emerged as an active field of research due to many novel properties of these materials (7–13). A growing interest has been developed in water-soluble polymers for their various applications potential in physical and biological systems (14–17). An increased general desire for hygiene in everyday life prompts a strong interest in antimicrobial modification of surfaces to prevent the growth of harmful micro organisms. Systems which show antimicrobial effects toward germs on contact without releasing toxic biocides are of strong current interest (18). Silver colloids have been known for a long time to possess antimicrobial properties, and also to be non toxic and environmentally friendly (19).

Silver nanoparticles prepared in hyperbranched polyurethane and in the presence of poly(vinyl pyrrolidone) are already reported (20–22). Also a few numbers of reports have been published on the development of silver nano particles in polyacrylamide matrix using different techniques. Saravanan et al. synthesized Ag nanoparticles containing polyacrylamide (PA) hydrogel composites by free radical crosslinking polymerization of acrylamide monomer in an aqueous medium containing Ag^+ ions (23). Polyacrylamide (PA)-silver nanocomposite materials have been synthesized by the reduction of silver salt in the polyacrylamide matrix (24).

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Authors, therefore, wish to report here the preparation and characterization of silver nanoparticles in polyacrylamide (PA) and polyacrylamide/hyperbranched polyamine (HB-PA) combined matrices. The antibacterial activity and dielectric constant of silver nanoparticles in the above matrices have also been studied.

2 Experimental

2.1 Materials

Acrylamide (SD Fine Chem.), potassium persulphate (or peroxydisulphate) (CDH), silver nitrate (Merck) sodium borohidride (Merck) were used as received. Hyperbranched polyamine was prepared by using the same method as reported earlier (25).

N,N′-dimethyl sulphoxide (DMSO, Merck, India) used after purification. Nutrient broth and nutrient agar (HIMEDIA, India) were used as received. *Bacillus Subtilis* bacteria strain was collected from the Department of Molecular Biology and Biotechnology, Tezpur University.

2.2 Preparation of Hyperbranched Polymer

The s-triazine based hyperbranched polyamine (Fig. 1) was synthesized by an $A_2 + B_3$ approach as in an earlier method (25) using 4,4′-(4,4-isopropylidenediphenyl-1,1′-diyldioxy) dianiline as an A_2 monomer and 2,4,6-trichloro1,3,5-triazine (CYC) as a B_3 monomer.

2.3 Preparation of Silver Nanoparticles

2×10^{-3} kg of acrylamide was taken in a three-necked round bottom flask and dissolved in 15×10^{-3} L of water with stirring 2×10^{-6} kg (0.1%) of potassium persulphate as initiator was added into the solution. The solution was then heated at 70 $\pm$ 2°C with constant stirring. After half an hour, the required amount of silver nitrate (2.5%, 5%, 10% with respect to acrylamide weight) aqueous solution was added dropwise into the pre-polymer solution. Then, after 10 min of stirring under the same conditions, sodium borohidride (equivalent amount to silver nitrate) aqueous solution was added. The solution color turns to a deep brown. After another 15 min, the reaction was stopped and the viscous solution was collected for further analysis.

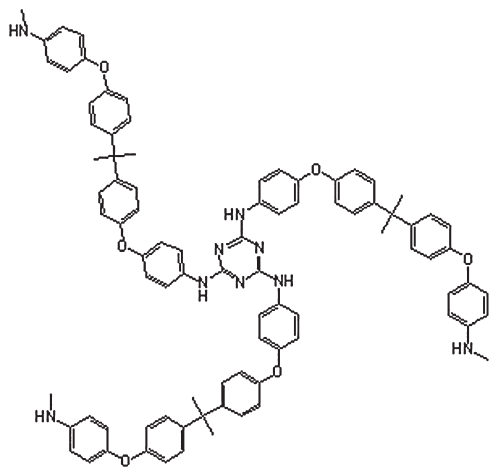

Fig. 1. Structure of hyperbranched polyamine.

In the case of the combined system (HB-PA), the silver nitrate solution was added, first in DMSO:water (1:1) colloidal solution of hyperbranched polyamine at room temperature with constant stirring. The rest of the procedure for the preparation of silver nanoparticles is the same as above, except the solvent used was DMSO: water (1:1) instead of only water.

2.4 Preparation of Culture Media for Incubation of Microorganisms

1.3×10^{-3} kg nutrient broth powder was dissolved in 0.1 L water by gently boiling in a 0.25 L conical flask stoppered by a cotton plug. The broth was sterilized by autoclaving at 6.8 kg pressure at 121°C for 15 min. Then, it was cooled in a laminar hood, which was disinfected beforehand by cleaning thoroughly with absolute alcohol, followed by UV irradiation for 20 min. A disinfected wire loop size stock microorganism of *Bacillus Subtilis* was transferred into the cold medium under laminar flow. After transferring the cultured microorganism, the conical flask was replugged and kept in an incubator oven for 24 h at 37°C with constant shaking. As the culture grows, the transparent medium becomes cloudy.

2.5 Testing of Antibacterial Activity

3.7×10^{-3} kg of nutrient agar powder was dissolved in 0.1 L of water by gently boiling in a conical flask. The agar solution was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 min. The solution was then cooled to 40–45°C in a laminar hood, which was disinfected beforehand following the same procedure as described earlier. 0.002 L of the above culture broth medium was added, after taking all precautions to avoid any contamination, and mixed thoroughly by hand shaking. The content was then poured into three Petri dishes with average equal agar thickness (2.5 mm). The dishes were cooled for a sufficient time (25–35 min) to solidify the agar medium, then five grooves in each dish were made by a sterilized crock borer (5×10^{-3} m diameter) and that medium was removed from the dish by help of a disinfected wire loop. 10×10^{-6} L of each test solution of at a particular concentration (10%, 15%, 20%) was poured in three grooves and in the other two grooves of the dish a positive control ampiciline and a negative control DMSO:water is poured. The test solutions contained PA silver nanoparticles and HB-PA silver nanoparticles. For each test solution, separate dishes were taken. In order to confirm that the antibacterial property is exclusively due

to silver nanoparticle a disc is prepared with the solutions of polyacrylamide, hyperbranched polyamine, a mixture of polyacrylamide and hyperbranched polyamine and DMSO: water mixture, however, no inhibition zone appeared. The dishes were then kept in an incubator oven at 37°C for 18 h to test the antimicrobial activity of the polymer solution. After the test, the inhibition zone diameter was measured from the clear zone of the agar dish.

2.6 Measurements

FTIR spectra for the compounds were recorded in a Nicolet (Impact 410, Madison, WI) FTIR spectrophotometer by using KBr pellets. X-ray diffraction studies were made on the film samples at room temperature (ca. 27°C) on a Rigaku X-ray diffractometer (Miniflex, UK). The scanning rate used was $5.0^\circ\ \mathrm{min}^{-1}$ over the range of $2\theta = 10–90^\circ$ for the above study. UV spectra of samples were recorded in a Hitachi (U-2001, Tokyo, Japan) UV spectrophotometer by using a 0.001% solution in DMAc. The size and distribution of silver nanoparticles were studied by using JEOL, model no. JEMCXII transmission electron microscope (TEM) at an operating voltage of 80 kV. The dielectric constant of the thin films was measured as discs of 10×10^{-3} m diameter (d) and 0.1×10^{-3}–0.2×10^{-3} m thickness (t) using a Hioki–3532-50 LCR Hi TESTER. The dielectric constant $= C_p t/\varepsilon_o a$, is calculated by using the capacitance (C_p) value obtained directly form the instrument, ε_o is the permittivity of vacuum and $a = \pi d^2/4$.

3 Results and Discussions

3.1 Preparation of Silver Nanoparticles

The silver nanoparticle is formed *in situ* in the matrix of PA and HB-PA by reduction of silver nitrate by sodium borohydride. Nanoparticles have very high surface energy and therefore have a great tendency to agglomerate (26). Polymers have been frequently employed as stabilizers for these particles in chemical synthesis of metal colloids, since they have the ability to prevent agglomeration and precipitation of the particles. The synthesis of such particles in the polymer matrix is also advantageous from the viewpoint of film casting and their use in various devices. In traditional methods, polymerization of organic monomer and formation of nanocrystalline metal particles are carried out separately, and the polymer matrix and metal particles are hybridized physically to form polymer-metal nanocomposites (27). It is, therefore, difficult to obtain homogeneously dispersed metal nanoparticles in the polymer matrix due to a high tendency of agglomeration of the nanoparticles. Thus, the size of such particles often exceeds 100 nm. In the present study, the metal salt is dispersed in the pre-polymer solution, which has low viscosity and hence, the particles are well-dispersed. Further, the amide groups of the polyacrylamide and the tertiary, secondary nitrogen, along with a large number of free active surface primary amine groups of hyperbranched polyamine, are forming stable complexes with the metal ions (Ag^+). These phenomena help in uniform dispersion and better stability. The study shows that at a particular ratio of Ag^+ to nitrogen (2.5% silver salt), Ag^+ formed the most stable complex (28) in the used polymer matrix. Thus, at this ratio, it is believed that the silver nanoparticles formed are uniform in size and dispersion, which are most desirable for such preparation.

Again, polyacrylamide is a crystalline, as well as film forming hydrophilic polymer. Therefore, macromolecular chains of polyacrylamide protect the surface of the Ag nanoparticles from being agglomerated after reduction. Again, the stability of silver nanoparticles in hyperbranched polyamine is much more than in the polyacrylamide. Therefore, hyperbranched polyamine is incorporated along with the polyacrylamide. The presence of three types of amine (1^o, 2^o and 3^o) in hyperbranched polyamine facilitates the formation of complex of Ag^+ ions with polymer chain and thus gives homogeneous dispersion of nanoparticles in the matrix. Thus, the present silver nanocomposite has the film forming ability along with high stability.

3.2 Characterization of Silver Nanoparticles

The formation of nanoparticle of silver in hyperbranched polymer matrix is first observed by UV-visible absorption spectral studies. No absorption peak was observed in UV-visible spectrum of Ag^+ solution in polymer before reduction. The peak for Ag^+ was not observed here is due to its d^{10} configuration (29). The optical absorption (UV-visible) spectra of Ag/PA nanocomposite at a different concentration of the Ag nanoparticles and Ag/HB-PA nanocomposite are shown in Figure 2. All the spectra for the nanocomposites show the presence of a peak around 400–410 nm (λ_{max}), which is characteristic of silvers surface plasmon resonance. The plasmon absorption peak shifts to a higher wavelength with the increase of aggregation of the particles, which happened with increasing the concentration of silver. In the case of 10% Ag-salt, the aggregation is likely to be

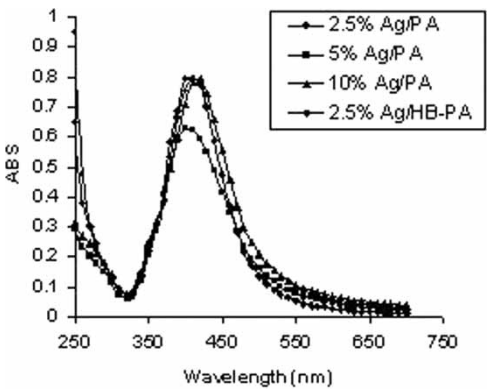

Fig. 2. UV spectra of silver nanoparticles.

more compared to the others. The sharpness of the peaks indicates the narrow size distribution of the nanoparticles in the polymer matrix, which is confirmed by the TEM micrographs (30).

In Figure 3, the main changes which occur in the IR spectrum of the Ag/PA nanocomposite were observed in the region of near 1650 cm^{-1} and near 1350 cm^{-1}. The little red shifting of the IR band near 1650 cm^{-1} is due to the >C═O stretching, -NH_2 bending and coupling modes between C-N stretching and >N-H bending (24). It is also due to the interaction between amide group and silver nanoparticle (24). Again the modification of intensity ratio of the two IR bands near 1350 cm^{-1} may be due to the changes in the C-N stretching modes (24). In the case of the Ag/HB-PA system, the changes occur in the region of 2900 cm^{-1} to 3000 cm^{-1}. The -CH_2 symmetric and asymmetric stretching bands appeared at 2870 cm^{-1} and 2965 cm^{-1}, respectively for the original hyperbranched polyamine, whereas in the Ag/HB-PA system, the bands appeared at 2900 cm^{-1} and 3000 cm^{-1}. This indicates the conformational changes occurred in the hyperbranched polyamine in the presence of nanoparticles.

The X-ray diffractrograms of the composite match the literature values of silver nanoparticles (Fig. 4). All the distinct peaks at 2 theta values of about 38, 44.5, 64.5, 77.5 and 82 representing the 111, 200, 220, 311 and 222 Braggs reflections of fcc structure of silver, confirms the formation of Ag nanoparticles in the colloid (31). The Skerrer diffraction formula was used to estimate the crystalline domain size (D):

$$D = k\lambda/\beta cos\theta$$

where $k = 0.9$ is for the Ag cubic structure, $\lambda = 0.1541 \times 10^{-9}$ m is the X-ray wavelength, β is the peak angular width and θ is the diffraction angle. The crystalline average domain size was found in the range of 8.9 to 10.9 nm and the size of the particles increases with the increasing amount of silver in the polymer matrix. A slight smaller average size (8.9 nm) of silver nanoparticles in HBPA matrix was observed compared to PA matrix (9.06 nm). The above results confirmed the silver nanoparticles formation in the used polymer matrices.

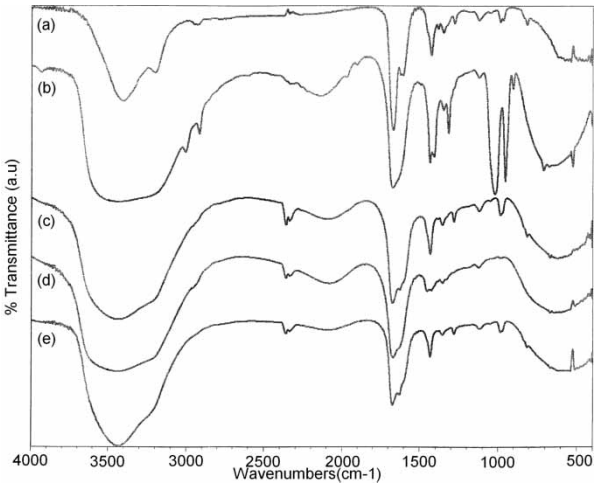

Fig. 3. FT-IR spectra of (a) PA; (b) Ag/HB-PA; (c) 2.5% Ag/PA; (d) 5% Ag/PA; (e) 10% Ag/PA.

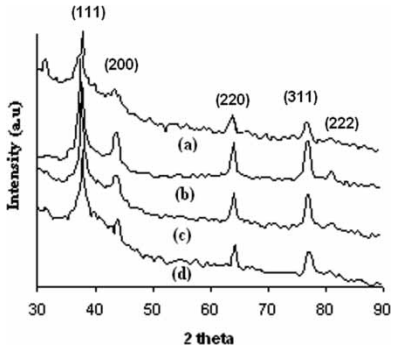

Fig. 4. X-ray diffractogram of (a) Ag/HB-PA (b) 2.5% Ag/PA (c) 5% Ag/PA (d) 10% Ag/PA.

The size and distribution of silver nanoparticles in the composite are shown in the TEM micrograph (Fig. 5) and histogram (Fig. 6). From the micrographs, it is clear that the silver nanoparticles are spherical in shape and dispersion is homogeneous in the polymer matrices. The histograms show the narrow size distributions of the nanoparticles. The average particle size is calculated from the histogram and found to be in the size range of 8.5–9.91 nm.

3.3 Antibacterial Property

Silver has long been considered a powerful and natural antibacterial. Silver works differently than most other substances in that it interferes with the enzyme that single cell bacteria, viruses and fungi need for their oxygen metabolism (32). This basically causes the cell to suffocate. Since no poisons or toxic elements are involved, the organisms do not develop a resistance to silver like they do to other agents. The extremely small size of silver nanoparticles means they exhibit enhanced properties when compared with bulk silver. This allows them to easily interact with other particles and increases their antibacterial efficiency (33). Thus, the development of new antibacterial substances is very significant. The results of this study (Fig. 7) indicate that the colloidal silver nanoparticles inhibited the growth of tested bacteria viz. *Bacillus Subtilis* even at very low total

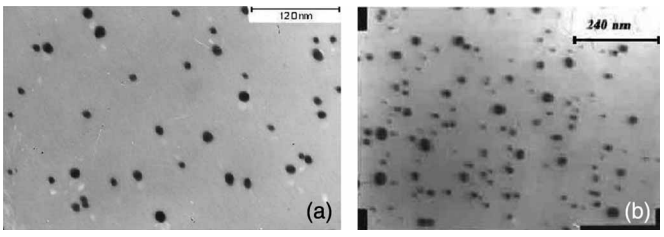

Fig. 5. TEM micrograph of (a) Ag/HB-PA (b) 2.5% Ag/PA.

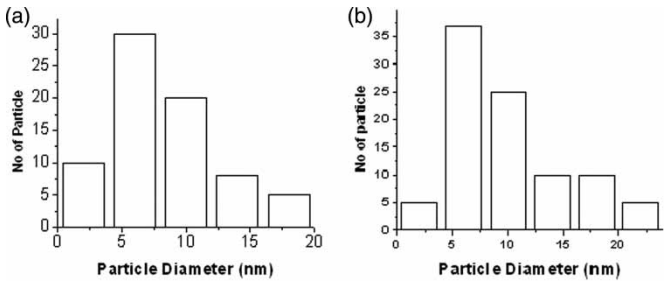

Fig. 6. Histogram for size distribution of (a) Ag/HB-PA, (b) 2.5% Ag/PA.

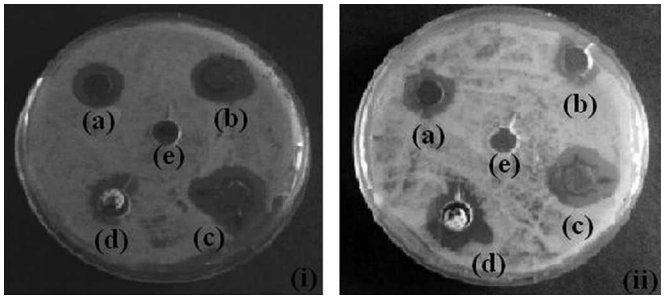

Fig. 7. Photographs of inhibition zone diameter of (i) [(a) 10%; (b) 15%; (c) 20%] of 2.5% Ag/HB-PA; (d) 100% ampiciline and (e) 1:1(v/v) DMSO:water, and (ii) [(a) 10%; (b) 15%; (c) 20%] of 2.5% Ag/PA; (d) 100% ampiciline; (e) 1:1 (v/v) DMSO:water for *Bacillus Subtilis* bacteria.

concentrations of silver ($0.5 \cdot 10^{-3}$ kg/L) The inhibition zone is slightly higher in the case of the Ag/HB-PA system than the Ag/PA system (Table 1). Furthermore, it is because of the relatively smaller size of the silver nanoparticle in the Ag/HB-PA system, as measured by TEM.

3.4 Dielectric Constant

Dielectric Constant is a number relating to the ability of a material to carry alternating current to the capability of a vacuum to carry alternating current. The capacitance created by the presence of the material is directly related to the Dielectric Constant of the material. If a material with a high dielectric constant is placed in an electric field, the magnitude of that field will be measurably reduced within the volume of the dielectric. This fact is commonly used to increase the capacitance of a particular capacitor design. The layers beneath etched conductors in Printed Circuit Boards (PCBs) also act as dielectrics. Thus, knowing the Dielectric Constant of a material is needed to properly design and apply instruments such as level controls using radar, RF admittance, or capacitance technologies.

Table 1. Inhibition zone diameter in different concentration of 2.5% Ag/PA and 2.5% Ag/HB-PA

	Inhibition zone diameter (10^3 m)		
Sample	10% solution	15% solution	20% solution
Ag/PA	15	16	19.5
Ag/HB-PA	16	19	21

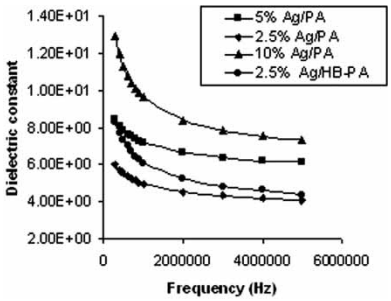

Fig. 8. Variation of dielectric constant with increasing frequency.

The variations of dielectric constant of the nanocomposite with frequency at different concentration have been studied. It has been observed in each case that the dielectric constant increases with the increase of silver concentration in the polymer matrix (Fig. 8). This may be due to the fact that at this Ag concentration, nano-Ag particles are scattered uniformly, and coulomb blockade effect arises (34). Again, when the Ag content is very high (12.5% silver salt) Ag nanoparticles agglomerate and become large in size, the capacitance between metal particles is also too large and coulomb blockade cannot occur. 2.5% Ag/HB-PA nanocomposite, the dielectric constant is a little higher than the 2.5% Ag/PA nanocomposite (Fig. 8). This may be due to the incorporation of a hyperbranched polyamine in the matrix, which slightly reduces the size of the nanoparticles (XRD and TEM results) and increases the capacitance of the matrix.

4 Conclusions

From this study, it can be concluded that well-dispersed and stable silver nano particles have been prepared by an *in-situ* reduction technique using hyperbranched polyamine and polyacrylamide as the matrix. The stability of colloidal solution of silver nanoparticles is higher in the combined matrix compared to polyacrylamide alone. The study shows that silver nanoparticles act as an effective antibacterial agent against *Bacillus Subtilis* bacteria. The dielectric constant of the matrices decreases with an increase in frequency but the values increase with the increase of concentration of silver nanoparticles in PA matrix.

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6 References

1. Zhao, H. and Ning, Y. (2000) *Gold Bull.,*33–103.
2. Halperin, W.P. (1986) *Rev. Mod. Phys.*, **58**, 533–606.
3. Mishra, L.C. *Scientific Basis for Ayurbedic Therapies*; CRC Press: Boca Raton, FL, 2003.
4. Cupp, M.J. and Tracy, T.S. *Dietary Supplements: Toxicology and Clinical Pharmacology*; Humana Press: New Jersey, 2003.
5. Zand, J., Spreen, A.N. and La Valle, J.B. *Smart Medicine for Healthier Living*; Avery: New York, 1999.
6. Wilsdon, J. (2004) *IEEE Technol. Soc. Mag.*, Winter, 16–21.
7. Sun, Y. and Xia, Y. (2002) *Science.*, **298**, 2176–2179.
8. Zhu, Y., Qian, Y., Lic, X. and Zhang, M. (1997) *Chem. Commun.,*1081–1082.
9. Yin, Y., Xu, X., Ge, X. and Zhang, Z. (1998) *Radiation Phys. Chem.*, **53**, 567–570.
10. Quaroni, L. and Chumanov, G.J. (1999) *J. Am. Chem. Soc.*, **121**, 10642–10643.
11. Bonet, F., Tekala-Elhsissent, K. and Sarathy, K.V. (2000) *Bull. Mater. Sci.*, **23**, 165–168.
12. Rogach, A.L., Shevchenko, G.P., Afanas'eva, Z.M. and Sviridov, V.V.J. (1997) *Phy. Rev.*, **101**, 8129–8132.
13. Mukherjee, M., Chakravorty, D. and Nambissan, P.M.G. (1998) *Phys. Rev.*, **B57**, 848–856.
14. Singh, A. and Mukherjee, M. (2005) *Macromolecules.*, **38**, 8795–8802.
15. Singh, A. and Mukherjee, M. (2004) *Phys. Rev.*, **E70**, 051608-1, 0516086-6.
16. Wang, C., Stewart, R.J. and Kopecek, J. (1999) *Nature.*, **397**, 417–420.
17. Kopecek, J., Kopeckova, P., Minko, T., Lu, Z.R. and Peterson, C.M.J. (2001) *J. Control Release.*, **74**, 147–158.
18. Stoimenov, P.K., Klinger, R.L., Marchin, G.L. and Klabunde, K.J. (2002) *Langmuir.*, **18**, 6679–6686.
19. Friedenthal, H. (1919) *Biochem.*, **Z94**, 47.
20. Lu, H.W., Liu, S.H., Wang, X.L., Qian, X.F., Yin, J. and Zhu, Z.K. (2003) *Mater. Chem. Phys.*, **81**, 104.
21. Chou, K.-S. and Lai, Y.-S. (2004) *Mater. Chem. Phys.*, **83**, 82.
22. Wang, H., Qiao, X., Chen, J., Ding, S. and Wang, X. (2005) *Mater. Chem. Phys.*, **94**, 449.
23. Sarvanan, P., Padmanabha Raju, M. and Sarfaraz Alam (2007) *Mater. Chem. Phys.*, **103**, 278–282.
24. Mukherjee, S. and Mukherjee, M. (2006) *J. Phys. Condens. Matter.*, **18**, 11233–11242.
25. Mahapatra, S.S. and Karak, N. (2007) *Polym. Degrad. Stab.*, **92**, 947–955.
26. Ball, P. and Garwin, L. (1992) *Nature.*, **355**, 761–766.
27. Brostein, L.M., Mirzoeva, E.SH., Seregina, M.V., Valetsky, P.M., Solodovnikov, S.P. and Register, R.A. (1996) *ACS Symp. Ser.*, **622**, 102.
28. Ottaviani, M.F., Valluazzi, R. and Balogh, L. (2002) *Macromolecules.*, **35**, 5105–5115.
29. Ulkur, E., Oncul, O., Karagoz, H., Yeniz, E. and Celikoz, B. (2005) *Burns.*, **31**, 874–7.
30. Khanna, P.K., Singh, N., Charan, S. and Kasi Viswanath, A. (2005) *Mater. Chem. Phys.*, **92**, 214–219.
31. Zhang, Z. and Han, M. (2003) *J. Mater. Chem.*, **13**, 641–643.
32. Panacek, A., Kvitek, L., Prucek, R., Kolar, M., Veceova, R., Pizurova, N., Sharma, V.K., Nevecna, T. and Zboril, R. (2006) *J. Phys. Chem.*, **B110**, 16248–16253.
33. Baia, L., Baia, M., Kiefer, W., Popp, J. and Simon, S. (2006) *J. Chem. Phy.*, **327**, 63–69.
34. Scott-Thomas, J.H.F., Field, S.B., Kastner, M.A., Smith, H.I. and Antoniadis, D.A. (1989) *Phys. Rev. Lett.*, **62**, 583–586.