Enzymatic Polymerizations Using Surfactant Microstructures and the Preparation of Polymer-Ferrite Composites

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ABSTRACT

Horseradish peroxidase has been used as a biocatalyst to synthesize a polymeric material from alkyl-substituted phenols. The synthesis is carried out in a surfactant-based microemulsion environment, with the monomer partitioned at the oil/water interface. The spherical nature of the microemulsion nanodroplets may be acting as a template for the polymer synthesis. The resultant polymer particles are spherical and typically in the submicron size range. The characteristics of the morphology development are described. The templating effect of the surfactant environment becomes more evident when the polymer particles are fully dissolved in a suitable solvent and refolded in the presence of surfactant. Interestingly, submicron-sized spherical particles are obtained only in the presence of surfactant, and particles of arbitrary morphology are seen in the absence of surfactant. Aspects of morphology development leading to the preparation of polymer-iron oxide composites are described.

Index Entries: Horseradish peroxidase; microemulsions; polymers; nanoparticles.

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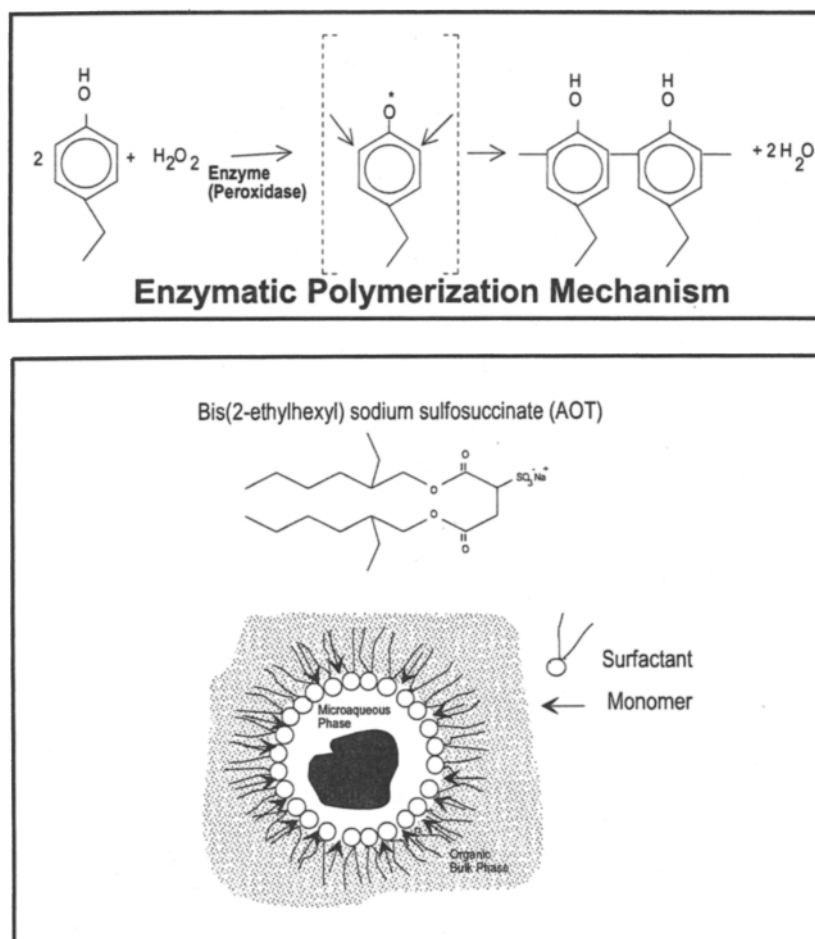
Fig. 1. Schematic showing a microemulsion droplet with the various components. The droplet is stabilized by the twin-tailed anionic surfactant (AOT). The monomer p-ethylphenol, which partitions to the interface, is indicated by the arrows. The enzyme horseradish peroxidase occupies the aqueous core of the droplet. A simplified polymerization reaction mechanism is shown at the top.

INTRODUCTION

Phenolic polymers are present in nature in the form of lignin in plants. Lignin is synthesized by the action of an enzyme, peroxidase, involving a one-electron oxidation of p-hydroxy-methoxy-substituted cinnamyl alcohol units (1). Analogous to the peroxidase reaction in plants, we have used horseradish peroxidase (HRP) to catalyze the reaction between p-ethylphenol monomer units as shown in Fig. 1. The reaction system is essentially a water-in-oil microemulsion that is formed when

water is added to a solution of surfactant, AOT (bis[2-ethylhexyl] sulfo-succinate, sodium salt), in isoctane. The enzyme being water-soluble is resident in the aqueous core of the dispersed nanodroplets. The polar nature of the hydroxyl moiety imparts surface-active properties to the monomer, causing the monomer to partition at the oil–water interface alongside the surfactant molecules as shown in Fig. 1. Reaction is initiated by the addition of H_2O_2 to 30% excess, based on a 1:1 stoichiometry of H_2O_2:monomer. The polymerization reaction is extremely fast, and rapid precipitation of the polymer is seen within a few minutes. The recovered polymer is seen to be spherical with particle sizes on the order of a micron. Detailed experimentation at various microemulsion compositions shows that the microemulsion droplet integrity needs to be maintained to obtain a final spherical morphology (2). In addition, the same polymer synthesis when carried out in essentially organic media (80/20 v/v dioxane/water) shows that the polymer does not possess any specific morphology. This observation demonstrates that the microemulsion environment has a templating effect on the polymer morphology.

During the polymerization process, intra- and intermolecular interactions in polymer solutions dictate the folding and conformation of the polymer chains. Once a certain degree of polymerization is achieved and the polymer chains begin to precipitate, these interactions manifest their influence on the morphology of the resultant polymer particles. The current understanding regarding the development of particle morphology is that the surfactant is intimately involved in shaping the polymer particles. FTIR studies indicate strong interaction between the surfactant and the monomer units (3). Specifically, hydrogen bonding between the carbonyl group on the surfactant and the hydroxyl group is seen. Considering the key role played by the surfactant in directing polymer folding, is it possible to refold polymers from solution into spherical morphologies? Refolding experiments, with poly(p-ethylphenol), indicate that spherical polymer particles can indeed be obtained by refolding the polymer chains from solution in the presence of surfactant. However, the particles obtained by such refolding techniques are somewhat different from those obtained by polymerization in microemulsion media. These aspects will be discussed in greater detail.

Applications for the phenolic polymers described above can be found in conventional areas, such as coatings and resins. In addition, owing to the unsaturation along the chain backbone, these polymers may possess novel electrooptical properties. In exploring the application potential for polymers synthesized in microemulsions, we have tried to exploit the morphology of the polymer particles. One such application that will be discussed here is the entrapment of nanoparticles into the polymer microspheres. Currently, there has been much interest in the synthesis of inorganic nanoparticles for industrial applications ranging from ceramics to magnetic recording and catalysis. Nanoscale materials exhibit novel optical, electronic, magnetic, and chemical properties because of their extremely

small dimensions. Owing to the high surface-area-to-volume ratio in such fine particle systems, particle aggregation is frequently encountered during processing. If these nanoclusters could be kept physically separated, the potential for these materials would be greatly increased. In addressing this issue, magnetic iron oxide particles synthesized in microemulsions have been incorporated into a polymer matrix resulting in the formation of an inorganic nanoparticle–polymer composite material. This is achieved by first synthesizing the magnetic particles in microemulsions and then conducting the polymerization reaction in the same medium.

In this article, we examine the morphological development of the polymer particles as more and more polymer chains are formed and the role of the surfactant in influencing the particle morphology. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used to examine the particle morphology. Synthesis of a new magnetic polymer composite material made up of iron oxides and poly(p-ethylphenol) is described, and some magnetic properties are also presented.

MATERIALS AND METHODS

The water-in-oil microemulsions were prepared using the anionic surfactant, bis(2-ethylhexyl) sodium sulfosuccinate (AOT). The size of the microemulsion droplets is directly related to the molar ratio of water to surfactant in the system, $w_0 = [H_2O]/[AOT]$, and is easily controlled by adjusting the amount of water added to the system. The notation w_0 10 reversed micelles refers to a solution containing 10 mol of water for every mole of AOT. Iron oxide particles were synthesized in w_0 10 reversed micelles by simply mixing individual reversed micellar solutions containing the reactants $FeSO_4$ and NH_4OH. The solution instantly turns to bluish-green and within minutes changes to a deep red color. The reaction mixture was kept stirred for about 2 h, and aliquots were ovendried at 40°C overnight. The dried aliquots were reconstituted to their original volume with isooctane to give a clear deep-red colored solution. To this solution, 0.01M HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) buffer, p-ethylphenol, and peroxidase in buffer were added to make up the reaction mixture to w_0 15 with 0.5M in AOT, 0.15M p-ethylphenol, and 0.5 mg/mL peroxidase. The reaction was initiated by the dropwise addition of hydrogen peroxide. The precipitated polymer was centrifuged and washed extensively with isooctane to remove the surfactant AOT and water. The recovered polymer composite was then ovendried at 40°C for 2 d before further analysis. In the refolding experiments, the polymer was synthesized as described above, but the solution did not contain any iron oxides. In refolding experiments, the polymer stock obtained after a 24-h reaction time was collected, washed three times with isooctane, dried, and stored for later use. This sample was dissolved in a suitable solvent, either acetone, benzene, or CCl_4, and
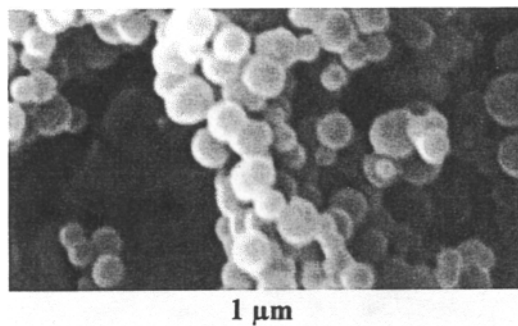
Fig. 2. SEM micrograph of polymer particles prepared in AOT-water-isooctane microemulsions.
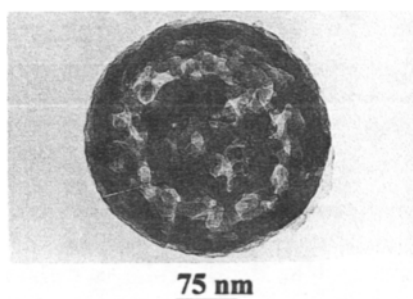

Fig. 3. TEM micrograph showing an isolated polymer particle. The particle is made up of a network of small polymer patches.

precipitated by diluting with 10 vol of either isooctane, or 10 vol of a $0.5M$ AOT in isooctane solution. The precipitates were centrifuged and washed before being analyzed through electron microscopy. Typical sample preparation for electron microscopy involved dispersing the polymer in isooctane with the help of mild sonication. A drop of the dispersed solution was placed on an SEM stub or a TEM grid. Although the SEM stub was coated with a 20 nm gold coating, the TEM sample was directly loaded onto the instrument for analysis.

RESULTS AND DISCUSSION

Templating Effect and Polymer Folding

When the polymerization reaction is conducted in the microemulsion environment, the resulting polymer morphology is spherical as illustrated by the SEM in Fig. 2 with particle size in the 0.3–0.8 μ range. This particle size is two to three orders of magnitude greater than the microemulsion droplet size (typically 3–10 nm) in the reaction system (4). Morphology characterization through TEM reveals some interesting features associated with the polymer particles. A TEM micrograph of a single polymer particle is shown in Fig. 3. In addition to being spherical, the polymer
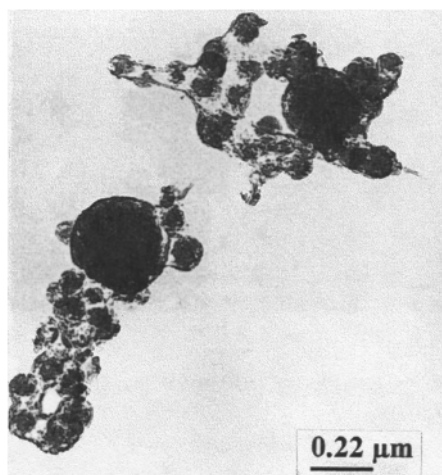
Fig. 4. TEM micrograph showing the morphology of the polymer particles at a stage of partial monomer conversion. H_2O_2 was added to 70% of the stoichiometric requirement.

particle is also partially transparent to the electron beam. The polymer particle appears to be made up of small patches of polymer, the size of which can be approximated to a few microemulsion droplets. We have determined through reaction time studies that the polymer particles become fully dense, electron opaque, if the reaction mixture is kept stirred for about 24 h.

A 1:1 stoichiometry exists between the amount of monomer consumed to the amount of H_2O_2 added to the reaction system. Thus, it is very easy to control the monomer conversion by simply adjusting the extent of H_2O_2 addition. By conducting partial conversion studies, it was possible to look at the role of the microemulsion environment in templating the polymer synthesis. At low conversions, a network of polymer chains can be seen with intermediate spherical nodules as illustrated by the TEM micrograph in Fig. 4. Such nodules may be the consequence of initial chain growth around the surfactant-stabilized microemulsion droplets. Collisons between droplets might then lead to interconnections between the nodules, which appear as the strands linking the nodules. When the reaction mixture is kept under agitation, these assemblies remain suspended in solution and thus come into further contact with the surfactant. Surfactant–polymer chain interactions eventually lead to the folding of these assemblies into the spherical particles observed in Figs. 2 and 3.

Since the formation of spherical particles occurs only under conditions of a well-defined microemulsion droplet, the question that arises next is the following: is the spherical morphology the sole result of the templating effect of the microemulsion environment, or is it contingent on simultaneous synthesis of polymer chains? To address this question, a couple of experiments were conducted using a stock of spherical polymer
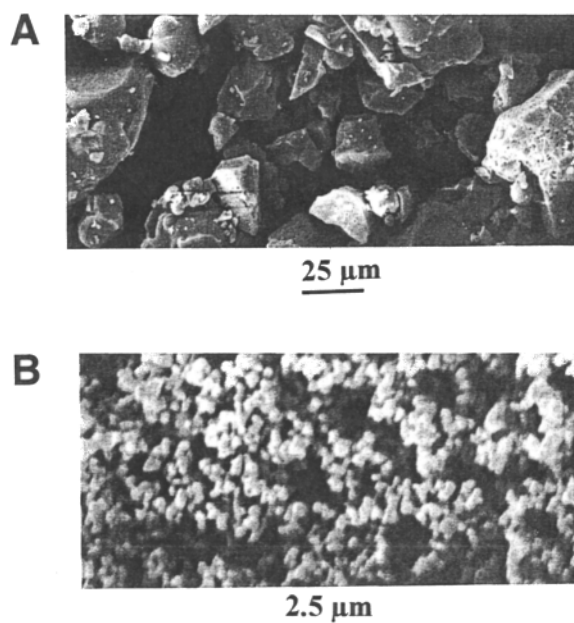
25 μm

2.5 μm

Fig. 5. SEM micrographs of polymer samples obtained by precipitation from solution: (A) in the absence of surfactant and (B) in the presence of 0.5M AOT.

particles. In the first, the polymer is dissolved in a solvent (acetone). Dry solid AOT was added to a concentration of 0.5M. After incubating the solution for about 3 h, 10 equivalent volumes of a 0.5M AOT/isooctane solution was added dropwise, with constant stirring. The precipitated polymer was recovered, washed, and imaged. As a control, the same precipitation was carried out in the absence of AOT. Interestingly, it can be seen that the particles formed in the absence of AOT exhibit no specific morphology or size (Fig. 5A), whereas the presence of excess AOT during precipitation leads to uniform spherical particles (Fig. 5B). Essentially identical results were obtained when the solvent was either benzene or CCl_4 instead of acetone. The concentrations of AOT employed are well above its critical micellar concentration (CMC) in all these cases. The bonds between the monomer units are flexible, and the rotation around each such biphenyl bond can lead to substantial changes in conformation of the polymer chain. Furthermore, the hydroxyl groups of the polymer backbone are strongly hydrogen bonded, especially in nonpolar solvents, as can be deduced from IR spectra. Thus, the polymer folding seems to occur largely by hydrogen bonding—both within the same polymer chain and between chains. The presence of excess AOT perhaps breaks such hydrogen bonding, because it can compete for the same protons. The precipitation of these polymer chains with the adsorbed surfactant leads to different kinds of interactions, giving rise to spherical polymer particles. We remark here that the size of the spherical particles obtained by the precipitation technique (ca.
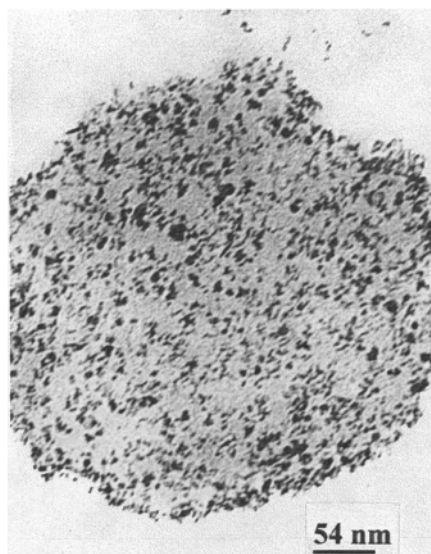
Fig. 6.   Microtomed TEM section of iron oxide–polymer composite showing the iron oxide particles dispersed in the polymer matrix.

0.3 μm) is smaller than that of the stock particles used. Thus, although the surfactant has the dominant role in folding of the polymer chains, the coupling of the folding process to the kinetics of polymerization may also be important in determining the particle size. Perhaps this difference is the result of the fact that nascent polymer chains are folded by the surfactant as opposed to the mature molecules or because of the larger size of the micelles in the polymerization system.

The technique is clearly applicable to other polymers that have polar substituents; in nonpolar solvents, AOT (and perhaps some other surfactants) can be expected to hydrogen bond naturally with the polymer chains and assist their folding to alternative structures.

Applications

In exploring potential applications for the polymer microspheres, nanometer-sized iron oxide particles were synthesized in the microemulsion environment. Because of the samll size of the microemulsion droplets, the growth of the iron oxide crystals is restricted to a certain size. These nanoparticles are incorporated into the polymer matrix by simply adding the enzyme and monomer to the iron oxide microemulsion and initiating polymerization via H_2O_2 addition. A thin-section TEM micrograph of an iron oxide–polymer composite particle is shown in Fig. 6. It is seen that the iron oxide particles, indicated by the dark spots, are uniformly distributed throughout the polymer matrix. Particle size analysis shows that most of the particles are smaller than 100 Å.

To determine the magnetic properties of the iron oxide–PEP composite material, a SQUID magnetometer was used. Field-dependent hysteresis loops were generated at 100 K and 2.0 K and are shown in Fig. 7A and B, respectively. The magnetization vs field data at 100 K shows that the data is perfectly superimposable as the field is cycled. This lack of hysteresis is characteristic of superparamagnetic particles of very small dimensions (5). However, as the temperature is significantly lowered to 4.5 K, the sample starts to display some hysteresis (data not shown). This hysteresis behavior is further amplified when the temperature is reduced to 2.0 K. The data from Fig. 7 indicate a very strong temperature-dependent behavior, especially at very low temperatures.

Temperature-dependent magnetization data for the sample, obtained by two different methods, are shown in Fig. 8. In the field-cooled (FC) case, the magnetic field is turned on at room temperature, the sample is cooled to very low temperatures, and the magnetization is measured at various equilibrium temperatures. For the zero field-cooled (ZFC) experiment, the sample is first cooled to the lowest temperature in the absence of any field, and then the field is turned on. The magnetization is then measured as the temperature is increased. At high temperatures, the FC and ZFC data exhibit the same trend. However, at low temperatures, they significantly diverge from each other. The FC curve reaches a plateau, and the ZFC curve shows a dramatic decrease in the magnetization. From the ZFC data in Fig. 8, it is apparent that a sharp maxima in the magnetization is seen as a function of temperature. This maxima is a characteristic trait of spin-glass materials (6) and occurs at 10 K. The temperature at which this maxima is observed is often referred to as the freezing temperature. Going back to the hysteresis loops shown in Fig. 7, it is worth noting that the sample exhibits hysteresis at temperatures below the freezing temperature of 10 K, with hysteresis vanishing at higher temperatures.

CONCLUSIONS

Synthesis of poly(p-ethylphenol) in microemulsions leads to the formation of micron-sized spherical polymer particles. TEM analysis shows that the polymer particles are partially transparent to the electron beam. This might be the result of a porous structure in the polymer particles. Fully dissolved polymer can be refolded into a spherical morphology by precipitation using surfactant-containing dilution solvent. The absence of surfactant in the dilution solvent results in the formation of morphologically ill-defined polymer particles. The polymer refolding process appears to be a general technique applicable to other types of polymer/surfactant systems. A potential application for these polymer particles has been developed through the incorporation of inorganic nanocrystals into the
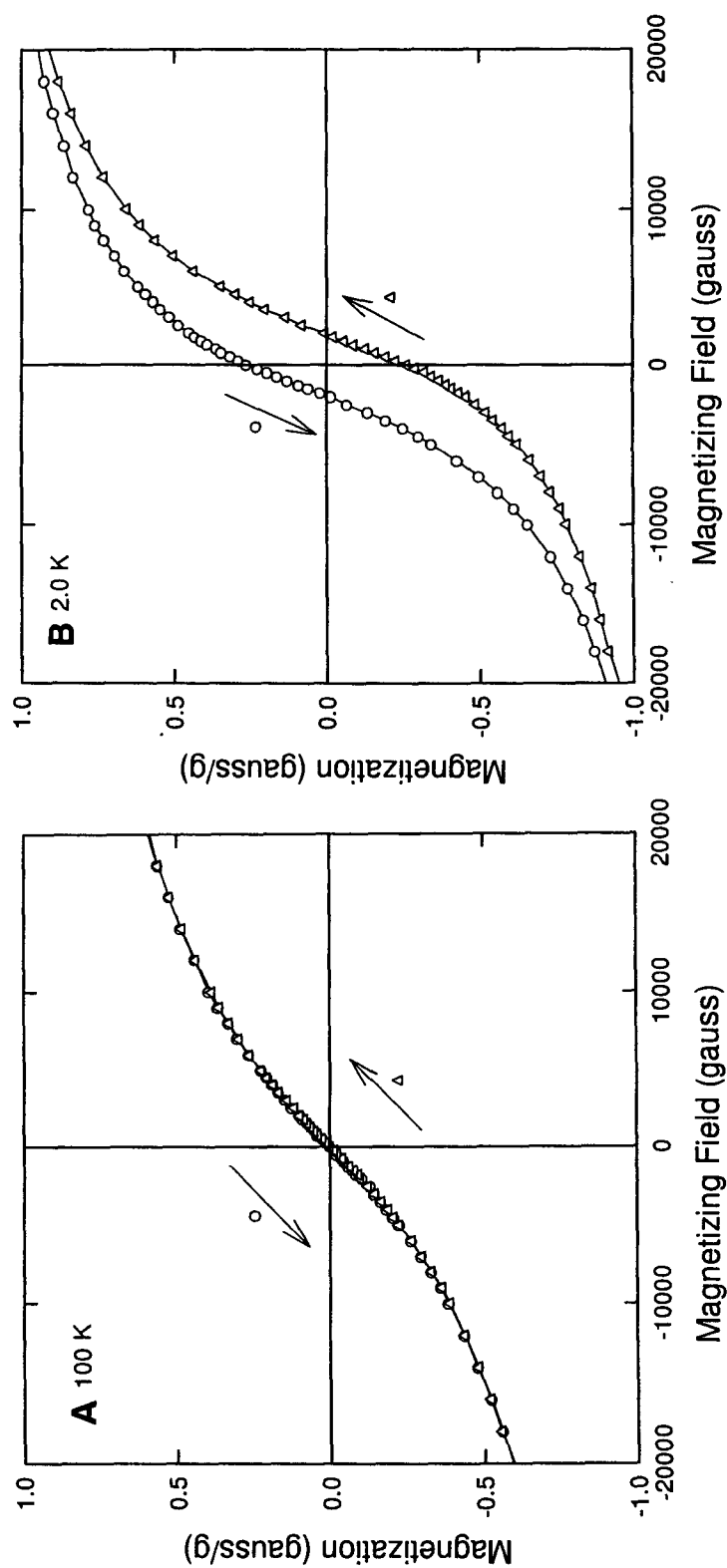
Fig. 7. Field-dependent magnetization data for the iron oxide–polymer composite at (A) 100 K, showing lack of hysteresis and (B) 2.0 K, showing hysteresis.
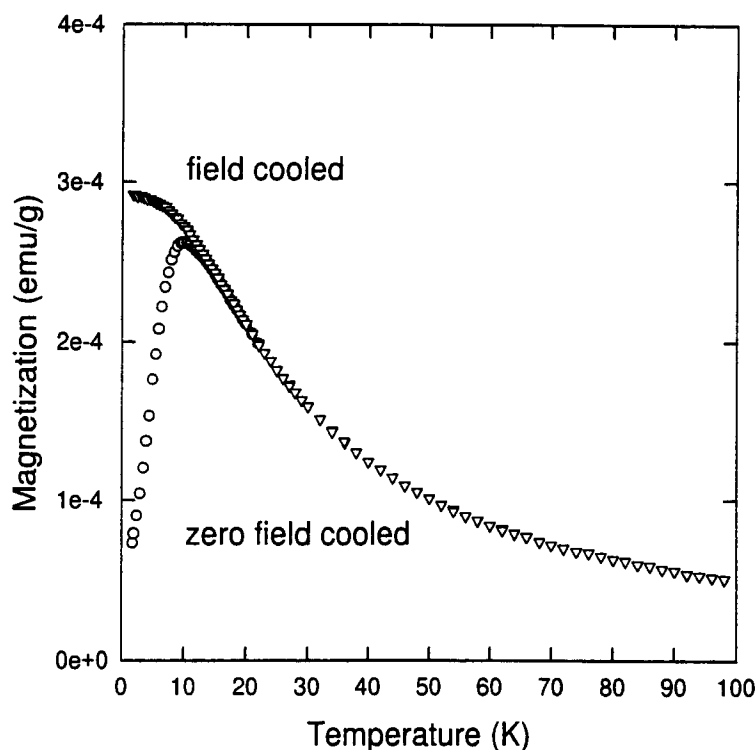
Fig. 8. Field-cooled (FC) and zero field-cooled (ZFC) data for the iron oxide–polymer composite illustrating the spin-glass-type behavior of the composite. Applied field = 1 kG.

polymer matrix. Excellent dispersion of the inorganic guest species is seen in this encapsulation technique. The iron oxide–polymer composite exhibits superparamagnetic behavior as seen through the lack of hysteresis at high temperatures. The composite also exhibits spin-glass-type behavior with a characteristic blocking temperature of about 10 K.

ACKNOWLEDGMENTS

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