Magnetic and Temperature-Sensitive Composite Polymer Particles and Adsorption Behavior of Emulsifiers and Trypsin

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Abstract: A combination of magnetic and temperature-responsive properties in the same polymer composites is expected to increase their potential applications in the biomedical field. Accordingly, micron-sized magnetite/polystyrene/poly(2-dimethylaminoethyl methacrylate-ethyleneglycol dimethacrylate), which are abbreviated as $Fe_3O_4/PS/P$ (DM-EGDM) composite polymer particles, were prepared by the seeded copolymerization of DM and EGDM in the presence of magnetite/polystyrene (Fe_3O_4/PS) particles. $Fe_3O_4/PS/P$(DM-EGDM) composite particles with magnetic properties showed a temperature-sensitive phase transition at approximately 31 °C. The adsorption behavior of the low molecular weight emulsifiers and trypsin (TR) as biomolecules were examined on $Fe_3O_4/PS/P$(DM-EGDM) composite polymer particles at different temperatures. The native conformation of TR was followed by measuring the specific activity under various adsorption conditions. The activity of the adsorbed TR on composite polymer particles was higher than those of the free TR and TR adsorbed on Fe_3O_4/PS particles.

Keywords: Fe_3O_4, temperature-sensitive, LCST, TR, specific activity.

Introduction

Magnetic polymer particles generated much interest among the researchers in the biomedical field because they can easily be separated from the dispersion by applying even low magnetic field instead of centrifugation, usually applied for separating classical non-magnetic particles. The latter process is time consuming and sometimes even reduces the colloidal stability. The magnetic polymer particles can be prepared via monomer polymerization using a number of techniques such as conventional emulsion polymerization,[1] dispersion polymerization,[2,3] suspension polymerization,[4-6] seed polymerization[7] and activated swelling method.[8] Of these methods seed polymerization is simple and widely accepted as it gives monodisperse polymer particles.

Poly(2-dimethylaminoethyl methacrylate) is an well-known temperature-responsive water soluble polymer that exhibits a sharp phase transition.[9] In a series of previous articles, H. Ahmad *et al.* prepared composite polymer particles comprising cross-linked shell of 2-dimethylaminoethyl methacrylate (DM) and ethyleneglycol dimethacrylate (EGDM).[10-15]

These composite polymer particles demonstrated an abrupt phase transition at temperature around the lower critical solution temperature commonly known as LCST (~32 °C). In other words, the surface of composite particles shrank above the LCST and swelled below it. This property was due to the reversible formation and rupture of hydrogen bonds between the amine group in the shell and surrounding water molecules. Composite polymer particles having such stimuli-responsive phase transition are considered to be useful in drug delivery, water treatment, bioseparator, bioreactor, cell activator and diagnostic reagent.[16-20]

The integration of magnetic responses into one such temperature-sensitive composite polymer particles would increase the application potential for magnetic separation, drug release systems, and for sensor and actuator purposes.[21] Sauzedde *et al.* reported the preparation of core-shell microspheres containing poly(*N*-isopropyl acrylamide) (PNIPAM), a kind of another widely used temperature-sensitive polymer, by physical adsorption of magnetite (Fe_3O_4).[22] In a relatively recent work, Cai *et al.* prepared multi-responsive core-shell Fe_3O_4/silica/P(DM-NIPAM) composite microspheres by a rather complicated multistep process after functionalizing the Fe_3O_4/silica with 3-(trimethoxysilyl)-propyl methacrylate.[23] In this work, authors mainly reported the effect DM

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comonomer on the swelling ratio at different pH values.

The objective of the present investigation is to prepare magnetically loaded composite polymer particles containing temperature-sensitive crosslinked PDM shell. Micron-sized Fe_3O_4/polystyrene (Fe_3O_4/PS) particles were prepared by seeded dispersion polymerization of styrene in presence of nano-sized Fe_3O_4 particles. Finally, seeded copolymerization of DM and EGDM was carried out in presence of Fe_3O_4/PS particles. The inclusion of nano-sized Fe_3O_4 particles was confirmed by magnetic susceptibility measurement and FTIR spectroscopy. Temperature-sensitive phase transition at temperature around the LCST was measured by studying the adsorption behavior of low molecular weight emulsifiers and trypsin (TR) as biomolecules. Additionally, specific activity of adsorbed/released TR was measured to check any conformational change.

Experimental

Materials. Styrene and 2-dimethylaminoethyl methacrylate (DM) of monomer grade (Fluka, Chemika, Switzerland) were distilled under reduced pressure. Ethyleneglycol dimethacrylate (EGDM) a crosslinking agent, purchased from Fluka, Chemika, Switzerland, was used without any purification. 2,2'-Azobisisobutyro nitrile (AIBN) and 2,2'-azobis(2-amindinopropane)hydrochloride (V-50) both from LOBA Chem., India were recrystalized from distilled water and ethanol, respectively. Poly(vinylpyrolidone), (PVP) of LOBA Chem., India, trycaprylylylmethyl ammonium chloride (Aliquate[336]), hexadecyl trimethyl ammonium bromide (HTABr) and sodium dodecylsulphate (SDS) all purchased from Fluka, Chemika, Switzerland were used as received. Trypsin (TR) from E Merck, Germany, was used without any purification. L-Lysine monohydrochloride purchased from BDH Chem., England was used for the preparation of lysine methyl ester hydrochloride (LME). Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron (II) chloride tetrahydrade ($FeCl_2 \cdot 4H_2O$), sodium hydroxide, oleic acid, and other chemicals were of analytical grade. Deionized water was distilled using a glass (Pyrex) distillation apparatus.

Characterization. Transmission electron microscope, TEM (Zeiss EM 912 Omega); Helios Gamma, single-beam UV- visible spectrophotometer (Unicam, UK); Burker proton NMR, 250 MHz; IR spectrophotometer (Shimadzu, FTIR-8900, Japan); refrigerated high speed centrifuge machine from Kokuson Corporation Tokyo, Japan; HI-9321 Microprocessor pH-meter and conductivity meter both from HANNA instruments, Portugal were used. NICOMP 380 (USA) particle sizer was used to measure hydrodynamic diameter of the polymer particles. Sherwood Scientific Magnetic Susceptibility Balance was used for susceptibility measurement.

Preparation of Fe_3O_4 Particles. Nano-sized Fe_3O_4 particles were prepared based on a reported method with minor mod-

ifications.[24,25] $FeCl_2 \cdot 4H_2O$ (4.87 g, 0.024 mol) and $FeCl_3 \cdot 6H_2O$ (15.89 g, 0.058 mol) were dissolved in 100 mL water separately and then the two iron solutions were mixed under vigorous stirring. 80 mL of 5 M aqueous sodium hydroxide solution was added immediately. The resulting black dispersion was continuously stirred at room temperature for 1 h and then heated under reflux at 80 °C for 2 h to obtain a brown dispersion. Oleic acid (2 g, 2.26 mmol) was slowly added towards the end of the process to stabilize the Fe_3O_4 dispersion. Finally Fe_3O_4 particles were washed by the replacement of dispersion medium with deionized distilled water, followed by 0.1 M HCl and again by deionized distilled water.

Preparation of Fe_3O_4/PS Particles. Micron-sized Fe_3O_4/PS particles were prepared by seeded dispersion polymerization of styrene (7 g, 0.067 mol) in presence of Fe_3O_4 particles (0.01 g) in 50 mL ethanol-water (4:1) dispersion media. PVP (0.4 g) and aliquate[366] (0.114 g) were used as stabilizer and co-stabilizer, respectively. Polymerization was carried out in a three-necked round bottom flask under a nitrogen atmosphere at 70 °C for 12 h. Conversion was almost 100%, measured gravimetrically. Fe_3O_4/PS particles were washed repeatedly by replacing the continuous phase with distilled deionized water.

Preparation of Fe_3O_4/PS/P(DM-EGDM) Composite Polymer Particles. Fe_3O_4/PS/P(DM-EGDM) composite polymer particles were prepared by second step seeded copolymerization of DM (0.98 g, 6.23 mmol) and EGDM (0.20 g, 1.08 mmol) with micron sized Fe_3O_4/PS particles (2.0 g) using V-50 (0.01 g, 0.036 mmol) as water soluble initiator under a nitrogen atmosphere in a three-necked round bottom flask maintained in a hot waterbath at 60 °C. Fe_3O_4/PS/P(DM-EGDM) particles were washed repeatedly by replacing the continuous phase with distilled deionized water.

Preparation of PS/P(DM-EGDM) Composite Polymer Particles. To compare the size distribution with magnetic Fe_3O_4/PS/P(DM-EGDM) composite polymer particles, non-magnetic PS/P(DM-EGDM) composite polymer particles were prepared. For this, PS particles were first prepared by dispersion polymerization of styrene (7 g, 0.067 mol) in 50 mL ethanol-water (4:1) medium under identical conditions as carried out for the preparation of Fe_3O_4/PS. Micron-sized PS/P(DM-EGDM) composite polymer particles were then prepared by seeded copolymerization of DM (0.98 g, 6.23 mmol) and EGDM (0.20 g, 1.08 mmol) with micron sized PS particles (2.0 g) using V-50 (0.01 g, 0.036 mmol) as water soluble initiator under a nitrogen atmosphere at 60 °C.

Measurement of Magnetic Susceptibility. The particles were separated from respective dispersion, dried in oven at 80 °C for several hours. The dried powders were then placed in a pre-weighed sample tube and measured the magnetic susceptibility (χ_g) using a Magnetic Susceptibility Balance.

Adsorption of Emulsifiers on Fe_3O_4/PS/P(DM-EGDM) Composite Polymer Particles. For each measurement, a

mixture of 20 mL was prepared from Fe_3O_4/PS/P(DM-EGDM composite dispersion (polymer solid, 0.1 g) and emulsifier aqueous solution, in such a way that the concentration of the emulsifier remained bellow the critical micelle concentration (CMC). The pH value of the mixture was adjusted at 10. The composite-emulsifier mixture was then allowed to stand for 2 h at 25 and 40 °C, respectively with occasional stirring. The conductance values were recorded at each 25 and 40 °C. The magnitude of adsorption was calculated by subtracting the emulsifier concentration in the medium from the initial concentration. The emulsifier concentration was obtained from calibration curves, representing the relationship between the concentration (mg/mL) and the conductance, assuming that the adsorbed emulsifier molecules don't contribute to the conductance.

Adsorption of Trypsin (TR) on Fe_3O_4/PS/P(DM-EGDM) Composite Polymer Particles. A mixture of 20 mL was prepared from purified composite dispersion (polymer solid, 0.1 g) and TR (200 mg/g of particles) aqueous solution. The pH value of the mixture was immediately adjusted at the isoelectric point (pH 10) using a buffer solution. In each measurement the dispersion-TR mixture was allowed to stand for 45 min. Polymer particles were then separated by centrifugation at 1,2000 rpm for 15 min. In order to remove dust particles completely, the supernatant was centrifuged once more. The concentration of the TR in the supernatant was determined by a UV-visible spectrophotometer at the wavelength of 280 nm. The amount of TR adsorbed was calculated by subtracting the concentration in the medium from that of the initial concentration. A calibration curve was used for this purpose.

Preparation of Lysine Methyl Ester Hydrochloride (LME). LME was prepared from lysine monohydrochloride by following the conventional method of ester preparation as reported elsewhere.[26] The product was recrystallized from ethanol by adding dehydrated ether and finally dried in a vacuum desiccator over anhydrous calcium chloride. LME was confirmed from its sharp melting point (196.5 °C), thin layer chromatography (TLC) measurement and ^1H-NMR (Chemical shift appeared at 3.9 ppm indicated the methyl protons, $-COOCH_3$, also no chemical shift is observed for the proton of $-COOH$).

Specific Activity of Trypsin (TR). Specific activities of free TR (TR aqueous solution) and adsorbed/released TR were measured at temperatures above and below the LCST (31 °C) by a pH stat method using LME as a substrate. The detailed procedure is outlined below:

Seed or composite dispersion was mixed with a known amount of TR aqueous solution and pH was immediately adjusted at 10, using 0.02 N KOH aqueous solution. In each measurement, TR concentration (45 mg/g) was kept constant. After maintaining the dispersion-TR mixture at certain temperature (variable 20 to 40 °C) for 45 min, 100 mL of 10^{-3} mol/L LME aqueous solution was added. Then the pH

of the mixture was quickly adjusted at 10 and maintained with 0.02 N KOH under magnetic stirring at the respective temperature for 3 min. The specific activity was calculated from the amount of KOH consumed to neutralize the acid liberated from the hydrolysis of LME.

Results and Discussion

Oleic acid coated Fe_3O_4 particles prepared by the co-precipitation of Fe(II) and Fe(III) in NaOH solution showed positive value for magnetic susceptibility (1.44×10^{-4}) indicating that the particles are paramagnetic. The particles were also moved under the applied magnetic field. In the FTIR spectra (Figure 1) the characteristic stretching vibrations due to Fe-O bonds of Fe_3O_4 particles appeared at 586 and 408 cm^{-1}, respectively, as also cited in other literatures.[27-29] The broad band at 3384 cm^{-1} can be assigned to the stretching vibration of surface water molecules or the envelope of hydrogen bonded surface OH groups.

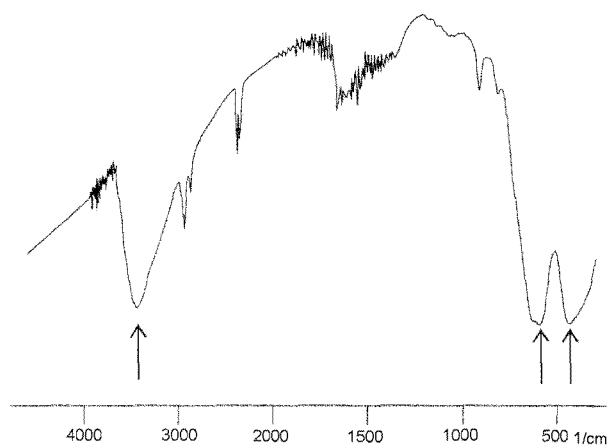The TEM photograph of nano-sized Fe_3O_4 particles shown in Figure 2 suggests that particles are smaller than 30 nm,

Figure 1. FTIR spectra of Fe_3O_4 particles prepared by coprecipitation of Fe(II) and Fe(III).
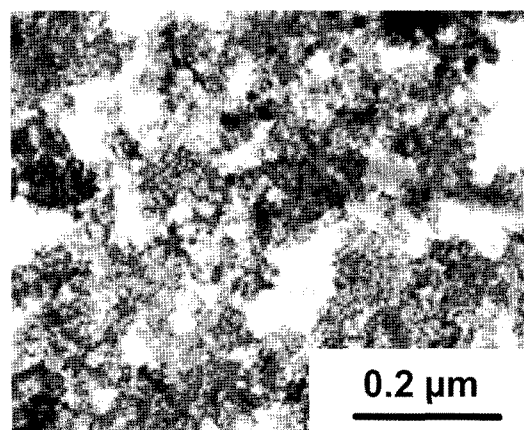

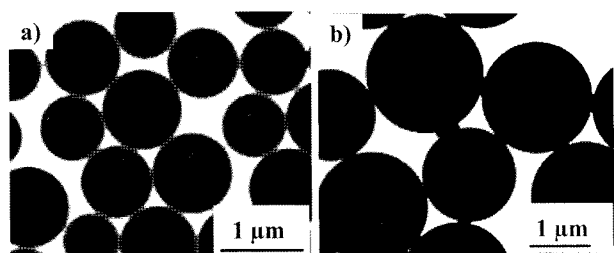**Figure 2.** TEM photograph of Fe_3O_4 particles prepared by coprecipitation of Fe(II) and Fe(III).

Figure 3. TEM photographs of PS (a) and Fe₃O₄/PS (b) particles prepared by dispersion and seeded dispersion polymerizations.

though, the average hydrodynamic diameter measured by DLS was around 200 nm. The formation of hydrogen bonded water molecules may be responsible for this large increase in hydrodynamic diameter. Other factors such as non-spherical shape and magnetic nature may also affect the measurement by DLS.

Figure 3(a-b) shows the TEM photographs of PS and Fe₃O₄/PS particles prepared by dispersion and seeded dispersion polymerizations, respectively. It is to be mentioned that both polymerizations were carried out with the same styrene content (14%) relative to the dispersion medium. The average sizes and coefficient of variations are 1.36 μm and 3.53% for PS; 1.86 μm and 3.29% for Fe₃O₄/PS particles, respectively. Compared to non-magnetic PS, the average size of Fe₃O₄/PS particles is relatively high. This indicates the possibility of Fe₃O₄ inclusion in PS particles by seeded dispersion polymerization. In the TEM photographs of Fe₃O₄/PS particles, small numbers of submicron-sized but spherical particles are observed. This suggests that only limited secondary nucleation may have occurred during seeded dispersion polymerization in presence of Fe₃O₄ particles.

Both PS and Fe₃O₄/PS particles were washed repeatedly by serum replacement, dried and then used for magnetic susceptibility measurement and FTIR spectra. PS particles showed negative value (-2.68×10⁻⁶) whereas Fe₃O₄/PS particles showed positive value (1.01×10⁻⁵) for magnetic susceptibility indicating the latter as paramagnetic particle. Relative to Fe₃O₄ particles, the paramagnetic intensity of Fe₃O₄/PS particles is reduced. This may be associated with the formation of PS layer over Fe₃O₄. FTIR spectra of PS and Fe₃O₄/PS particles are shown in Figure 4. In both spectra C-H and C=C stretching vibrations of the aromatic ring appeared at around 3050 and 1450-1500 cm⁻¹, respectively. But in Fe₃O₄/PS particles an extra absorption band due to Fe-O is observed at around 540 cm⁻¹. The above results suggest that most of the Fe₃O₄ particles have successfully been encapsulated by seeded dispersion polymerization.

In order to compare the size distribution and appearance, both PS/P(DM-EGDM) and Fe₃O₄/PS/P(DM-EGDM) composite polymer particles were prepared by seeded copolymerization of DM and EGDM in presence of PS and Fe₃O₄/PS seed particles. Figure 5 shows the TEM photographs of

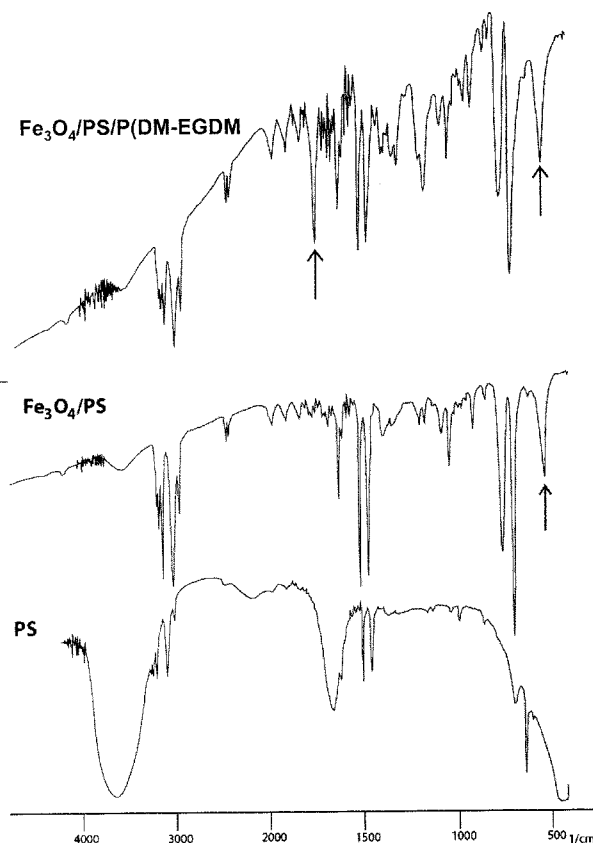**Figure 4.** FTIR spectra of washed PS, Fe₃O₄/PS and Fe₃O₄/PS/P(DM-EGDM) particles prepared by dispersion, seeded dispersion and seeded polymerizations.
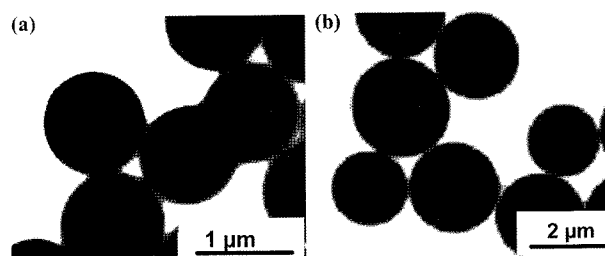
Figure 5. TEM photographs of PS/P(DM-EGDM) (a) and Fe₃O₄/PS/P(DM-EGDM) (b) composite polymer particles prepared by seeded copolymerization.

PS/P(DM-EGDM) and Fe₃O₄/PS/P(DM-EGDM) composite polymer particles. The averaged sizes and coefficient of variations are 1.67 μm and 4.21% PS/P(DM-EGDM); 2.1 μm and 4.17% for Fe₃O₄/PS/P(DM-EGDM) composite particles. Both particles are spherical and ignoring the high dispersity index it is observable that average size of each composite increased slightly from the corresponding seed particles. The relative appearance of both composite particles in the TEM photographs indicates that Fe₃O₄/PS/P(DM-EGDM) composite particles are more stable under the electron beam. In the FTIR spectrum (Figure 4) for

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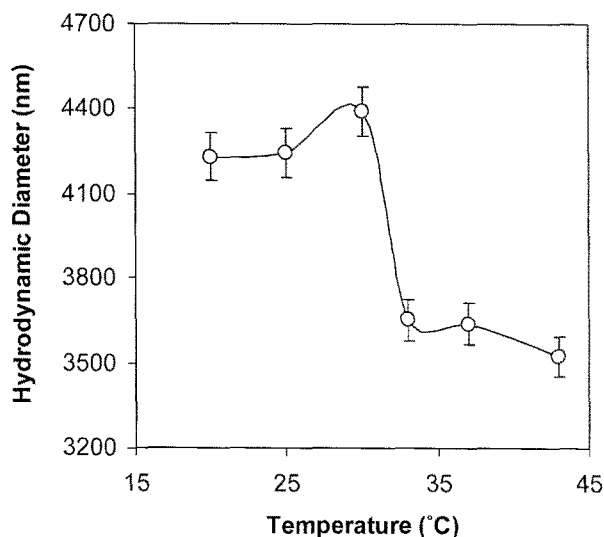**Figure 6.** Variation of average hydrodynamic diameter of washed Fe$_3$O$_4$/PS/P(DM-EGDM) composite polymer particles with temperature at pH 9.
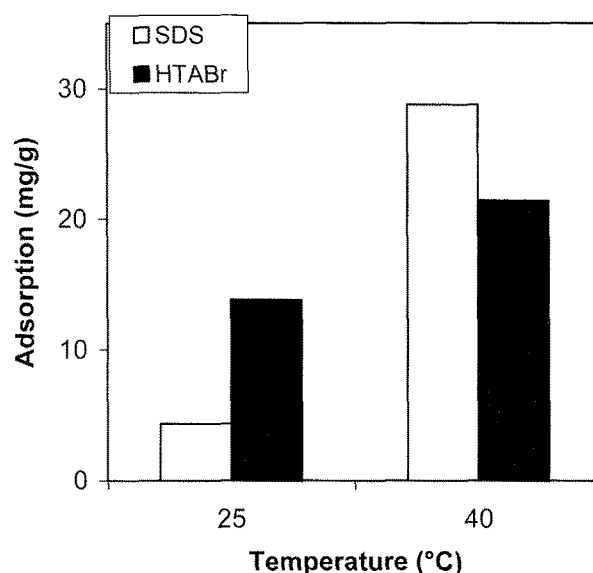

Figure 7. Magnitude of sodium dodecyl sulphate (SDS) and hexadecyl trimethyl ammonium bromide (HTABr) adsorbed on Fe$_3$O$_4$/PS/P(DM-EGDM) composite polymer particles at pH 10, measured under constant concentration against the total polymer solid. SDS: 1.03×10^{-3} mol L^{-1} and HTABr: 8.21×10^{-1} mol L^{-1}; polymer solid: 0.1 g; immobilization time: 2 h; temperature: 25 and 40 °C.

washed Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles, a strong absorption band due to C=O stretching of the ester group was observed at 1718 cm^{-1}. This absorption band was not observed for either PS or Fe$_3$O$_4$/PS particles. Washed Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles showed positive magnetic susceptibility (7.5×10^{-7}) and the paramagnetic intensity was lower than the corresponding Fe$_3$O$_4$/PS seed particles. The particles were also visibly attracted towards the magnetic field in both emulsion and dried states.

The temperature-sensitive property of PS/P(DM-EGDM) composite particles has already been studied and reported elsewhere.[10,11,30] In this article, temperature-sensitive property of magnetic Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles will be discussed.

Figure 6 shows the variations of hydrodynamic diameters of Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles measured at pH 9. Despite the limitation of the instrument for measuring the particle size distribution of micron-sized latex, it is observed that the hydrodynamic diameters decreased sharply at around 31 °C which is close to the LCST (33 °C) of PS/P(DM-EGDM) composite particles.[30] Hence, P(DM-EGDM) copolymer layer lies near the surface of composite particles dehydrated and collapsed with increasing temperature above the LCST. Contrary to this, hydrodynamic diameters of PS and Fe$_3$O$_4$/PS particles remained almost unchanged with temperature (data not shown). The slight deviation in the LCST for Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles is possibly associated with the change in molecular environment due to the incorporation of Fe$_3$O$_4$ particles. Relative to the average diameter (2.1 μm) of Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles measured from the TEM photograph, the higher hydrodynamic diameter (~3.5 μm) at 45 °C implied

that particles dispersed in aqueous medium are still enough hydrophilic. However, the partial coagulation of Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles at 45 °C can not be ruled out as the shell layer of composite particles turned into hydrophobic. The phase transition was reversible i.e. hydrodynamic diameter returned to almost the original value on reducing the temperature to 20 °C.

Figure 7 shows the adsorption behaviors of anionic SDS and cationic HTABr emulsifiers on Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles at 25 and 40 °C, respectively. All the measurements were carried out at pH 10. The magnitude of adsorption for both emulsifiers at 25 °C is lower than that at 40 °C. It is already known that the magnitude of adsorption depends on the intensity of hydrophobic and electrostatic interactions between the adsorbent and adsorbate. Since the particles surface is largely electrically neutral at pH 10, the higher magnitude of adsorption at 40 °C is expected to be related to the increased hydrophobic interaction. Hence, the surface of Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles swelled with water and became hydrophilic at temperature below the LCST, whereas with increasing temperature above the LCST the same surface deswelled and turned hydrophobic. The change in the amount of adsorption between SDS and HTABr is attributed to the difference in molecular characteristics such as hydrophobicity, molecular weight and ionic property.

Figure 8 shows the magnitude of adsorption of TR on Fe$_3$O$_4$/PS/P(DM-EGDM) composite particles as a function
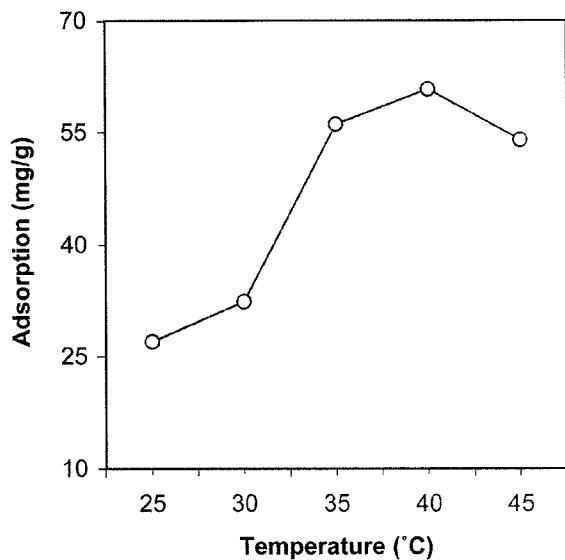
Figure 8. Magnitude of trypsin (TR) adsorbed on Fe₃O₄/PS/P(DM-EGDM) composite polymer particles at different temperatures measured at pH 10 under constant concentration against the total polymer solid. TR: 200 mg g⁻¹ of particles; polymer solid: 0.1 g; immobilization time: 45 min.

of temperature. This measurement was carried out at the isoelectric point (pH 10) as TR is electrically neutral at this pH. The magnitude of adsorption increased sharply at around the LCST (31 °C), indicating a phase transition from hydrophilic to relatively hydrophobic with increasing temperature.

Specific activity of any biomolecule measured under identical conditions is largely dependent on the native conformation. Figure 9 shows the variations of specific activi-
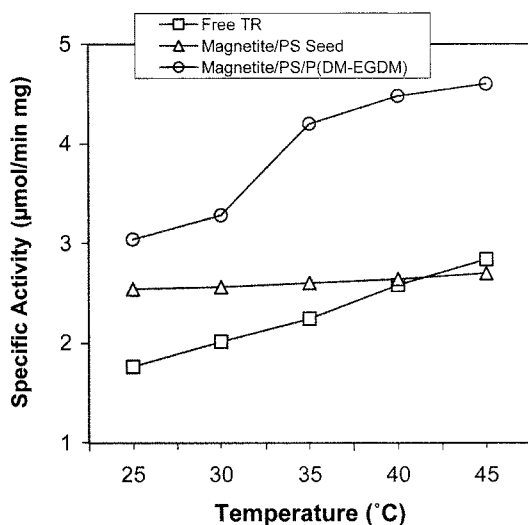
ties of free TR (TR aqueous solution) and TR in presence of Fe₃O₄/PS and Fe₃O₄/PS/P(DM-EGDM) composite particles as a function of temperature. All the measurements were carried out under constant concentration of TR (45 mg g⁻¹ of particles) at pH 10. Specific activities of free TR increased steadily as the hydrolysis rate of LME increased with increasing temperature. This is the general characteristic of any enzyme catalyst. Comparatively, free TR has the lowest specific activity because self digestion occurred rapidly due to the frequent contact among themselves as the solution was kept standing for 45 min before each measurement. Specific activities of TR in presence of Fe₃O₄/PS particles are relatively high as compared to free TR but remained unchanged with increasing temperature. Under the measurement conditions, TR is expected to be completely adsorbed on Fe₃O₄/PS particles. Adsorption of TR on Fe₃O₄/PS reduced the self digestion but since the surface of Fe₃O₄/PS particles is strongly hydrophobic, the adsorbed TR may have denatured. On the contrary, specific activities of TR in presence of Fe₃O₄/PS/P(DM-EGDM) composite particles are much higher as compared to those of both free TR and adsorbed TR on Fe₃O₄/PS particles. Moreover, specific activities of TR in presence of composite particles increased sharply at temperature around the LCST (31 °C). Based on the results shown in Figures 8 and 9, it can be assumed that a part of the added TR remained free at temperature below the LCST, whereas, at temperature above the LCST most of the added TR are adsorbed as the particles surface gradually changed from hydrophilic to hydrophobic. The higher specific activity of adsorbed TR on Fe₃O₄/PS/P(DM-EGDM) composite particles at temperature above the LCST than

Figure 9. Variations of specific activities of free trypsin (TR), adsorbed TR on F₃O₄/PS and Fe₃O₄/PS/P(DM-EGDM) particles with temperature measured under a constant concentration of TR (45 mg g⁻¹ of particles) at pH 10 and immobilization time of 45 min.
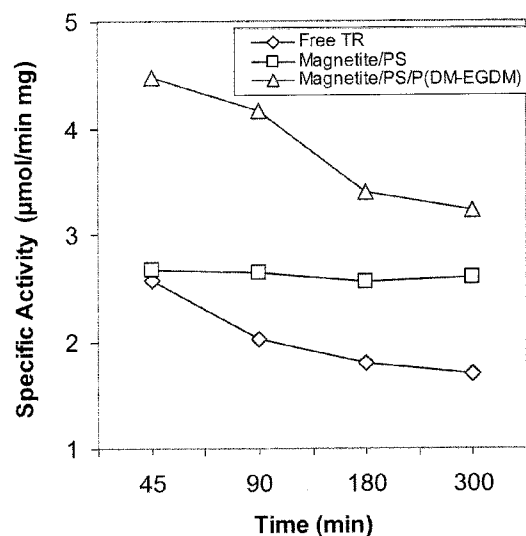
Figure 10. Variations of specific activities of free trypsin (TR), adsorbed TR on Fe₃O₄/PS and Fe₃O₄/PS/P(DM-EGDM) particles with time measured under a constant concentration of TR against the total solid content. TR: 45 mg g⁻¹ of particles; polymer solid: 0.1 g; temperature: 40 °C, and pH: 10.

that on Fe_3O_4/PS particles suggests that the surface of composite particles is comparatively less hydrophobic.

Figure 10 shows the effects of immobilization time on the specific activities of free TR and TR adsorbed on Fe_3O_4/PS and Fe_3O_4/PS/P(DM-EGDM) composite particles at 40 °C. Specific activities of adsorbed TR on Fe_3O_4/PS/P(DM-EGDM) composite particles remained almost steady until 90 min adsorption time but after that they decreased rapidly. Since the decrease in specific activity is associated with conformational change, it may be assumed that an adsorption time of more than 90 min at 40 °C is not suitable. Though the specific activities of adsorbed TR on Fe_3O_4/PS/P(DM-EGDM) composite particles decreased considerably but they still remained much higher than those of both free TR and adsorbed TR on Fe_3O_4/PS particles. Specific activities of free TR decreased rapidly with time indicating enhanced self digestion at 40 °C. Compared to this, specific activities of adsorbed TR on Fe_3O_4/PS particles remained steady with adsorption time. Adsorption of TR on Fe_3O_4/PS particles prevented the self digestion but the relatively strong hydrophobic surface may have produced so much conformational change of adsorbed TR that specific activities did not decrease with time.

Conclusions

Magnetically loaded temperature-sensitive PS/P(DM-EGDM) composite polymer particles were prepared by two steps seeded copolymerization. The average hydrodynamic size of the magnetic PS/P(DM-EGDM) composite particles decreased sharply at around the LCST (31 °C). This suggested that P(DM-EGDM) copolymer shell swelled with water at temperature below the LCST and dehydrated, turned hydrophobic at temperature above the LCST. The adsorption behaviors of emulsifiers and TR confirmed the temperature-sensitive phase transition of composite polymer particles surface. The specific activities of TR in presence of magnetic PS/P(DM-EGDM) composite polymer particles measured under different adsorption conditions showed that surface of composite polymer particles are hydrophilic enough to reduce the conformational change.

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