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Multiresponsive polymeric particles with tunable morphology and properties based on acrylonitrile (AN) and 4-vinylpyridine (4-VP)

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ABSTRACT

We report the synthesis of amphiphilic, pH and magnetic field sensitive polymeric particles obtained from the modification of poly(acrylonitrile-co-4-vinylpyridine) (p(AN-c-4-VP)) core-shell nanoparticles. The magnetic metal nanoparticles were encapsulated in the microemulsion during the polymerization to achieve magnetic-p(AN-c-4-VP)-composites with various morphology. We further chemically modified each component of p(AN-c-4-VP) particles and its composite to tune the hydrophilicity of the particles. Modification of nitrile (hydrophobic) groups to amidoxime (hydrophilic) groups by amidoximation reaction on AN, and quarternization of nitrogen on pyridine ring of 4-VP were carried out to tune the hydrophilicity and the charge of the particles. The modification also performed on magnetic responsive composites after inclusion of separately prepared magnetic Fe_3O_4 nanoparticles. It was further demonstrated that these multiresponsive particles can be used as drug carrier. A nonsteroidal and anti-inflammatory drug Naproxen was used as a model active agent for drug loading and the release studies from (p(AN-c-4-VP) based particles in phosphate buffer solution (pH = 7.4) at ambient temperature.

1. Introduction

Hydrogels are three dimensional hydrophilic polymer networks that can be swollen to many times of their original mass and volume in aqueous environments. They can be made to responsive to various environmental factors such as pH, temperature, electric and magnetic fields [1–3]. More recently, increasing interest has been devoted to exploration of stimuli sensitive polymeric particles that are also called smart or intelligent materials which can be used, due to their unique physical and chemical properties, in biomedical applications such as controlled drug delivery, biolabelling, separation, catalysis [4–6] and so on. During the past few years, iron oxide nanoparticles both magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) have been investigated for potential biomedical applications, especially in drug delivery system because of their nontoxic nature. Controlled drug delivery system is getting an excitement by utilization of magnetic nano or micro particles with polymeric micro and nanogels as composites. The main idea of behind the magnetic responsive particle is to target drug carriers with their therapeutics in vivo to the specific cells or organs with aid of externally applied static magnetic field [7,8].

Acrylonitrile (AN) based polymeric materials show fiber forming properties which have high strength, stiffness and abrasion resistance. In addition, the cyano groups in polyacrylonitrile are suitable for conversion of hydrophobic nitrile groups to hydrophilic amidoxime groups that may improve the chemical, physical and biological properties of the polymeric materials as well as increasing the adsorption amount of active agents in controlled drug release applications. Polymers carrying amidoxime groups have very interesting properties as it stimulate high hydrophilicity. Amidoxime group containing polymers are also very important in biological and environmental applications [9]. By the same token, poly(4-vinylpyridine) (p(4-VP)) is a neutral polymer, but have very intriguing properties coming from the hydrophobic vinyl and benzene like pydridinyl structure and hydrophilic nature of nitrogen atom on the ring. Moreover, pyridine has novel amphoteric properties (acidic and basic character) and hydrophilic–hydrophilic balance. Additionally, p(4-VP) can be quaternized to form positive charge on the nitrogen atom with different alky chain length containing reagents presenting it as an antimicrobial material [10,11]. P(4-VP) and p(4-VP) based materials can be used in many filed as actuators, metal binding materials, chromophore forming, and as bactericidal materials and so on. Furthermore, p(4-VP) is versatile material to be tuned chemically to introduce different charges on their backbones. Therefore, a material derived from resourceful monomers such as

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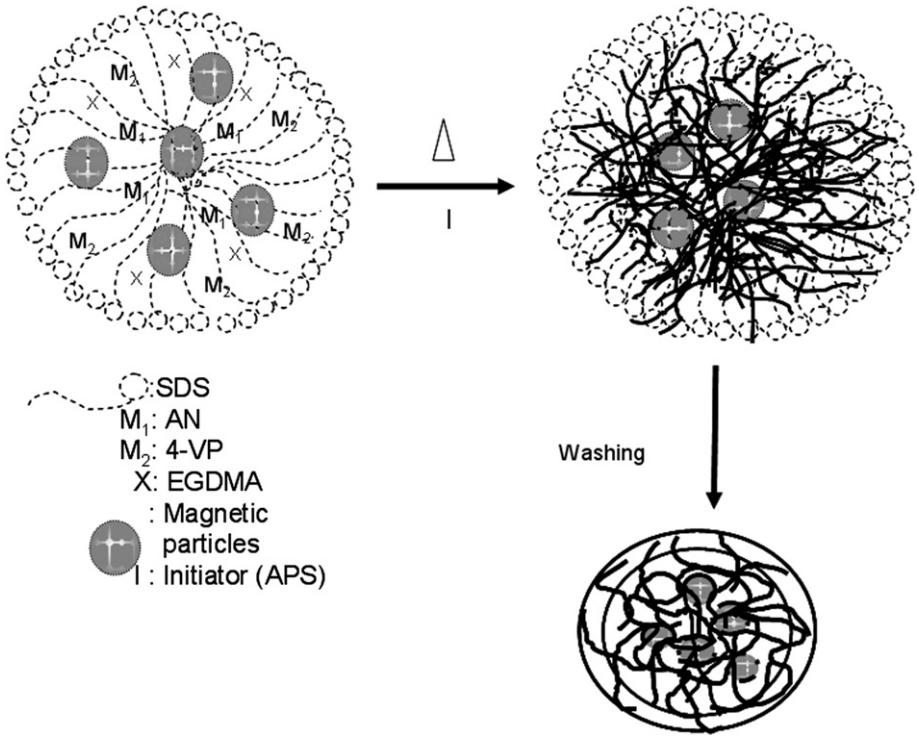

Fig. 1. The schematic representation of the magnetic particle containing p(AN-c-4-VP) composite material synthesis.

AN and 4-VP provide exceptional tool in the resultant material properties. Previously, we reported the functional core-shell polymeric particles with controllable shell thickness based on AN and a thermoresponsive N-isoproply acrylamide (NIPAM) for delivery purpose [11,12].

Here, the present work focuses on the synthesis and characterization poly(acrylonitrile-co-4-vinylpyridine) (p(AN-c-4-VP)) core-shell nanoparticles and their modification to introduce additional functional properties such as adaptable hydrophilic/hydrophobic balance, charge, pH and even magnetic responsiveness. The magnetic filed responsive behavior was afforded by physically entrapping separately prepared ferrite (Fe_3O_4 and Fe_2O_3) magnetic nanoparticles inside polymeric materials that exhibit considerable sensitivity to the external stimuli. The modifiable particles were further utilized for the potential drug delivery devices (DDD) utilizing a water soluble model drug, Naproxen, for the drug delivery studies.

2. Metarials and methods

2.1. Materials

4-vinylpyridine (4-VP) (%99, Acros), and acrylonitrile (AN) (% 99, Sigma–Aldrich, Milwaukee, Wisconsin) were used as monomers in the polymeric particle synthesis. Ethylene glycol dimethacrylate (EGDMA), as a crosslinker, and ammonium persulfate (APS) as redoks initiator, were also obtained from Aldrich. And sodium dodecyl sulfate (SDS) (%96, Merck) as an anionic surfactant was used as micelle forming stabilizing agent. Hydroxylamine hydrochloride ($NH_2OH.HCl$) (98%, Merck) and sodium hydroxide (NaOH, Aldrich) for amidoximation reactions were used. Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) (Acros) and iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$) (Fluka) were used as received in the preparation of magnetic particles. Aqueous ammonia (%26) (NH_3), and NaCl, Merck) were used in formation

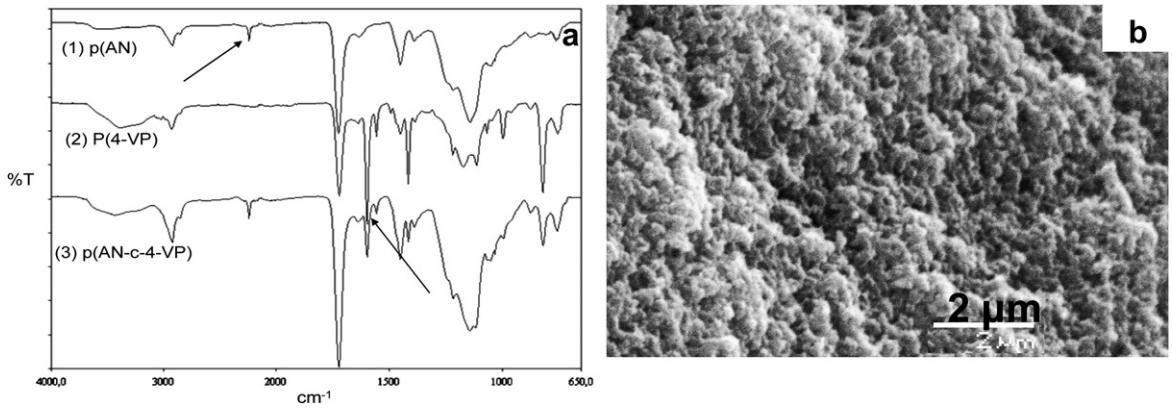

Fig. 2. (a) The FT-IR images of the synthesized particles, (1) p(AN), (2) p(4-VP),and p(AN-c-4-VP) and, (b) SEM images of p(AN-c-4-VP) particles.

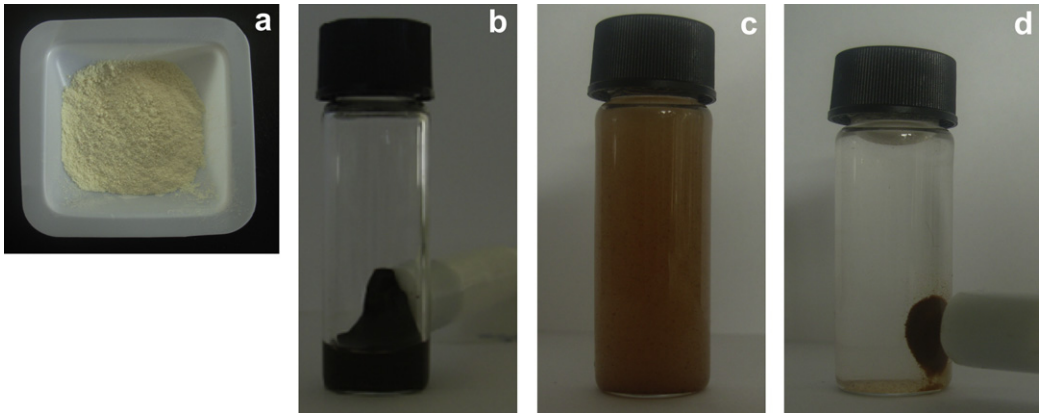

Fig. 3. Digital camera images of (a) p(AN-c-4-VP) particles, (b) ferrite particles (Fe3O4), (c) ferrite containing p(AN-c-4-VP) composite suspended in DI water before, and (d) after applied magnetic field.

of magnetic nanoparticles. 2- bromoethylamine hydrobromide (BEA) (99% from Alfa Aesar), and HCl were used as a charge forming agents. All the solvents, acetone and ethanol are the highest purity available. Naproxen as a model drug was a gift from a local vender. DI water with 18.2 MΩ cm conductivity (Milli pore milli Q) was used throughout the experiment. All of the chemicals used in this study were used without further purification.

2.2. Synthesis of core-shell nanoparticles

p(AN-c-4-VP) core-shell nanoparticles were synthesized by microemulsion polymerization by the modification of previously reported method [11,12]. Briefly, polymerization and simultaneous crosslinking was accomplished in a 30 ml vial equipped with a magnetic stir bar using 0.1 M 20 ml SDS solution in water. In a typical one pot one-to-one (1:1) mole ratio of AN to 4-VP core-shell nanoparticles synthesis as follows: 0.175 ml AN was dissolved in 0.1 M 20 ml SDS solution and 10 mol % EGDMA with respect to total monomer amounts was added. After vortex mixing, an equal amount of 4-VP (2.658 mmole) was added to the solution and mixed thoroughly to obtain an isotropic clear solution. The mixture was then placed in temperature controlled oil bath at 75 °C under constant stirring (550 rpm) for 10 min, and the polymerization was initiated by addition of 1 ml of 1 mol % APS solution in water with respect to total monomer amount. The polymerization was carried out for 8 h unless otherwise stated. All nanoparticles were purified by centrifugation (10 000 rpm for 20 min) at 20 °C, followed by removal of the supernatant solution and redispersing with copious DI water and centrifugation at least three times. Then the product was dried in air and kept in closed container for further use. The conversion was low and found as % 45.3 ± 3.8 measured by gravimetrically.

2.3. Synthesis of ferrite coated p(AN-c-4-VP) nanoparticles

The magnetic ferrite metal particles were synthesized according the recipe reported in the literature with some modifications [12]. In brief, both $FeCl_2$ (0.43 g) and $FeCl_3$ (1.168 g) were dissolved in 20 mL DI water with the aid of ultrasonic bath. The mixture was vigorously stirred under nitrogen gas at 85 °C and 1.5 mL aqueous ammonia (26%) was added slowly into the solution under vigorous stirring to obtain good dispersion of the particles. The color of solution turned from orange to black immediately. Stirring was then stopped and strong magnet was used to settle the black precipitate. The magnetite precipitates were washed twice with deionized water and once with 0.02 M sodium chloride by magnetic decantation. The magnetic Fe_3O_4 nanoparticles obtained were in the average size of 8–10 nm as reported [13]. Nanosized magnetite particles was dispersed in 10 ml deionized water with ultrasonic bath and 100 μl of this ferroliquid was used for composite core-shell particle preparation by adding to the particle precursor (1:1 mol ratios of AN:4-VP containing EGDMA in SDS solution at 75 °C) before the addition of APS solution to initiate the polymerization and crosslinking reaction. The polymerization was carried out 8 h and the composite particles were washed as described earlier (2.2).

2.4. Amidoximation reaction

Amidoxime group containing p(AN-c-4-VP) based core-shell particle were obtained by conversion of hydrophobic nitrile groups to hydrophilic amidoxime groups. The excess amount of 10 wt.% of $NH_2OH.HCI$ aqueous solution (10 fold of nitrile groups in the nanogel based on the 50% conversion of synthesized particle) was neutralized by 1: 1 mol ratio of NaOH solution, and reacted with the polymeric particle with stirring continuously at 300 rpm for 24 h at ambient temperature.

2.5. Quaternization of p(AN-c-4-VP) based particles

The p(AN-c-4-VP) based particles were reacted with 2-bromoethylamine in ethanol to quarternize (to form positive charge on nitrogen of 4-VP) the particles at ambient temperature. Briefly, the excess amount of $NH_2CH_2CH_2Br.HBr$ solution was neutralized by 1:1 mol ratio of KOH solution in ethanol with stirring continuously at 500 rpm and reacted with p(AN-c-4-VP) particles for 24 h at ambient temperature. The particles were denoted as p(AN-c-4-VP)-BEA and purified as described earlier (2.2).

BEA functionalized p(AN-c-4-VP) based particles were further reacted with 0.1 M HCl to protonate the amine groups at the one end of BEA to form p(AN-c-4-VP)-BEA-H. Magnetic p(AN-c-4-VP) based particles were also reacted with BEA ascribed earlier to form magnetic particles with positive charge. And again, secondary protanation was done by excess amount HCl to develop more charges on the modified 4-VP moieties.

2.6. Drug loading and in vitro release

To load the drug, Naproxene, a weighed amount (0.5 g) of the 4-VP based particles (unmodified, modified, or magnetic

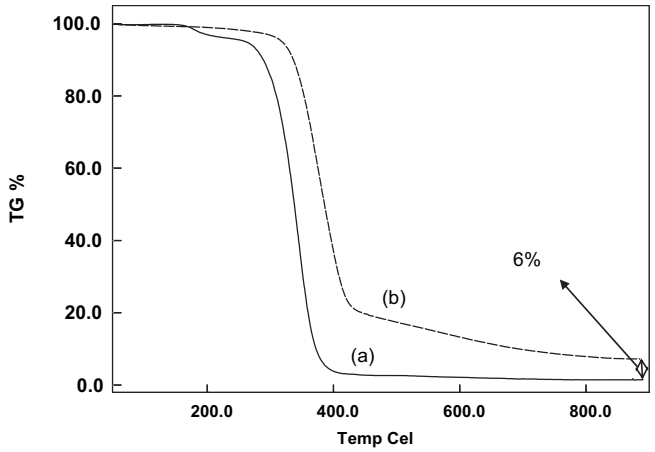

Fig. 4. The thermograms of p(AN-c-4-VP) (a), magnetic particles containing p(AN-c-4-VP) composites (mag- p(AN-c-4-VP) (b).

composites) were placed in 200 ml 2000 ppm naproxen aqueous solution for 24 h at ambient temperature. After loading period, the particles were purified with centrifugation and dried at 35 °C for 24 h. To investigate the release characteristic, 100 mg naproxene loaded nanoparticles were suspended in 5 ml of phosphate buffered saline (PBS) at pH = 7.4 and transferred to a dialysis membrane (molecular weight cut off <12 000 Da, Aldrich). The release sack was then situated into a flask containing 95 ml of PBS. Released amount of the drug into the PBS buffer under constant stirring (500 rpm) was evaluated by UV–spectrometer (T80 + UV/VIS Spectrometer, PG Ins. Ltd.) at 330 nm as a function of time. All experiments were conducted in triplicates and the data presented were averaged values with standard errors. At certain time intervals, samples were withdrawn from the release medium and the release amount was determined from a previously constructed calibration curve at 330 nm in PBS considering the dilution effect.

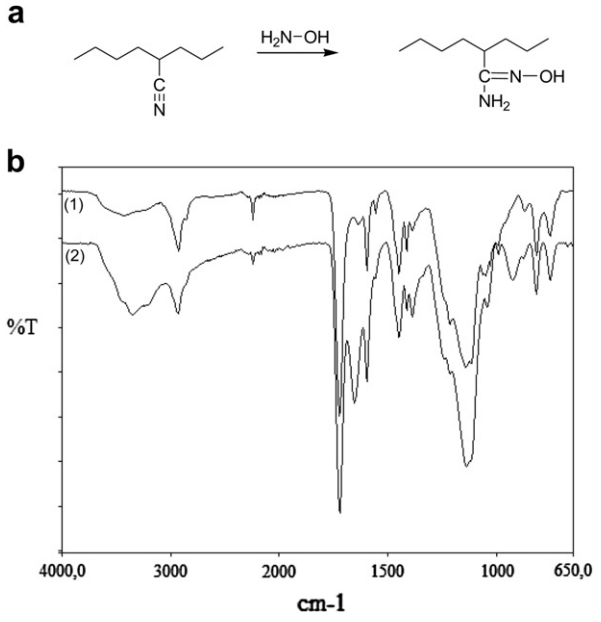

Fig. 5. (a) The schematic representation of the amidoximation reaction mechanism, (b) FT-IR spectra of (1) p(AN-c-4-VP) and (2) amid-p(AN-c-4-VP).

2.7. Particle characterization

2.7.1. Dynamic light scattering (DLS) and SEM Analyses

The sizes and the polydispersities of the synthesized nanogel particles were determined by using particle size analyzer (Dynamic Light Scattering, Brookhaven Ins. & cor. 90 plus). Nanoparticle samples were diluted with 0.01 M KNO_3 solution in water and allowed to equilibrate at each temperature for 1 min before data collection. Each data presented in this investigation was the average value of five consecutive measurements with 20-s integration time. All measurements were made at 90° angle and used 35 mW solid state laser detectors with operating at 658 nm.

The SEM images of the particle were acquired from powder samples deposited sample on carbon tape attached on aluminum SEM stubs after Au coatings to a few nm under vacuum with SEM (Jeol JSM-5600 LV) operating with 20 kV.

2.7.2. FT-IR Spectroscopy studies

The FT-IR spectra of p(AN-c-4-VP) and its modified particles were recorded with a FT-IR spectrometer (Perkin Elmer spectrum 100), in the spectral range 4000–650 cm^{-1} with a resolution of 4 cm^{-1} using with ATR technique.

2.7.3. Thermogravimetric analysis

Thermal behavior of p(AN-c-4-VP) and its particles were investigated using a thermogravimetric analyzer (SII TG/DTA 6300, Japan). About 4 mg of powdered sample was placed in ceramic crucibles and analyzed over the temperature range of 100–900 °C at 10 °C min^{-1} heating rate under the dry flow of N_2 at the rate of 100 mL min^{-1}.

3. Results and discussion

The reaction scheme shown in Fig. 1 represents the magnetic p(AN-c-4-VP) (sub)micron particle formation in SDS micelles upon temperature induced free radical polymerization of the corresponding monomers with APS at 75 °C. Due to their unique properties of p(4-VP), there has been an increasing interest in the 4-VP based particles and polymers [14]. Therefore here, copolymeric nanoparticles containing AN and 4-VP as p(AN-c-4-VP) were synthesized and made responsive to various environmental factors. The particles were chemically modified to tune amphiphilicity to induce multiresponsiveness, and made magnetic field sensitive by enclosing magnetic metal nanoparticles. The main focus of this study was to design core-shell type particles and its' composite with different morphology amenable to external magnetic field, with tunable chemical structures in terms of charge and hydprohilicity.

As illustrated in Fig. 2(a) the distinct peaks at 2240 cm^{-1} for p(AN) and 1598 cm^{-1} for p(4-VP) are exist in p(AN-c-4-VP) FT-IR spectra conforming the copolymer particle formation. And Fig. 2(b) is the SEM images of p(AN-c-4-VP) particles (1:1 mol ratio). Since SEM images were obtained in dry stated the dimension of the particles are smaller that swollen states that will be reveal in detail with modification and DLS studies in the following sections.

The Fe_3O_4 nanomagnetic particles were prepared using precipitation technique which involves the Fe^{+2} and Fe^{+3} salts in the ratio of 1:2 under alkaline and inert condition:

$$Fe^{2+}(aq) + 2Fe^{+3}(aq) + 8OH^- \xrightarrow[NH_3]{H_2O} Fe_3O_4 + 4H_2O$$

Fig. 3 illustrates the digital camera images of (a) p(AN-c-VP) particles, (b) the magnetic Fe_3O_4 particles, and Fe_3O_4 containing p(AN-c-VP) particles as composite (mag-p(AN-c-VP) in water. As can

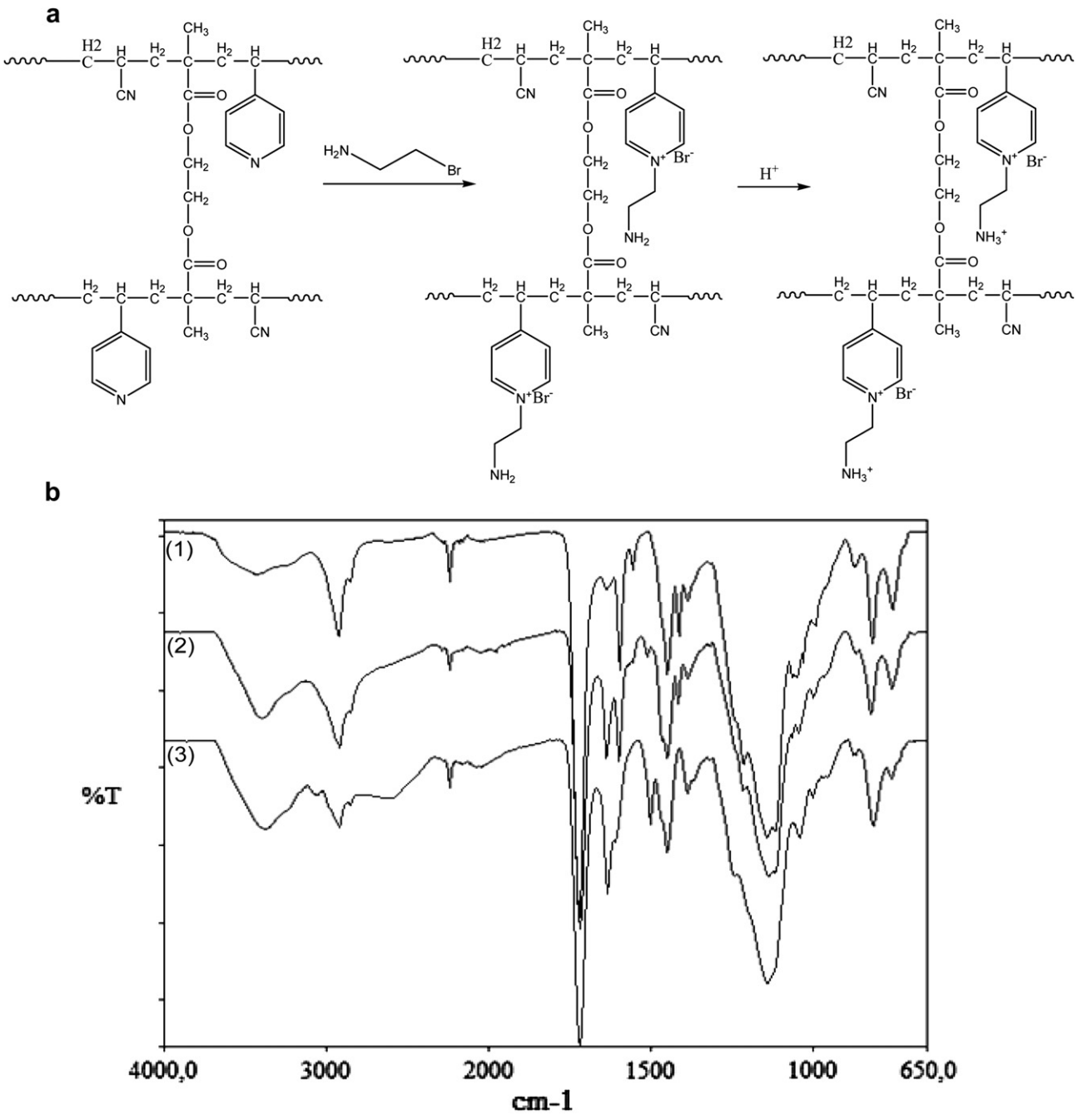

Fig. 6. (a) The schematic of the quarternization reaction mechanism, (b) the FT-IR spectra and of p(AN-c-4-VP) (1), functionalized p(AN-c-4-VP) with 2-bromoethylamine (2) as (p(AN-c-4-VP)–BEA), further functionalization of (2) with hydrochloric acid as (3) (p(AN-c-4-VP)–BEA-H).

be seen from (d) of the same figure, mag-p(AN-c-VP) composites can be directed with and externally applied magnetic field.

To determine the amount of metal nanoparticles inside p(AN-c-VP)-composites, thermogravimetric studied were carried out on bare p(AN-c-VP) and p(AN-c-VP)-composites as shown in Fig. 3.

The weight loss of both p(AN-c-VP) and mag-p(AN-c-VP) composites were measured with heating up to 900 °C with 10 °C/min heating rate under nitrogen atmosphere. The TG analysis revealed that the composites particles contain almost 6 wt.% ferrite particles as shown in Fig. 4. The TG measurements were performed under nitrogen atmosphere to prevent oxidation of further oxidation of magnetic particles and to prevent reaction with polymeric materials.

One of the components of p(AN-c-4-VP) is AN which can be chemically modified to have different chemical structure resulting in considerable change in the physical and chemical properties of the modified p(AN-c-4-VP). The hydrophobic nitrile groups in AN of p(AN-c-4-VP) were readily converted to hydrophilic amidoxime groups that are pH responsive [11,12]. The scheme of the corresponding amidoximation reaction is shown in Fig. 5 (a),) and the FT-IR spectra of p(AN-c-4-VP) as (1), and its amidoximated form (amid-p(AN-c-4-VP were shown in (b) as (2). As can be seen from the spectra of p(AN-c-4-VP), the bands for nitrile groups at 2240 cm^{-1} and pyridine ring at 1598 cm^{-1} peak can clearly be seen. After amidoximation reaction, (2) in Fig. 5(b), the nitrile peak was dramatically reduced. It is also clear from Fig. 5(b) (2) that the

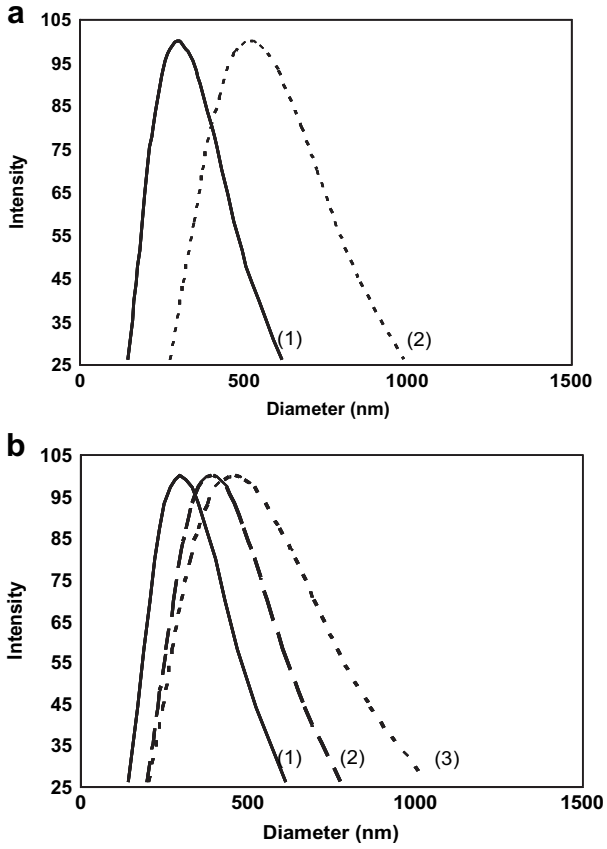

Fig. 7. (a) particle size distribution of p(AN-c-4-VP) before (1) 300 nm, and after (2) 520 nm with amidoximation reaction as amid-p(AN-c-4-VP), (b) particle size distribution of p(AN-c-4-VP) (1) 300 nm, with 2-bromoethylamine functionalization (2) 394 nm to form (p(AN-c-4-VP)-BEA), and further functionalization of (2) with HCl (3) 460 nm to form (p(AN-c-4-VP)-BEA)-H).

appearance of a new adsorption peaks at about 1654 cm^{-1} corresponds to the stretching vibration of −C═N bonds and splitting about 1550−1650 for stretching frequencies for newly formed amid groups (amide-I and amide-II). The results evidently show the conversion of the original nitrile groups to amidoxime groups through the treatment with hydroxylamine.

The other components in p(AN-c-4-VP) is 4-VP that can also be modified to induce unique chemical and physical properties to the modified form of p(AN-c-4-VP) [14]. Fig. 6(a) illustrates the

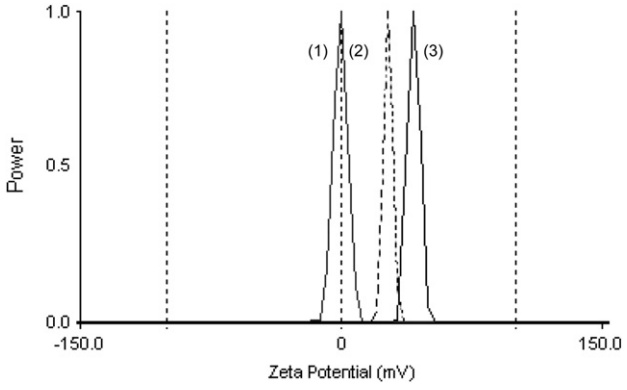

Fig. 8. Zeta potential measurements of p(AN-c-4-VP) (1), p(AN-c-4-VP)-BEA (2), and further functionalized (2) as p(AN-c-4-VP)-BEA-H (3). The zeta potentials are 0.68, 26, and 40 mV, respectively.

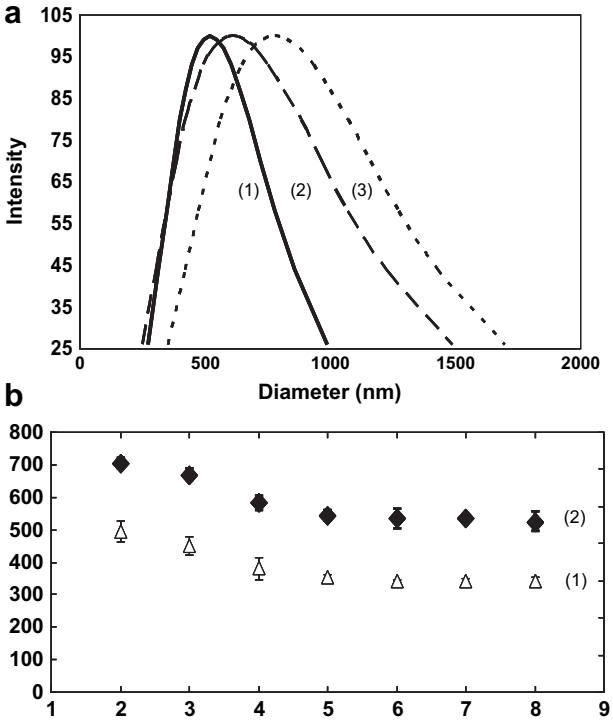

Fig. 9. (a) Particle size distribution of (1), amid-p(AN-c-4-VP) = 520 nm, (2) amid-p(AN-c-4-VP)-BEA = 613 nm, and (3) amid-p(AN-c-4-VP)-BEA-H = 779 nm (b) the pH dependence of hydrodynamic diameters of p(AN-c-4-VP) (1), amid-p(AN-c-4-VP) (2) particles.

chemical modification of 4-VP moieties in p(AN-c-4-VP) particles with 2-BEA to produce p(AN-c-4-VP)-BEA (generation of one positive charge) and further treatment of p(AN-c-4-VP)-BEA with HCl to produce p(AN-c-4-VP)-BEA-H (development of second positive charges) on the pyridinyl groups. Fig. 6(b) shows the FT-IR spectra of p(AN-c-4-VP) (1), and p(AN-c-4-VP)-BEA as formation of one positive charge on every 4-VP ring as p(AN-c-4-VP^{+}) in (2) and, further functionalization of p(AN-c-4-VP^{+}) with hydrochloric acid treatment to form p(AN-c-4-VP)-BEA-H as p(AN-c-4-VP^{++}) in (3).

The most important distinction between these three spectra is the increasing intensity of a band at 3400 and 2589 cm^{-1} (N−H stretching vibrations) with the quarternization of p(AN-c-4-VP) particles. It is clear from (2) spectrum that upon the introduction 2-BEA onto 4-VP moieties of p(AN-c-4-VP) particles, the peak formation at 1636 for the bending vibration and the two bands for amide I and amide I between 1650 and 1500 are obviously noticed. With the further treatment with HCl to generate double charges on p(AN-c-4-VP)-BEA resulted $-NH_3^+$ formation of the free amine. The increase in the intensity of corresponding peaks were shown in (3) of Fig. 6(b). A new peak was formed about 1500 cm^{-1} for $-NH_3^+$ was noticeable. The other peaks were confirmed with FT-IR atlas are consistent with the functional groups on both p(AN-c-4-VP) based particles.

To validate the effects of chemical modifications of each components of p(AN-c-VP) particles on the particle size and charge, particle sizes and zeta potential measurements were performed before and after modification reactions. Fig. 7(a) illustrates the change in size p(AN-c-VP) particles after AN modification (amidoximation reaction). DLS measurements revealed that particle size distribution of p(AN-c-4-VP) before amidoximation reaction was 300 nm (1) from the Fig. 7(a), and upon amidoximation reaction the particle sizes were increased to 520 nm due to formation hydrophilic amidoxime groups that are also responsive to pH.

The other component, 4-VP, modifications of p(AN-c-VP) particles were also performed and found out that the modified p(4-VP) derivatives greatly influenced the particles sizes and charges. As demonstrated in Fig. 7(b) particle size distribution of p(AN-c-4-VP) (1), 300 nm change to 394 nm with 2-BEA functionalization by the formation of a charge (p(AN-c-4-VP)BEA) shown as (2), and after further treatment of this p(AN-c-4-VP)-BEA with HCl to obtained p (AN-c-4-VP)-BEA-H particle size increased to 460 nm shown as (3) in the same figure.

Zeta potential measurements also confirm the positive charge formation on the p(AN-c-4-VP) particles as shown in Fig. 8. The protonation of the pyridinyl ring, as demonstrated with FT-IR spectra, can be conveniently monitored by zeta measurements due to positive charge formation on the particles. Without any treatment, p(AN-c-4-VP) particles are almost neutral, 0.68 mV as illustrated in Fig. 8 (1). And upon reaction of 2-BEA with p(AN-c-4-VP) (2) to generate p(AN-c-4-VP)-BEA, the charges of the particles increased to +26 mV. The further treatments with HCl to protonate $-NH_2$ groups of (2) with produce more positive charges on the particles as p(AN-c-4-VP)-BEA-H were successful and the zeta potential value of this particles were increased significantly to +40 mV.

To investigate the simultaneous effect of both component modifications on p(AN-c-4-VP) particles, the DLS measurement were also performed on amid-p(AN-c-4-VP) and its 2-BEA treated form as amid-p(AN-c-VP)-BEA particles and then further treatment with HCl to form amid-p(AN-c-VP)-BEA-H. The increase in the particle size of modified p(AN-c-VP) were demonstrated in Fig. 9(a) as follows: The amid- p(AN-c-4-VP) particle size was 528 nm indicated as (1), and amidoximated p(AN-c-4-VP)-BEA particle size was 613 nm designated as (2), and the amidoximated p(AN-c-4-VP)-BEA-H particle size was 779 nm appointed as (3). It is apparent that the 300 nm p(AN-c-VP) particle sizes can be tuned up to 779 nm and the charges from almost 0 mV–40 mV by chemical modification. Due to the hydrophilic nature of amidoxime groups and the charge–charge interactions on the polymeric particle network this additive increase in particle sizes were anticipated.

The modification of functional groups on the nanonetwork provides an extra benefit to the materials such as fine tunability of hydrophilicity and hydrophobicity of the particle network. Therefore, the nitrile (hydrophobic) groups were converted to amidoxime (hydrophilic) groups by amidoximation reaction increased the swelling degree of the particles and made responsive to pH. Fig. 9 (b) demonstrated the change in particle size with pH of the medium obtained by DLS measurements. As very well known p(4-VP) is a weak base (K_b for pyridine is 1.5×10^{-9}) dissociating electrolytes and the degree of dissociation depends on pH of the solution. Thus, the change in pH of the solution where particles are formed can affect the size and the net charges of the nanogels. The variations in the size of the nanoparticles in different pHs of the solutions were measured and demonstrated in Fig. 9(b) as (1) for p (AN-c-VP) and as (2) for amidoximated p(AN-c-VP). The pH of the solutions were arranged by addition of HCl and NaOH in 100 ml 0.01 m KNO_3 in which all the DLS measurements were conducted. As can be seen from the figure, in acidic media (lower pHs) the particles are protonated and sizes were increased due to charge repulsions. And with the amidoximation reaction the particle size shifted almost 200 nm higher than the original size. The variations on the particle sizes of p(AN-c-4-VP)-BEA and p(AN-c-4-VP)-BEA-H with pH were also observed (data is not shown).

To demonstrate the potential use of p(AN-c-4-VP) based particles as guidable and modifiable avenue in the application of drug delivery devices (DDD), the magnetic metal nanoparticles were embedded in polymeric p(AN-c-4-VP) nanoparticles and utilized in the release studies of a model drug. Drug release from nanoparticles with magnetic feature as a targetable drug carrier can be accomplished and controlled outside of body by applying external magnetic field [7,8]. Naproxen used as a water soluble model drug which is a nonsteroidal anti-inflammatory active agent and the chemical structure is given in Fig. 10(a). The particles were placed in concentrated Naproxene solution in DI water to load the particles with drug as described in Section 2.6, and than placed in semi-permeable membrane (MW cut off <12 000) in PBS for release studies. Briefly, 100 mg of the nanoparticles were resuspended into 5 ml of PBS solution (pH = 7.4) and transferred to a dialysis membrane then membrane was positioned into 95 ml PBS solution. The released amount of drug was determined at 330 nm with previously obtained calibration curve at the same wavelength in PBS. Every measurement was repeated three times and their averaged values with standard deviations were given. The released amount of Naproxen with time from both p(AN-c-4-VP), and mag-p(AN-c-4-VP) was demonstrated as (1) and (2) in Fig. 10(b).

Both particles released very little amounts of drug almost 4 and 5 mg per gram dry particles. However, as the p(AN-c-4-VP) are chemically modifiable, the amidoximated particles showed almost

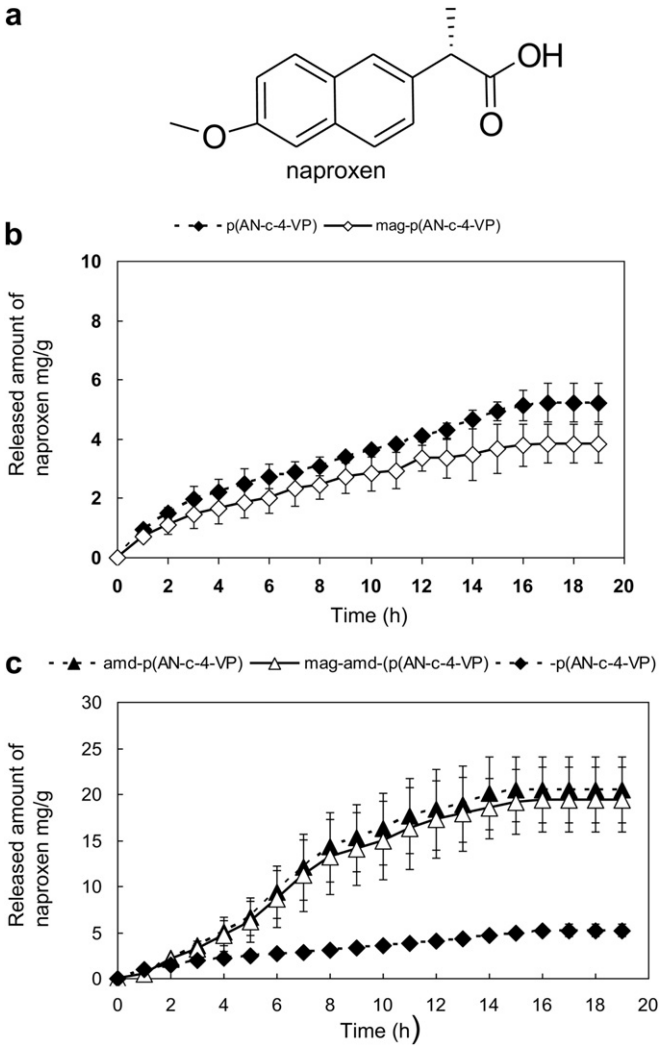

Fig. 10. (a) The chemical formula of a model drug, Naproxene. (b) and (c) the Naproxen release profile from p(AN-c-4-VP) based particles. [Naproxen loading: 500 mg particles in 200 mL 2000 ppm naproxen aqueous solution for 24 h at ambient temperature. Release medium: 100 mL PBS, pH 7.4 with 100 mg drug loaded particles].

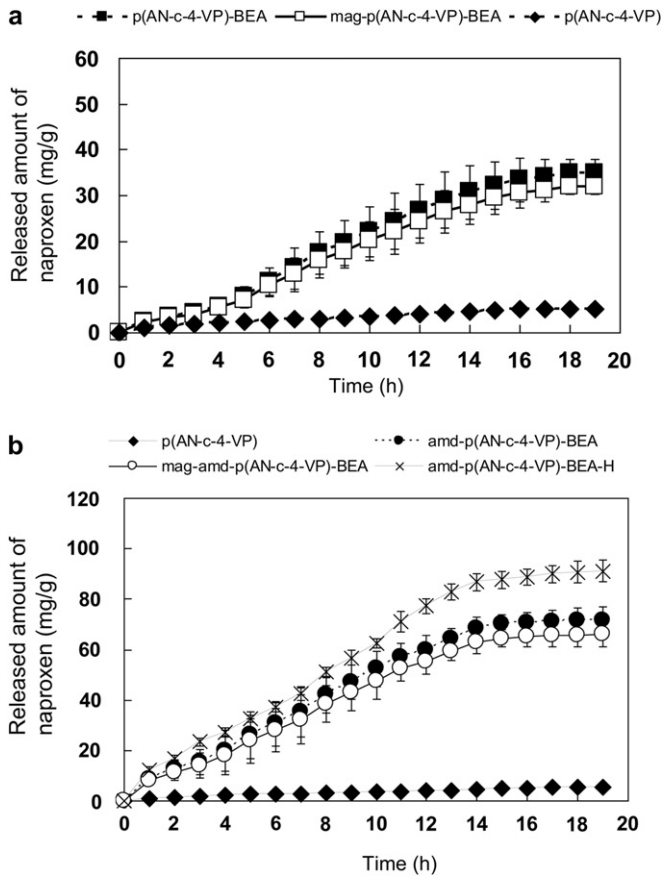

Fig. 11. (a) and (b) Naproxen release profile from modified p(AN-c-4-VP) based particles. [Naproxen loading: 500 mg particles in 200 mL 2000 ppm naproxen aqueous solution for 24 h at ambient temperature. Release medium: 100 mL PBS, pH 7.4 with 100 mg drug loaded particles].

4 fold (20 mg Naproxen per gram particle) increase in the released amounts as illustrated in Fig. 10(c). And it took almost 15 h to release the loaded hydrophilic drug in PBS. It is important to note that the same drug loading procedure was applied to all particles studied in this investigated. Therefore, due to the hydrophilicity of the particles after modification, more amounts of drug could be incorporated into particles nanonetwork.

To validate further usage of these tunable particles as targetable and modifiable DDD, we also studied the drug release of further modified particles obtained with BEA treatments. Fig. 11 emphasizes that the released amount of drug was increased from the following order: p(AN-c-VP) (placed in every drug release graphs for comparisons) < mag-amid-p(AN-c-VP)-BEA < amid-p(AN-c-VP)-BEA < amid-p(AN-c-VP)-BEA-H. The most sticking feature observed from this investigation is the increased amount of Naproxen release with the increase amount charge and the hydrophilicity from the modified particles. It is very important to note that bare p(AN-c-VP) particles can only release almost 5 mg drug per gram particle whereas after modification this amount was increased to almost 90 mg (~14 fold). This is undoubtedly a significant accomplishment and clearly demonstrates the versatility and resourcefulness of these particles.

4. Conclusions

Here, we demonstrated p(AN-c-4-VP) particles synthesis in a single step and made responsive to magnetic field by inclusion of previously prepared magnetic Fe_3O_4 particles during particle polymeric preparation. The obtained particles were expected to assume core-shell morphology as AN is more hydrophobic than 4-VP. During the polymerization, AN was located in the core of SDS micelles and more hydrophilic monomers were presumed to be at the periphery of the micelles [11,12]. In addition to the newly introduced magnetic behavior, every component of the synthesized p(AN-c-VP) particles were subjected to modification to tune hydrophilicity and the charge of the particles. The particles were amendable in terms of chemical and physical properties as AN and 4-VP chosen for particle forming monomers. Both of these components in p(AN-c-4-VP) and magnetic p(AN-c-4-VP) particles were successfully modified to various charges, and hydrophilic degrees making these particles tunable in dimensions, hydrophilic/hydrophobic degree, and made multiresponsive against pH, ionic strength, solvent and so on conferring new physical and chemical properties. Furthermore, it was demonstrated that a hydrophilic drug, naproxene loading into bare p(AN-c-4-VP) particles was trivial (only about 5 mg per gram particles), but, after chemical modification the loading of this drug increased almost 14 fold (almost 90 mg per gram particles).

Polymeric particles capable of modifications and containing amidoxime groups and various charges with magnetic property is one of the most important and useful feature of these types of particles and have great potentials in biomedical fields.

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