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Colloidal drug carries from (sub)micron hyaluronic acid hydrogel particles with tunable properties for biomedical applications

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ABSTRACT

Hyaluronic acid (HA) hydrogel particles were synthesized in a single step employing water-in-oil microemulsion system. The HA particles were formed in the micro-environments of aqueous HA solution in oil by chemical crosslinking with divinyl sulfone (DVS). To produce magnetic field responsive HA-composite particles, iron magnetic nanoparticles were introduced into microemulsion system during synthesis to obtain HA-magnetic composites. For this purpose, iron nanoparticles were separately synthesized and mixed with linear HA followed by chemical crosslinking of linear HA with DVS in the micro-environments to envelope magnetic metal nanoparticles in the emulsion system. Scanning electron microscopy (SEM), dynamic light scattering (DLS) studies, and zeta potentials measurement were performed for particle size, charge and morphological characterization. Additionally, HA particles were chemically modified to induce desired functional groups on the particle surface and utilized for potential drug delivery vehicles. Trimethoprim (TMP) a bacteriostatic antibiotic drug were used as a model drug for the release studies in phosphate buffer solution (PBS) at pH 7.4 from bare HA, magnetic HA-composite and modified HA hydrogel particles.

1. Introduction

Due to their unique properties, such as excellent biocompatibility, high water retention ability and permeability, hydrogels have found a variety of applications especially in the biomedical fields including drug delivery systems, replacement of blood vessels, wound dressings, soft tissue substitutions, contact lenses and in many other bio-related areas (Francis, Kumar, & Varshney, 2004; Hoffman, 2002). The natural polymer, hyaluronic acid (HA) is a linear polysaccharide composed of alternating disaccharide units of D-glucoronic acid and N-acteyl-D-glucosamine with β-(1,4)interglycosidic linkage. The functions of HA is immense in human, and have many important roles such as in pressure stability of cartilage, in cell adhesion and cancer metastasis, and as a lubricant in joints, and so on. Many synthetic and naturally derived materials have been reported to form well characterized hydrogels. One of these natural materials is HA. HA is polyanionic biopolymer and is one of the main components of the extracellular matrices found in many tissues. HA has been widely studied as hydrogel forming polymer in the applications of pharmaceuticals, drug delivery systems, cosmetics, and tissue engineering and even for regenerative therapy (Lin, Ren, & Ji 2009; Luo and Prestwich, 2001; Rouse, Whateley, Thomas, & Eccleston, 2007; Tian et al., 2005). Due to its unique chemical, physicochemical characteristics, and distinctive biological functions, HA is intriguing and could be an ideal biomaterial to design advanced systems as it plays beneficial roles in cell adhesion, motility, inflammation, wound healing, cancer metathesis and so on (Enstwistle, Hall, & Turley, 1998). In addition, chemical modification of HA could provide new functions with desired traits and physicochemical properties for compound applications i.e., devising drug delivery systems and designing templates for tissue engineering (Bodnar et al., 2009; Luo, Kirker, & Prestwich 2000; Prestwich, Marecak, Marecek, Vercruysse, & Ziebell, 1998).

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During the past few years, iron oxide nanoparticles both magnetite (Fe_3O_4) and magnetite (γ-Fe_2O_3) have been investigated for potential biomedical applications, especially in drug delivery system (Dobson, 2006; Yan, Li, Zhang, Liu, & Yang, 2009). Due to the magnetic responsiveness of ferrites (Fe_3O_4 and γ-Fe_2O_3) that can be guided with the applied external magnetic field to deliver composite magnetic particles in vivo to specific sites for targeted and controlled delivery of their cargos. Modifiable hydrogel-composites particle are of great significance (Dobson, 2006; Luo & Prestwich 2001; Rouse et al., 2007).

HA has carboxylic acid and hydroxyl groups that can be easily modified under mild conditions. In general, various methods have been developed to obtain HA with different functional groups in various forms such as in gels, films and particles (Hahn, Jelacic, Maier, Stayton, & Hoffman, 2004; Jha et al., 2009; Luo, Kirker, & Prestwich 2001; Rouse et al., 2007; Sahiner, Jha, Nguyen, & Jia, 2008; Yan et al., 2009). In this investigation, we synthesized HA and HA-composites, crosslinked to form hydrogel particles using microemulsion system in a single step to expand the usage HA in biomedical field. To the best of our knowledge, as there is only one report for HA-magnetic nanoparticles and their use for targeted delivery of peptides (Kumar et al., 2007); here, we demonstrated a facile method for magnetic metal particle encapsulation to attain magnetic responsive HA-composites by introducing magnetic metal nanoparticles inside HA hydrogel particles and utilized as drug carrier that can have potential use as targetable delivery systems under externally applied field in certain organisms. Additionally, we report the post-chemical modification of HA particles to acquire different chemical functional groups on the surface of HA particles. We also demonstrated that bare HA, magnetic HA-composite and modified HA particles can be used as drug carriers. Drug release studies were performed in phosphate buffer saline solution (pH = 7.4) at ambient temperature using trimethoprim (TMP) as a model drug.

2. Materials and methods

2.1. Reagents

Hyaluronic acid sodium salt (HA, 98%, Fluka, MW = 1.3 M Da), divinyl sulfone (DVS, 98%, Merck) as a chemical crosslinker, and sodium bis(2-ethylhexyl) sulfosuccinate (AOT, 96%, Fluka) as a surfactant, and 2,2,4-trimethylpentane (isooctane) as a solvent were used as received. Sodium (meta) periodate as a oxidizing agent and sodium hydroxide (NaOH, Sigma–Aldrich), hydrochloric acid (HCl, 37%, Riedel-de haen), Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, Acros), and iron(II)chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, Fluka) and ammonium hydroxide (26%, from Riedel-de haen) were used for the preparation of magnetic particles. 2-Bromoethylamine hydrobromide (99%, Alfa Aesar) and hydrazine monohydrate (Sigma–Aldrich) were utilized for modification of HA. Potassium iodide (Riedel-de haen) and iodine (Fluka) were used for aldehyde test. All the solvents, acetone and ethanol are the highest purity available. Ultrapure distilled water 18.2 MΩ cm (Millipore Direct-Q UV3) was used throughout out the studies. Trimethoprim (TMP) as model drugs were used and obtained from Pfizer.

2.2. Synthesis and modifications of hyaluronic acid particles

HA particles were synthesized using water-in-oil microemulsion system according to the previously reported procedure with some modifications (Sahiner & Jia, 2008). A solution was prepared by dissolving HA in 0.2 M NaOH at a concentration of 0.05 g/mL. Then 0.54 mL of this HA solution was dispersed in 15 mL 0.2 M AOT solution in isooctane. The mixture was immediately vortexed until a clear suspension was obtained. Varying amounts of DVS (20–70% by mole relative to the HA repeating unit) were subsequently added to the HA solution, and the mixture was vortex mixed again to obtain an isotropic solution and dispersion of DVS in microemulsion system. The reaction was allowed to proceed for 1 h at ambient temperature under constant stirring (1200 rpm). The obtained HA particles were precipitated with excess of acetone. The particles were purified by centrifugation (Hermle Labortechnik Z 383 K) at 10,000 rpm for 10 min at 20 °C followed by removal of the supernatant solution and redispersing with acetone and water and recentrifugation at least three times for washing purpose. Then, the product was dried in room temperature and kept in closed container for further use (yield: 90%).

2.3. Chemical modification of HA particle

Crosslinked HA hydrogel particles were treated with $NaIO_4$. It is known that peridoate oxidizes the proximal hydroxyl groups to form two aldehyde groups on HA (Jia et al., 2004). Therefore, to generate aldehyde groups on HA particles, 1 g of HA particles were dispersed in 100 mL 10 wt% of $NaIO_4$ solution in water. The reaction mixture was stirred for overnight at ambient temperature. The particles were purified by washing as described earlier and denoted as A-HA.

To confirm the formation of aldehyde groups on HA particles, an aldehyde assay was carried out. For that goal, 50 mg modified A-HA particles were placed in 1 mL of 5% NaOH solution under continuous mixing, and KI–I_2 indicator is added dropwise until the appearance iodine gas. The solution is mixed 1 h and the formation of yellow precipitates is the positive result for aldehyde groups (indicator: 2 g KI and 1 g I_2 in 10 mL water).

To introduce different functional groups on A-HA particles, further chemical modification experiments were conducted. For this purpose, 1 g of A-HA particles was dispersed in 100 mL of 10 wt% of 2-bromoethylamine hydrogen bromide solution in water. The reaction was stirred for overnight at ambient temperature. The particles were purified by centrifugation as previously described. And the same HA particles treated with 10 wt% hydrazine monohydrate solution, following the treatments with 0.5 M excess HCl to form positive charges on the particles. The modified HA particles were washed several times by centrifugation and stored in clean containers for characterization and drug loading experiments.

2.4. Synthesis of Fe_3O_4 coated HA particles

The magnetic Fe_3O_4 particles were synthesized according the recipe reported in the literature with some modifications and then encapsulated with HA particles (Yan et al., 2009). In brief, both $FeCl_2$ (0.2 g) and $FeCl_3$ (0.61 g) were dissolved in 30 mL deionized water in ultrasonic bath. The mixture was vigorous stirred under nitrogen gas at 50 °C, and 2.5 mL aqueous ammonia (26%) was added slowly into the solution under vigorous stirring to obtain good dispersion of the metal nanoparticles. The color of bulk 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. The obtained Fe_3O_4 nanoparticles average size was reported as of 6–15 nm (Yan et al., 2009). Nanosized magnetite particles were dispersed in 30 mL deionized water with ultrasonic bath. 1 mL of oleic acid was added to stabilize nanoparticles under vigorous stirring for 20 min. 200 μL of this ferro-liquid was used for HA-composite particle preparation by adding to the precursor solution (HA solution) before the addition of DVS to initiate the crosslinking of linear HA. The crosslinking reaction was carried out 1 h and the composite particles were washed by centrifugation.

2.5. Drug loading and in vitro release

A weighed amount (50 mg) of HA based particles were placed in 250 ppm 100 mL drug solution, TMP, in DI water overnight at ambient temperature under constant stirring. After loading procedure, HA hydrogels particles were separated by centrifugation from drug solution and dried at 25 °C. These drug loaded nanoparticles were resuspended into 5 mL of phosphate buffered saline (PBS, pH = 7.4) and transferred to a dialysis membrane (molecular weight cut-off ≥ 12,000 Da, Aldrich) and placed into a releasing flask containing 95 mL of PBS. Released amount of the drug into the PBS buffer was evaluated by UV–vis spectrometer (T80 + UV/vis Spectrometer, PG Ins. Ltd.) at 280 nm as a function of time. All experiments were carried out in triplicates and the results were averaged given with standard deviation. A calibration curve was previously constructed at 280 nm with five different drug solutions obtained by sequential dilution of the drug solution in PBS samples. The loading capacities of TMP to bare, magnetic and functionalized HA particles were also determined by using UV–vis spectrometer. For this goal, UV–vis measurements were also carried out in DI water and the loading amounts were calculated from drug solutions before and the absorption by the HA based particles from the previously prepared calibration curve in DI water.

2.6. Particle characterization

The hydrodynamic diameters, polydispersities and zeta potential of crosslinked HA based particles were determined using particle size analyzer and with zeta potential measurements. DLS (Brookhaven Ins. & Cor. 90 plus) is equipped with 35 mW solid state laser detector at an operating wavelength of 658 nm. Measurements of the average size of particles were obtained at 25 °C with 90° angle detection in PS cuvettes. The samples were prepared with 0.01 M KNO_3 solution in water and were sonicated for 20 min to obtain good dispersion of the particles. Each sample was measured three times and average serial data were calculated with standard deviation.

Scanning electron microscopy (SEM) images were obtained with HA hydrogel particles on SEM stubs at ambient temperature after gold sputtering to a few nm (2–5 nm) thickness, with operating voltage of 10–15 kV (Hitachi S3000 N).

Drug loading and release studies were performed using UV–vis spectrometer.

All of the HA based particles' and newly formed functional groups analysis were confirmed by FT-IR spectrometer with 4 cm^{-1} resolution (Perkin Elmer Spectrum, 100 equipped with ATR).

The TG analyses were performed under nitrogen atmosphere with 100 mL/min flow rate with 10 °C/min heating rate using up to heating 1000 °C with a thermo gravimetric analyzer (SII TG/DTA 6300, Japan).

3. Result and discussion

Hyaluronic acid (HA) as one of the most important components in living organism and found in the different parts of the human body performs various biological functions in cells and tissues. HA plays vital roles in the organization of the extra cellular matrix, cell signaling and proliferation, joint lubrication and cell surface protection and promoting wound healing. As a natural polymer, HA is an attractive material due to its biocompatible and biodegradable nature. Although, HA is an abundant non-sulfated glycosaminoglycan component of synovial fluid and extracellular matrices in human body, it has poor mechanical properties and suffer from short in vivo residence times (Bodnar et al., 2009; Burdick, Chung, Jia, Randolph, & Langer, 2005; Hoffman, 2002).

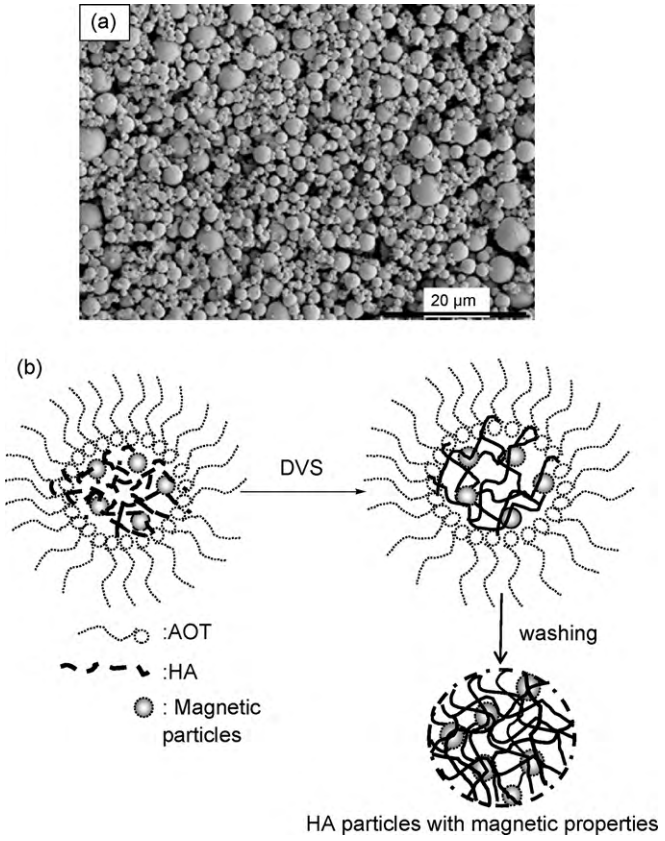

Fig. 1. (a) SEM images of (sub)micron (micron and nano) size HA particles synthesized from natural HA with 50% DVS crosslinker. (b) Schematic illustration of magnetic particle containing HA-composite material synthesis in AOT reverse micelles.

Earlier, we employed one pot synthesis of different HA based hydrogel particles by the crosslinking of linear HA molecules with DVS under basic conditions in reverse micelles (Sahiner & Jia, 2008; Sahiner et al., 2008). It was demonstrated that HA particles, prepared in AOT reverse micelle system, produce nonporous spherical shape particles with sizes ranging from few micrometer to tens of nanometers (Jha et al., 2009). Here, we prepared HA particles and modified chemically to generate different charges by chemical treatment and also endowed magnetic properties by encapsulating magnetic iron particles inside nonporous HA particles in single step in which previously prepared magnetic iron nanoparticles were embedded inside reverse micelle containing linear HA before crosslinking with DVS. Fig. 1(a) demonstrates SEM images of HA particles synthesized inside AOT reverse micelles, whereas Fig. 1(b) shows the schematic presentation of magnetic nanoparticle containing HA-composite particles preparation route. To demonstrate visually that these composite materials can be used as potential guided delivery vehicles, digital camera images of (a) magnetic particles under a magnetic field, (b) HA hydrogel particles, (c) magnetic HA-composite particles in aqueous environment and (d) under magnetic field were illustrated in Fig. 2. As can be seen, these composite materials can interact with magnet and potentially be targeted with an externally applied magnetic field. These kinds of natural and biocompatible magnetic composite materials with tunable characteristic may find important applications in biomedical fields. It is also noteworthy to mention that the ferrite particles were not eluded from the HA particles as the HA matrix is not porous under this synthesis conditions (Jha et al., 2009). The amount of ferrite metal particles inside HA particle can be controlled during the composite material preparation

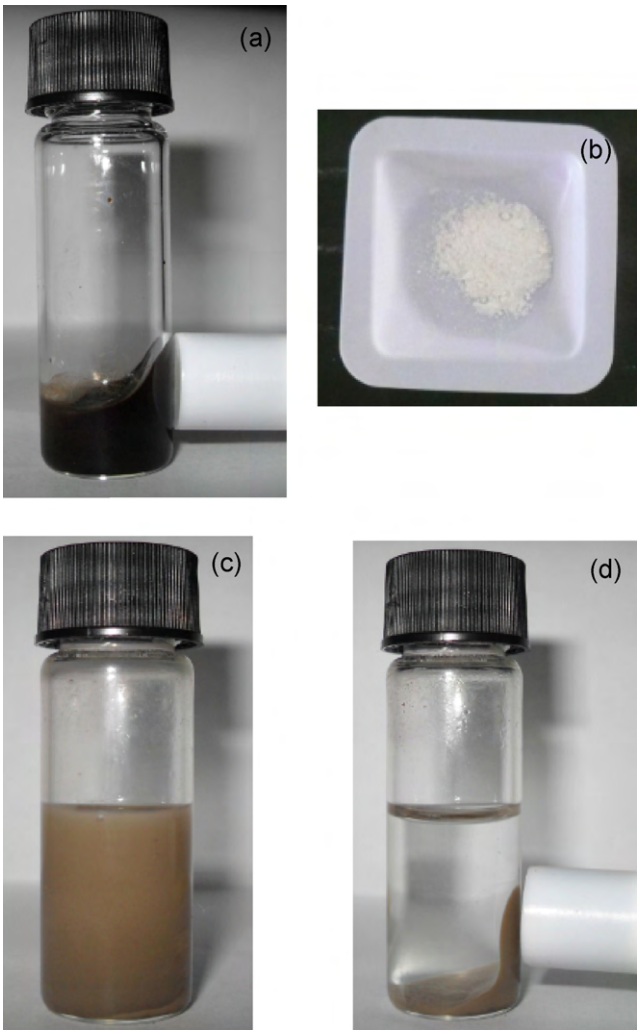

Fig. 2. Digital camera images of (a) ferrite particles, (b) HA particles, (c) ferrite containing HA-composite in suspension, and (d) same composites after externally applied magnetic field.

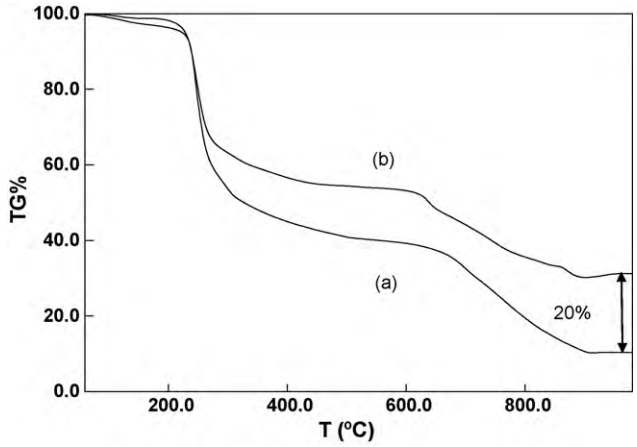

Fig. 3. The TG thermogram of (a) HA particles, and (b) ferrite containing HA-composites.

with the adjusting added amount of ferrite during HA synthesis (before crosslinking with DVS, see Fig. 1(b)). To investigate thermal behavior and to determine the amount of magnetic metal nanoparticles, bare HA particles (a), and ferrite (iron magnetic nanoparticle) containing HA-composites thermogravimetric analysis were performed and their TG thermograms were taken by 10 °C/min heating rate up to 1000 °C as demonstrated in Fig. 3. As illustrated in Fig. 3, the metal nanoparticle (ferrite) content is almost 20 wt%. As seen from the thermograms, there are two distinct degradation temperatures for HA based materials. Whether it is bare HA or HA-composite, both particles degradations start around 200 °C loosing roughly their 40 and 60 wt% at about 250 °C, respectively. Second degradations start again for both particles little over 600 °C and continue up to 900 °C under nitrogen atmosphere. Around 1000 °C bare HA leaves almost 10 wt% waste whereas HA-composite giving off almost 30% waste, therefore; it can be interpreted that the magnetic ferrite content of these composite materials is almost 20% by weight. Previously, we have reported optimum particle synthesis process depending upon the MW of HA, reaction time, feed ratio (Sahiner & Jia, 2008). In this investigation, the MW of HA was 1.3 MDa, and the amount of crosslinker (DVS) was tuned varying from 20 to 70 mol% based on the HA repeating units for magnetic composite syntheses and for other post-chemical modifications. To illustrate the possible usage of HA particles as drug delivery vehicle, a model drug, trimethoprim (TMP), an antibacterial antibiotic medicine was used in the release studies from both bare HA and magnetic HA particles in PBS at physiological pH (7.4). The TMP loading was accomplished from a concentrated aqueous TMP solution whilst the release experiments were performed in PBS buffer at pH 7.4. The amounts of drug loadings were determined from a calibration curve constructed in DI water at 280 nm for all HA based particles. The loadings were found as 100 ± 10, 24 ± 6 mg/g for bare HA, and magnetic HA, respectively which corresponds to 20%, and 4.8% loading efficiencies using UV–vis spectrophotometer. The chemical structure of TMP was shown in Fig. 4(a) and the relative % release profile with time from bare HA and magnetic HA-composites were shown in Fig. 4(b). As can be seen TMP release was faster for bare HA then ferrite containing HA-composite particles. Almost all the loaded TMP, 100 ± 10 mg drug per gram particle, was released from bare HA particles within 200 min, whereas it took HA-composite almost 1200 min to release even lesser amount of loaded drugs, 24 ± 6 mg drug per gram particle. This could be due to interaction of drug molecules with ferrite particles inside HA particles. Additionally, the longer release time can also be attributed

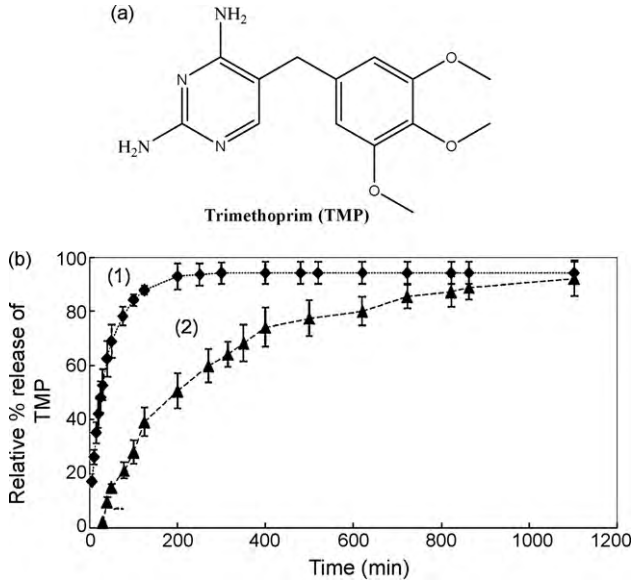

Fig. 4. (a) The chemical structure of the model drug, trimethoprim (TMP). (b) Relative % TMP release profile from bare HA particles (1), and magnetic HA particles at ambient temperature in PBS.

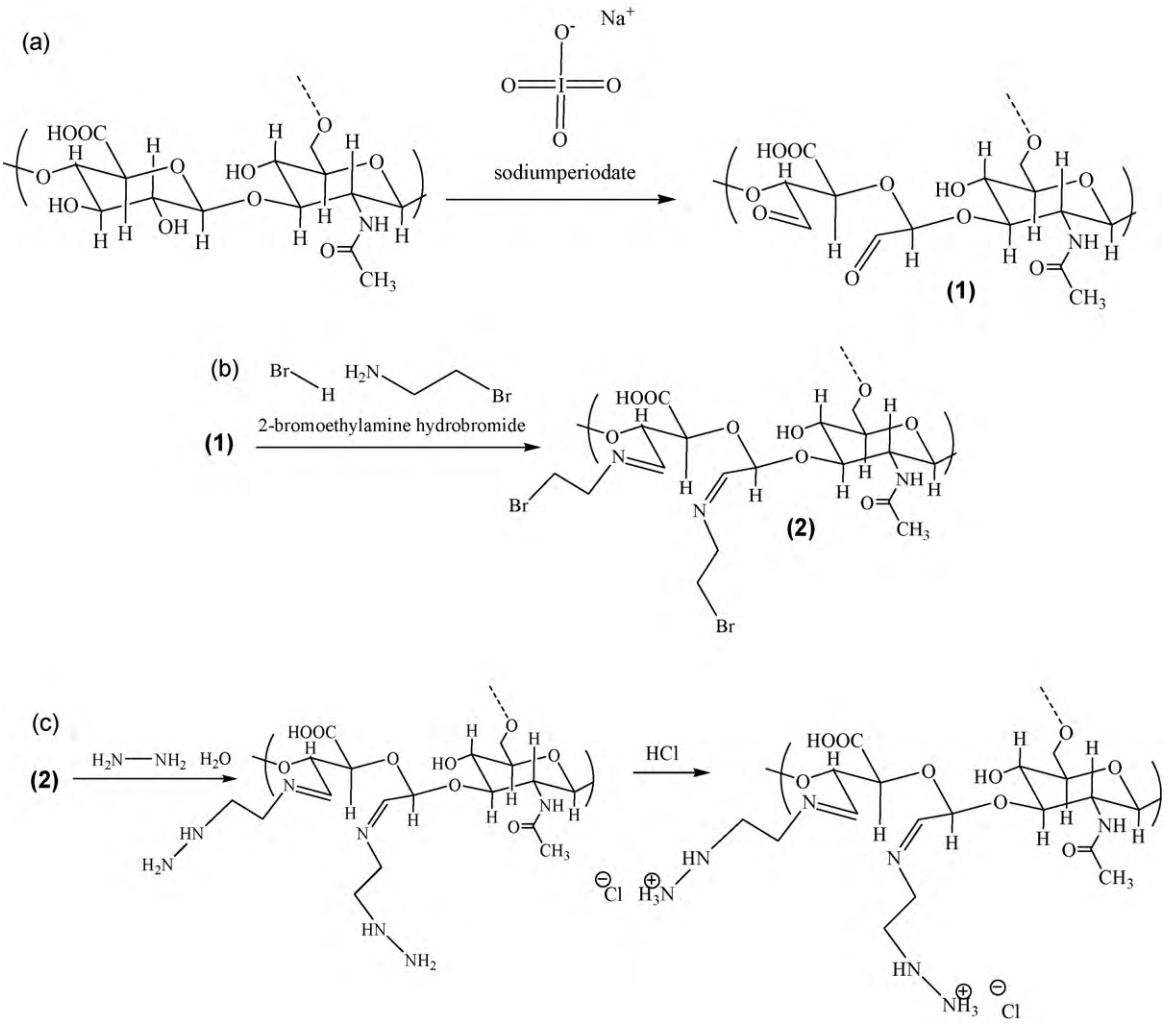

Fig. 5. (a) Oxidation of HA particles with sodiumperiodata to form dialdehyde groups on HA particles. (b) The reaction of aldehyde group containing HA with 2-bromoethylamine in the presence of hydrogen bromide. (c) Hydrazide reaction (N_2H_4) of the modified HA particles to remove bromide and the quaternization reaction to form positive charges on HA particles.

to the formation of additional pores inside the HA particles due to the presence of magnetic ferrites as was reported bare HA particles are not porous (Jha et al., 2009). It is also worth mentioning that the relative % drug release calculations were made based on the loaded amount of TMP. Even though bare HA has more TMP loading capacity (100 ± 10 mg/g), the magnetic HA-composite released less amount of drugs (24 ± 6 mg/g) in a prolonged release time. This is an important surplus asset for in the usage of these composite materials as drug carriers. The released amount per gram HA particles will be compared after chemical modifications of these particles, subsequently.

In addition to providing magnetic property to HA particles, another very important aspect of this investigation is the chemical modification of HA particles. As it is very well known, the surface charge and the existence of functional groups are the key characteristic for any carriers used in biomedical applications. To form additional functional groups on HA particles, post-chemical modification (after formation of HA particles) experiments were carried out. New derivatives of HA by chemical modification can present desired biomechanical and physicochemical properties for spatial and temporal arrangements of biologically active agents such as drugs, proteins, genes, peptides and so on. As HA is inherently biocompatible and biodegradable, the usage of HA with tunable functionality and magnetic properties to engineer new materials is a significant contribution to the bio-related sciences. HA has free carboxyl groups in every repeating unit and free hydroxyl groups some of which were already used for the formation of particles. Also, the vicinal hydroxyl groups allow easy modifications under mild condition (Burdick et al., 2005; Jia et al., 2004; Luo et al., 2001; Prestwich et al., 1998). It is known that aldehyde groups can be readily formed on HA particles by chemical treatment of HA with sodium periodiate that afford oxidation of the proximal hydroxyl groups to aldehyde opening the sugar rings (Jia et al., 2004). The reaction scheme for the generation aldehyde groups in Fig. 5(a), and further treatment with 2-bromoethylamine in Fig. 5(b), and sequential hydrazine and hydrochloric acid treatments to generate positive charge on HA particles were shown in Fig. 5(c). The FT-IR spectra were taken and confirmed the newly formed band stretching frequencies of the functional groups as shown in Fig. 6(a). The peaks that verifies the quaternization reaction 1730 cm^{-1} for 1 min (C=N) stretching and 1550 cm^{-1} for asymmetric bending for $-NH_3^+$, and broad band about 3000 cm^{-1} for N–H in and asymmetric stretching frencies for NH_3 are existed in the quaternized HA particles. Upon quaternization reaction, the particle size was expected to increase due to the positive charge–positive charge interactions. The increase in particle size was corroborated with DLS measurements and the HA particles corresponding size distributions were shown in Fig. 6(b). As can be seen the particles

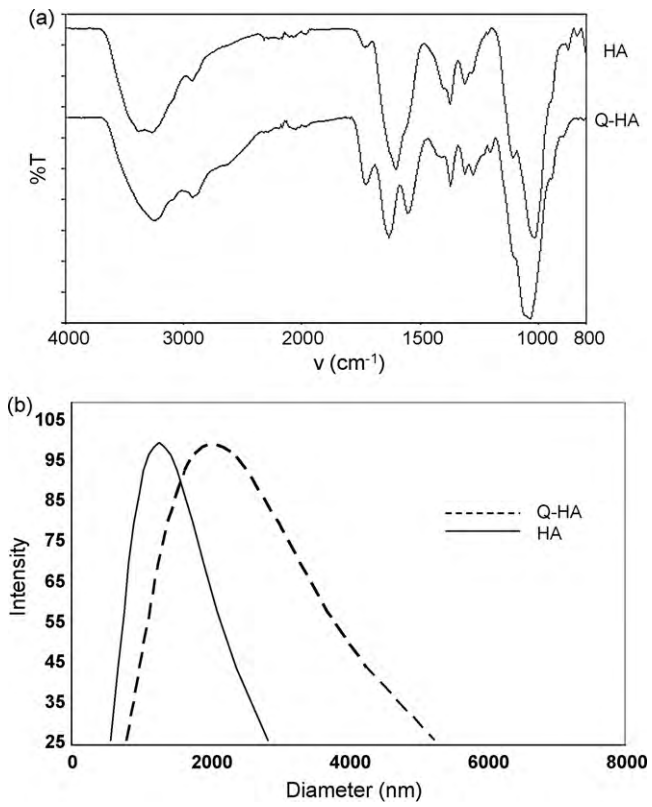

Fig. 6. (a) FT-IR spectra of the bare HA and quaternized HA particles, and (b) the change in size distribution of HA particle after quaternization [HA: 1261 nm and Q-HA 2037 nm].

size distribution was increased from 1261 to 2037 nm by the quaternization reactions (positive charge formation). This is very important for a carrier that desired charges and functional groups can be generated in addition to induced magnetic behaviors for specific applications. Especially natural and biodegradable polymers such as HA, investigated here with tunable characteristic provide additional benefit in their biomedical use.

The zeta potential measurements further substantiate the positive charge formation as demonstrated in Fig. 7(a). The surface charge of bare HA particles were found as −32 mV with zeta potential measurements. After the positive charge formation (quartenization reaction), the surface charge of the modified HA was increase to −11 mV conforming the positive charge formation on the HA particles. The reason for not having zero charges on the HA could be the incomplete reaction of chemical modification and quartenization reactions. Although, hydrazine can readily evolve nitrogen upon heating leaving an alky chain, as proof of concept it was revealed here that positive charge can be obtainable on HA particle surface. This evidence reveals that HA particle can have both positive and negative charges concurrently. Therefore, different charge containing active agents can also be carried by the same carrier as proven with this investigation. To demonstrate the usage of these particles further, a drug, TMP release from positive charged HA particles was investigated and compared with the released amounts of bare HA and magnetic HA-composite. As illustrated in Fig. 7(b), the chemically modified HA released much more TMP than both bare HA and magnetic HA-composite keeping in mind that all the carriers were exposed to same loading procedure (50 mg particles in 250 ppm 100 mL TMP solution in aqueous solution with overnight loading time). The chemically modified HA can load loading 132 ± 7 mg drug per dram of modified HA particle with the loading efficiency of 26.4%. As can be recognized from the chemical structure of TMP (Fig. 4(a)), the drug has basic amine groups that can interact with the positive charges on HA particles in addition to the interactions with free carboxyl groups, therefore; this drug loaded much more onto positively charged HA particles than the other forms of HA. On the other hand, unmodified HA particle shown as (2) in Fig. 7(b) can load much more drug molecules than magnetic HA-composite particles per gram (100 mg/g) shown as (3) in the figure. This is plausible as the number of free carboxyl groups on bare HA particles are greater in number than magnetic HA-composites particles. Therefore, the TMP adsorption capacity of bare HA is much more than magnetic HA particles. The reason for the least amount of drug was absorbed and released from magnetic HA-composites could be due to lesser number of the available sites which was occupied with magnetic metal ferrites. And 20% of the weight of the composite is magnetic particles. Due to the some probable interactions between carboxyl groups and ferrite particles, the accessible numbers of the free carboxyl groups that can also interact with drug molecules are reduced in numbers. Additionally, some the carboxyl groups may interact with ferrites inside HA particles. As can be seen, the modified particle can provide additional advantages for the release of other active agents in term of cumulative release amount of the drug. On the other hand, the magnetic HA-composites absorbed and release lesser amount of drugs with prolonged release time offering another additional advantage. Additionally, the magnetic HA-composite particles are very stable and kept their magnetic behavior up to 6 months. The particles were kept in DI water in a closed container. Modifiable, biocompatible and versatile HA based particles with such a longer storage time capability are appealing and their tunable characteristic make these kinds of materials very promising candidates especially for in vivo targeted delivery endeavors.

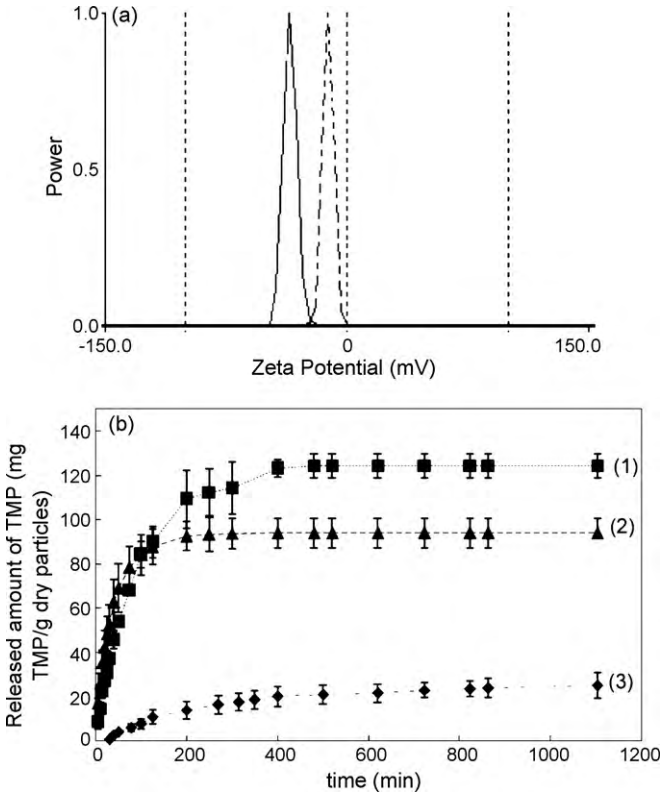

Fig. 7. (a) Zeta potential measurements of bare HA (−35 mV, solid line), and after positive charge formation with modification of HA (−11 mV, dashed line). (b) Drug (TMP) release profile from (1) positively charged HA particle (mg/g), (2) bare HA particles and (3) magnetic HA-composite particles at ambient temperature.

4. Conclusions

In addition to single step synthesis of HA particles, we have demonstrated that HA particles can be made responsive to an external magnetic field and can be used as potential guided drug delivery devices. We further demonstrated that additional functional groups on HA nanoparticles can be formed and employed for the adsorption and release of active agents. These types of carrier based on natural, biocompatible and biodegradable HA with tunable additional characteristics i.e., magnetic responsiveness and new chemical functional groups represent a novel type superior drug delivery devices (DDD) for multitask assignments. For example, various active agents (drug, proteins, DNA, etc) with different charges and hydrophilic/hydrophobic balance can be loaded on these DDD simultaneously, and guided under externally applied magnetic field and release their payloads at the specified sites. In fact, our current investigation involves such research in vitro and in vivo for different active agent carrying systems. The HA carrier presented here have great potentials in biomedical and pharmaceutical fields for targeted delivery systems.

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