Magnetic Nanoparticles Grafted with Cyclodextrin for Hydrophobic Drug Delivery

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A novel magnetic nanocarrier, cyclodextrin (CD)−citrate−gum arabic modified magnetic nanoparticles (GAMNPs), for hydrophobic drug delivery was fabricated by grafting the citrate-modified CD onto the GAMNPs via carbodiimide activation. The analyses of the transmission electron microscopy and the dynamic light scattering revealed that the product had a mean diameter of 14.6 nm and a mean hydrodynamic diameter of 26.2 nm. The CD grafting was confirmed by Fourier transform infrared spectroscopy, and the amount of CD grafted on the GAMNPs was determined to be 28.7 mg/g by the thermogravimetric analysis. The feasibility of using CD−citrate−GAMNPs as a carrier for hydrophobic drug delivery was demonstrated by investigating the formation of the inclusion complex and the in vitro release profile using ketoprofen as a model hydrophobic drug. It was found that CD−citrate−GAMNPs exhibited a considerable adsorption capability for ketoprofen as compared to GAMNPs. The complexation of CD−citrate−GAMNPs with ketoprofen was found to be exothermic and follow the Langmuir adsorption isotherm. Also, the presence of surfactant (sodium dodecyl sulfate, SDS) led to a decrease in the inclusion of ketoprofen because the linear structure of SDS made it easier to enter the cavity of CD as compared with the less linear ketoprofen. The results of the ketoprofen inclusion and the release experiments indicate that this system seems to be a very promising vehicle for the administration of hydrophobic drugs.

Introduction

Fundamental research encompassing chemistry in nanoscience/nanotechnology as they relate to achieving new developments in biomedicine is important because nanomedicine has the realistic potential to bring a paradigm shift in the way diseases in humans are diagnosed and treated.[1] Drug targeting has emerged as one of the modern technologies for drug delivery. The possibilities for the application of iron oxide magnetic nanoparticles (MNPs) in drug targeting have drastically increased in recent years.[2] MNPs in combination with an external magnetic field and/or magnetizable implants allow the delivery of particles to the desired target area and fixing them at the local site while the medication is released and acts locally (magnetic drug targeting, MDT).[3–5] The surfaces of these particles are generally modified with organic polymers and inorganic metals or oxides to make them biocompatible and suitable for further functionalization by the attachment of various bioactive molecules.[6]

Gum arabic (GA) is a natural polymer with a mucoadhesive property that has some desirable features of a controlled drug delivery system like localization in specified regions that improve and enhance the bioavailability of drugs.[7] On the other hand, cyclodextrins (CDs) form a whole new family of pharmaceutical excipients that have a doughnut-shaped structure with a hydrophilic outer surface and a lipophilic cavity, where poorly water-soluble molecules can shelter their most hydrophobic parts.[8,9] Therefore, grafting CD molecules on the GA-modified magnetic nanoparticles (GAMNPs) may lead to a drug carrier that possesses the following properties: (1) allows a controlled release of a bioactive substance; (2) can form noncovalent inclusion complexes with a wide variety of lipophilic drug molecules allowing the solubilization, stabilization, and transport of hydrophobic drugs; and (3) can be magnetically guided to the local site at the specified time for dosage and elimination.

The new drug carrier was fabricated by grafting the citrate-modified CD onto GAMNP via carbodiimide activation as is illustrated in Scheme 1. Its size, morphology, and binding with CD were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential meter, Fourier transform infrared (FTIR) spectroscopy, and thermogravimetric analysis (TGA). In addition, to demonstrate the feasibility of using CD-grafted GAMNPs (CD−citrate−GAMNPs) as the carrier of hydrophobic drugs, the formation of the inclusion complex between ketoprofen and CD−citrate−GAMNPs as well as the in vitro release profile of ketoprofen was studied. Ketoprofen is a poorly soluble nonsteroidal anti-inflammatory drug that requires

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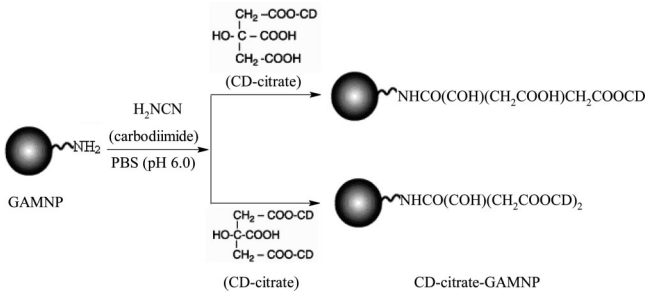
Scheme 1. Fabrication of CD-Grafted GAMNPs Using CD−Citrate as a Source



frequent dosing for therapeutic maintenance because of its fairly fast elimination from the body. However, exposure of the stomach to high levels of ketoprofen can cause gastric damage such as ulceration or bleeding.[10] The use of CD−citrate−GAMNPs as the carrier might be a promising strategy for the dosing of hydrophobic drugs like ketoprofen. The newly fabricated nanocarrier injected into the patient via the circulatory system could serve a major purpose through drug targeting to inflammation sites to maintain appropriate concentrations while at the same time reducing cost, overall dosage, and unwanted side effects.

Experimental Section

Materials. β-Cyclodextrin (CD) was procured from Aldrich Chemical Co. (St. Louis, MO). Citric acid was obtained from Riedel-deHaën (Seelze). Ketoprofen and carbodiimide were procured from Sigma-Aldrich Chemical Co. (Germany). The water used throughout this work was the reagent grade produced by a Milli-Q SP ultrapure-water purification system from Nihon Millipore Ltd., Tokyo. All other chemicals were of analytical grade and used without further purification.

Preparation of CD−Citrate. CD−citrate was prepared using a semidry reaction method as reported by El-Tahlawy et al.[11] First, 3.0 g of CD was mixed with 1.8 mL of water containing 1.02 g of citric acid. Then, the reaction mixture was allowed to react in a circulating air oven at 105 °C for 3 h. The cured sample was purified by repeated washing with isopropanol to remove unreacted components and soluble fragments or byproducts. Finally, the resultant CD−citrate was dried at 60 °C for 24 h and stored in an airtight container for further use.

Fabrication of CD−Citrate−GAMNPs. GAMNPs were prepared by the coprecipitation of Fe^{2+} and Fe^{3+} ions with ammonia solution followed by surface modification with GA under sonication according to our previous work.[12,13] Fe_3O_4 MNPs were prepared by coprecipitating Fe^{2+} and Fe^{3+} ions by ammonia solution and treating under hydrothermal conditions. For the modification of GA, 100 mL of a solution containing 5 mg/mL of GA was mixed with 1.0 g of MNPs in a stoppered bottle. The reaction mixture was sonicated for 20 min, then mixed on a vortex mixer for 5 min, and again sonicated for another 10 min. The product GAMNP was recovered magnetically from the reaction mixture, then washed three times with distilled water to remove the loosely bound GA, and finally dried in an air oven at 50 °C for 24 h.

Linking of CD−citrate onto GAMNPs was done by reacting the carboxylic groups of CD−citrate with the amino groups of GAMNPs via carbodiimide activation. First, 1.0 g of Fe_3O_4 nanoparticles were added to 10 mL of buffer A (0.003 M phosphate, pH 6, 0.1 M NaCl) and sonicated for 10 min. Then, 5.0 mL of carbodiimide solution (0.025 g/mL in buffer A) was added, and the reaction mixture was sonicated for another 10 min. Finally, 0.5 g of CD−citrate dissolved in 10 mL of buffer A solution was added to the above reaction mixture and sonicated for a further 45 min. The CD−citrate−GAMNPs were recovered from the reaction mixture by placing the bottle on a permanent magnet with a surface magnetization of 6000 G. The magnetic particles all settled within 1–2 min, were then washed repeatedly with water and ethanol, and finally were vaccuum-dried.

Characterization. TEM analysis was carried out using a Hitachi model H-7500 at 120 kV. FTIR spectra were recorded on a Varian FTS-1000 FTIR spectrometer. The hydrodynamic diameter was measured by DLS using a Malvern Autosizer 4700/PCS100 spectrometer equipped with an Ar ion laser operating at 488 nm. The zeta potentials were measured on a Malvern ZEN2600 Zetasizer Nano Z. TGA was done on the dried samples in air with a heating rate of 10 °C/min on a Shimadzu TA-50WSI TGA.

Loading of Ketoprofen. The stock solution of ketoprofen in water (1.25 mM) was prepared by first dissolving the drug in water of pH 11.0 and then decreasing the solution pH to 6.8 by adding a few drops of 0.1 M hydrochloric acid. The formation of the ketoprofen−CD complex was investigated by putting 25 mg of CD−citrate−GAMNPs into 5 mL of ketoprofen solutions with different concentrations (0.0125–1.25 mM) at pH 6.8 and 398 K. The solution was stirred by a horizontal laboratory shaker at 200 rpm to achieve the equilibrium, and then, the MNPs were removed magnetically from the solution. It was found that the adsorption equilibrium was reached in 24 h. The concentration of ketoprofen was determined by the spectrophotometric method at 260 nm on a Jasco model V-570 UV–visible spectrophotometer. Each experiment was repeated twice, and the reproducibility was found to be fairly good. The solution pH was adjusted by NaOH or HCl.

In Vitro Release. The release of ketoprofen from the CD−citrate−GAMNPs was investigated in the phosphate buffer at pH 7.4 and 310 K. For each experiment, 250 mg of CD−citrate−GAMNPs containing a known amount of ketoprofen was suspended in 50 mL of the release medium with stirring at 310 K. Samples (1 mL) were periodically removed from the supernatant solution and assayed after settling the nanoparticles by magnetic application. The volume of each sample withdrawn was replaced by the same volume of a fresh medium. The amount of released ketoprofen was analyzed spectrophotometrically at 260 nm. Each drug release study was performed in duplicate to confirm the reproducibility.

Results and Discussion

Properties of CD−Citrate−GAMNPs. The typical TEM images and particle size distributions of the GAMNPs and CD−citrate−GAMNPs are shown in Figure 1A,B, respectively. According to our previous work,[12] the MNPs had a mean diameter of 13.2 nm and the surface modification with GA led to the formation of secondary particles with a mean diameter of 34.2 nm. From Figure 1B, the mean diameter of the CD−citrate−GAMNPs could be estimated as 14.6 nm, which was smaller than that for the GAMNPs while close to that for the MNPs. This revealed that the CD grafting had resulted in the deagglomeration of secondary particles. This might be due to the change in the surface functional groups of the GAMNPs by the grafting of the CD.[13]

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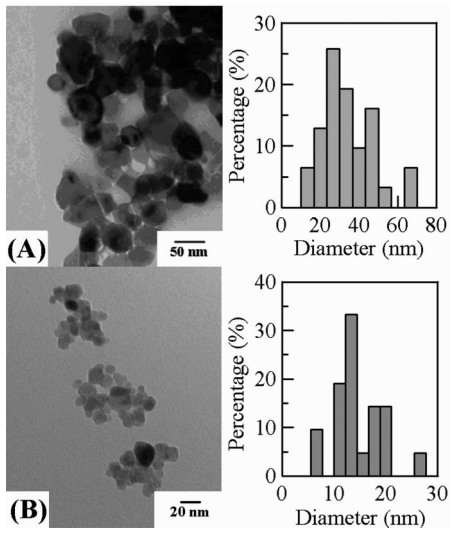

Figure 1. Typical TEM images and particle size distributions of GAMNPs (A) and CD−citrate−GAMNPs (B).

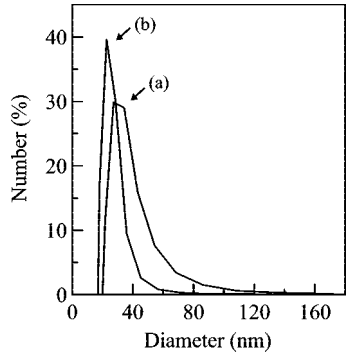

Figure 2. Hydrodynamic diameter distributions of GAMNPs (a) and CD−citrate−GAMNPs (b).

The hydrodynamic diameter distributions of the GAMNPs and CD−citrate−GAMNPs in water are shown in Figure 2. According to our previous work,[12] the GAMNPs had a mean hydrodynamic diameter of 36.8 nm. In this study, the resultant CD−citrate−GAMNPs were mostly in the ranges of 18.0–50.0 nm, with the rest accounting for less than 2.0%., and their mean hydrodynamic diameter could be estimated as 26.2 nm, which was significantly larger than the mean diameter observed by TEM (14.6 nm). This revealed that the agglomeration of the CD−citrate−GAMNPs, mainly attributable to the interaction between the outer hydroxyl group of CD and the carboxylic group of GA, must occur in water.[14] As for the smaller mean hydrodynamic diameter as compared to that of the GAMNPs, it could be attributed to their different surface states which were confirmed by zeta potential measurements.

Figure 3 indicates the pH-dependencies of the zeta potentials for the GAMNPs and CD−citrate−GAMNPs (0.1 mg/mL) in 0.01 M NaCl solutions at pH 1.60–10.0. Obviously, the zeta potentials of all nanoparticles increased with the decrease in pH because of the protonation of the hydroxyl, carboxyl, and amine groups of the GAMNPs and CD−citrate−GAMNPs. It was seen that the isoelectric point (pI) of the GAMNPs shifted drastically from 3.6 to 2.1 after

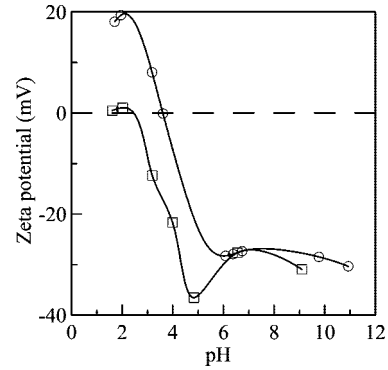

Figure 3. Zeta potentials of GAMNPs (○) and CD−citrate−GAMNPs (□) at various pH values.

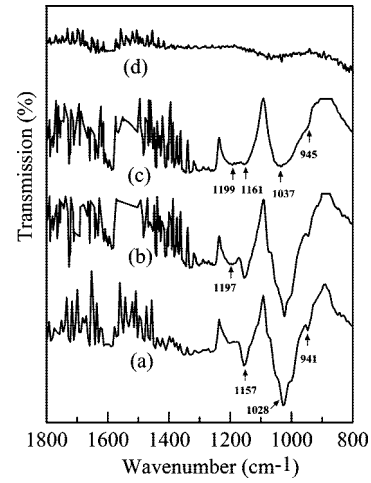

Figure 4. ATR-IR spectra of CD (a), CD−citrate (b), CD−citrate−GAMNPs (c), and GAMNPs (d).

treating with CD−citrate. The shift in pI was mainly due to some of the unreacted carboxylic groups of the citrate and the hydroxyl groups present on the outer surface of the CD, which influences the surface charge with the change in solution pH.

To further demonstrate the grafting of CD, the FTIR spectra of CD, CD−citrate, CD−citrate−GAMNPs, and GAMNPs were analyzed using the attenuated total reflection (ATR)-IR technique as shown in Figure 4. The spectra of CD and CD−citrate were quite similar, with all the characteristic peaks of CD at 941, 1028, and 1157 cm^{-1} in both spectra. The peak at 941 cm^{-1} was due to the R-1,4- bond skeleton vibration of CD. The peaks at 1028 and 1197 cm^{-1} are related to the antisymmetric glycosidic ν_a(C−O−C) vibration and the coupled ν(C−C/C−O) stretch vibration. The prominent peak in the spectrum of CD−citrate at 1197 cm^{-1} could be referred to the CD linkage to citrate. Furthermore, it was obvious that the spectrum of the CD−citrate−GAMNPs showed a drastic change as compared to that of the GAMNPs. All the significant peaks of CD in the range of 1200–900 cm^{-1} were present in the spectrum of the CD−citrate−GAMNPs with a small shift. On the basis of all this evidence, it could be concluded that CD has been grafted on GAMNPs.[15,16]

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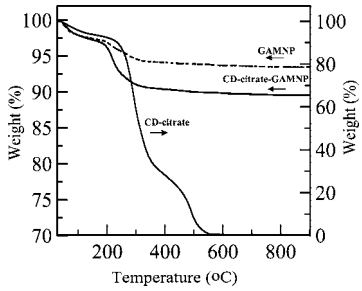

Figure 5. TGA curves of GAMNPs, CD−citrate, and CD−citrate−GAMNPs.

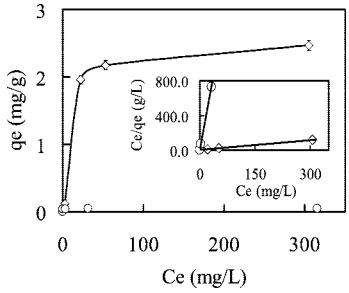

Figure 6. Equilibrium isotherms for the adsorption of ketoprofen by GAMNPs (○) and CD−citrate−GAMNPs (□). The inset illustrates the corresponding linear dependences of C_e/q_e on C_e.

The amount of CD grafted on the surface of GAMNPs was estimated from the TGA of GAMNPs, CD−citrate, and CD−citrate−GAMNPs. As shown in Figure 5, the unbound CD−citrate could be thermally decomposed completely above 550 °C. The TGA curve of GAMNPs showed two steps of weight loss over the temperature range of 30 to 900 °C. The first weight loss step of about 2.22% in the range of 30–150 °C might be due to the loss of residual water in the sample. The second weight loss step of about 3.52% in the range of 150–340 °C was due to the decomposition of GA. Similarly, the TGA curves for CD−citrate−GAMNPs showed two steps of weight loss, contributed from the loss of residual water in the sample in the range of 30–150 °C and the loss of CD−citrate−GA in the range of 150–400 °C. The first and second weight losses were 2.45% and 6.64%, respectively. From the second weight loss, the amount of CD−citrate was evaluated by deducting the weight loss attributable to GA, the value of which was obtained from the GAMNP analysis, and then the amount of CD was calculated from the weight loss obtained attributable to the CD−citrate. The amount of CD grafted on the GAMNPs could be calculated as 28.7 mg/g.

Inclusion Complexation with Ketoprofen. Figure 6 shows the capabilities of GAMNPs and CD−citrate−GAMNPs for ketoprofen loading. Obviously, CD−citrate−GAMNPs exhibited a considerable inclusion complexation capability for ketoprofen, but the ketoprofen loading by GAMNPs was quite low. This revealed that the CD grafted on the GAMNPs indeed could enhance the adsorption of ketoprofen effectively because of its special hydrophobic cavity structure. As for the ketoprofen loading by the GAMNPs, it could be attributed

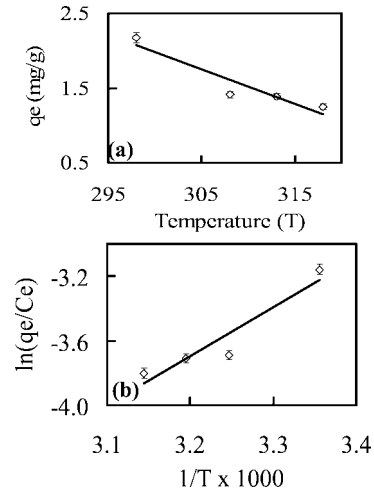

Figure 7. Effect of temperature on ketoprofen inclusion by CD−citrate−GAMNPs (a) and the corresponding plot of $\ln(q_e/C_e)$ against $1/T$ (b).

to the weak hydrophobic interaction between ketoprofen and GA in aqueous solution. In addition, to gain more insight into the phenomenon of ketoprofen complexation by the CD−citrate−GAMNPs, the adsorption equilibrium data were fitted with a Langmuir isotherm equation, which can be expressed as

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m K_L} \quad (1)$$

where q_e is the equilibrium adsorption capacity of ketoprofen (mg/g), C_e is the equilibrium ketoprofen concentration in solution (mg/L), q_m is the maximum adsorption capacity (mg/g), and K_L is the Langmuir adsorption equilibrium constant (L/mg). As indicated in the inset of Figure 6, the plots of C_e/q_e versus C_e yielded straight lines, revealing that the adsorption of ketoprofen on GAMNPs and CD−citrate−GAMNPs followed the Langmuir adsorption isotherm. From the slopes and intercepts, the values of q_m and K_L could be determined as 2.72 mg/g and 0.04 L/mg for CD−citrate−GAMNP and 0.043 mg/g and 12.1 L/mg for GAMNP. Obviously, the maximum loading of ketoprofen by the CD−citrate−GAMNPs was more than 60 times that by the GAMNPs.

Effect of Temperature. The effect of temperature on the adsorption of ketoprofen by CD−citrate−GAMNPs was investigated in the temperature range of 278–318 K and at an initial ketoprofen concentration of 0.25 mM. As shown in Figure 7a, the adsorption capacity of the CD−citrate−GAMNPs for ketoprofen decreased with the increase in temperature, revealing that the inclusion process was exothermic. The corresponding enthalpy and entropy changes (ΔH and ΔS) could be determined from the van't Hoff plots,[17] $\ln(q_e/C_e)$ versus $1/T$, as shown in Figure 7b. From the slope ($-\Delta H/R$) and intercept ($\Delta S/R$), the values of ΔH and ΔS were calculated to be −25.01 kJ/mol and −110.89 J/(mol K), respectively. Thus, the inclusion complexation of ketoprofen by the CD−citrate−GAMNPs had a favorable enthalpic term ($\Delta H < 0$) and an unfavorable entropic term ($\Delta S < 0$).

Effect of the Presence of Surfactant. For solubilization of drugs, the most common techniques employed by a

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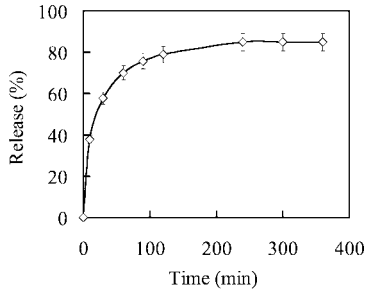

Figure 8. Release profile of ketoprofen from CD−citrate−GAMNPs.

formulation scientist to enhance the solubility of a hydrophobic drug involve in situ salt formation (pH adjustment) or use of additives such as complexing agents, surfactants, and cosolvents. However, very few studies on the combined impacts of surfactants and CD on drug loading have been reported.[18] So, the effect of the presence of surfactant on the inclusion of ketoprofen by CD−citrate−GAMNPs was studied by taking a solution of surfactant (sodium dodecyl sulfate, SDS) and ketoprofen and varying the concentration of SDS (0.05–0.20 mM) while keeping the concentration of ketoprofen constant (0.25 mM). The concentration of SDS was kept below the critical micelle concentration to avoid the possibility of solubilization of ketoprofen in the solution by formation of micelles.

It was observed that the inclusion of ketoprofen by CD−citrate−GAMNPs was 2.17 mg/g in the absence of SDS but decreased to 1.28, 1.11, and 0.50 mg/g when 0.05, 0.10, and 0.20 M of SDS were present, respectively. The decrease in the inclusion of ketoprofen with increasing concentration of SDS could be due to the different shapes of the SDS and ketoprofen. Catena and Bright[19] reported that the CD molecule preferred to approach the substrate equatorially as opposed to axially; hence, the more linear compound found the entry into the hydrophobic cavity by the equatorial approach more favorable than the less linear compound, which was hindered during the inclusion process. SDS molecules are more linear as compared to ketoprofen, so they can enter the cavity of CD more easily; hence, the majority of the cavity of the CD may be occupied by SDS molecules whose hydrophobic chain tends to reside by coiling in the hydrophobic cavity of CD.

In Vitro Release. Figure 8 shows the release profile for the ketoprofen-loaded CD−citrate−GAMNPs (2.47 mg/g) as a function of time. The profile showed an initial rapid release in the first 30 min and a slow and steady release in the following period until equilibrium was reached after about 5 h. Complete release of the drug was not observed, probably because of the thermodynamic equilibrium that existed between the drug−CD complex of the CD−citrate−GAMNPs and the released drug in the solution, which retarded the complete drug release. From this result, we could speculate that, following administration, some of the encapsulated drug molecules will be rapidly released from the CD−citrate−GAMNPs whereas some will remain associated to CD−citrate−GAMNPs. At this level, the degradation of the particles, probably driven by the enzyme lysozyme,[20] could facilitate the delivery of the associated drug at the absorption site.

To our knowledge, this is the first work that describes the synthesis of a CD-grafted GA modified magnetic nanocarrier and a further scope of study exists. Modification of the synthesized nanocarrier to tune it for controlled drug release is a possibility by grafting CD derivatives,[21] which will be a topic of our future research.

Conclusions

A novel magnetic nanocarrier for hydrophobic drugs was successfully fabricated by grafting CD on GAMNPs that inherits the cumulative effects of the inclusion and transport properties of CD, the bioadhesive property of GA, and the magnetic properties of Fe_3O_4 for MDT. The grafting was confirmed by ATR-IR spectroscopy, and the product was characterized using TEM, DLS, and TGA. It was shown that the resultant CD−citrate−GAMNPs had a mean diameter of 14.6 nm and a mean hydrodynamic diameter of 26.2 nm. The ability of the newly synthesized magnetic nanocarrier to form complexes with a model hydrophobic drug, ketoprofen, was studied; it was found that the loading capacity of the CD−citrate−GAMNPs was significantly higher than that of GAMNPs. The complexation of the CD−citrate−GAMNPs with ketoprofen was exothermic and obeyed the Langmuir adsorption isotherm. Also, the presence of SDS led to the decrease in the inclusion of ketoprofen because the linear structure of SDS made it easier to enter the cavity of CD as compared with the less linear ketoprofen; hence, the majority of the cavity of CD might be occupied by SDS. The results of ketoprofen inclusion and release experiments indicate that this system seems to be a very promising vehicle for the administration of hydrophobic drugs.

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