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Research Article

pH-Triggered Magnetic-Chitosan Nanogels (MCNs) For Doxorubicin Delivery: Physically vs. Chemically Cross Linking Approach

Somayeh Sadighian[1], Hassan Hosseini-Monfared[1]*, Kobra Rostamizadeh[2,3], Mehrdad Hamidi[2,4]

[1] *Faculty of Science, Department of Chemistry, University of Zanjan, Zanjan, Iran.*
[2] *Zanjan Pharmaceutical Nanotechnology Research Center, Zanjan University of Medical Sciences, Zanjan, Iran.*
[3] *Department of Medicinal Chemistry, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.*
[4] *Department of Pharmaceutics, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.*

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Abstract

Purpose: This paper evaluates the impact of cross linking strategy on the characteristics of magnetic chitosan nanogels (MCNs) as targeted drug delivery system for doxorubicin.

Methods: Sodium tripolyphosphate (TPP) and glutaraldehyde were used as physical (electrostatic) and chemical (covalent binding) cross-linker agents, respectively. MCNs were characterized by means of X-ray diffraction (XRD), Scanning electron microscopy (SEM), fourier transform infrared (FT-IR) spectroscopy and vibrating sample magnetometer (VSM). Scanning electron microscopy (SEM) indicated the formation of spherical nanostructures with the final average particle size of around 35-40 nm.

Results: The finding proved the superparamagnetic properties of the MCNs with relatively high-magnetization values which indicate that the MCNs were enough sensitive to external magnetic fields as a magnetic drug carrier. To understand the differences between the drug delivery properties of chemically and physically cross linked MCNs, the drug release studies were also conducted. Altogether, the results of this study clearly indicate that, however, both MCNs exhibited sustained drug release behaviour, the chemically cross linked MCNs provided enhanced controlled drug release characteristics in comparison to physically cross linked MCNs. Besides, according to the drug release behaviour of MCNs in buffer solutions in two different medium with the pH values of 5.3 and 7.4, it was clear that both nanoparticles exhibited pH sensitivity where the extent of drug release in the acidic media was significantly higher than neutral media.

Conclusion: It can be concluded that chemically cross linked MCNs may serve as an ideal carrier for stimuli-triggered and controlled anticancer drug delivery.

Introduction

Polymeric nanocomposites consisting of inorganic nanoparticles and organic polymers as a new class of materials have attracted wide attention in different fields. They offer unique features with the combination of both inorganic nanoparticles and organic polymers characteristics, which are not available in each individual constituent.[1] The nanocomposites provide significant improvements in several properties, such as optical, mechanical, and magnetic properties.[2] Magnetic nanoparticles (MNPs) are a rapidly evolving materials contributing to their multifunctional properties including small size, superparamagnetism and low toxicity.[3] Particularly in the pharmaceutical field, they have now attracted a lot of interests owing to their intrinsic magnetic properties for guided delivery of drugs.[4] In order to improve MNPs characteristics for drug delivery propose such as colloidal stability and prolong circulation kinetics, it is demonstrated that their coatings with hydrophilic polymers, namely chitosan, could be considered as an adequate strategy. Chitosan, a naturally-derived co-polymer of N-acetyglucosamine and d -glucosamine, has been extensively studied as a biodegradable and biocompatible polymer in drug delivery systems, gene therapy, and membranes for ultrafiltration.[5,6] It seems that chitosan-encapsulated Fe_3O_4 nanoparticles would most likely improve magnetite nanoparticles characteristics in terms of biodegradability and long circulation time. Moreover, the amino groups on the chitosan structure can also be used for further functionalization with specific components, such as targeting agents, various drugs, or other functional groups for the purpose of achieving carriers with advanced characteristics. We reported on our recent study the synthesis and in vitro characterization of a dual targeted drug delivery system using a core–shell magnetite nanoparticulate system conjugated with doxorubicin. In order to enhance the biocompatibility of carrier and minimize unwanted side effects of doxorubicin and Fe_3O_4 nanoparticles, conjugated magnetite core–shell nanoparticles were encapsulated within chitosan.[7]

Doxorubicin and its bioactive derivatives are among the

***Corresponding author:** Hassan Hosseini-Monfared, Tel: +98 (241) 5152576, Fax: +98 (241) 2283203, Email: monfared@znu.ac.ir

most widely used anticancer drugs in chemotherapy treatment,[8] however, it suffers from disadvantageous side effects. Drug targeting, therefore, shows an interesting motivation to prevent side effects and enhance cytotoxicity of doxorubicin. The majority of attempts to associate doxorubicin to nanoparticulate carriers have used anionic or neutral polymers. For instance, Janes et al.[9] synthesized chitosan/ TPP nanoparticles to encapsulate doxorubicin as an efficient drug carrier with possibility of targeting to specific site, reduced side effects and controlled drug release.

Following our previous results, in this contribution we aimed to investigate the impact of crosslinking strategy on the chitosan modified magnetic nanoparticles characteristics for the purpose of designing novel drug carrier with improved properties in terms of targeting to specific site, reduced side effects and controlled drug release. To achieve the goal, chitosan modified magnetic nanoparticles were prepared by two different cross linking methods; (1) physically by ionotropic gelation method using tripolyphosphate (TPP) and (2) chemically via glutaraldehyde.[10]

Materials and Methods

Materials

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), sodium tripolyphosphate (TPP), glutaraldehyde and glacial acetic acid were from Merck and purchased locally. Chitosan of molecular weight in the range of $10^5 - 3\times10^5$ g/mol and degree of deacetylation ≥75% was obtained from Sigma. Doxorubicin hydrochloride was purchased from Celonlabs (Andhra Pradesh, India).

Preparation of TPP cross linked magnetic chitosan nanogels (TPP/MCNs)

TPP cross linked magnetic chitosan nanogels (TPP/MCNs) were prepared by the co-precipitating method. Briefly, ferric chloride hexahydrate (2 mmol) and ferrous sulfate heptahydrate (1 mmol) were dissolved in 50 ml of acetic acid buffer solution of chitosan (chitosan solution was prepared by dissolving 0.5 g chitosan powder in acetate buffer (100 mL, pH=4.8) at room temperature). Next, 3 mL of NH_4OH solution (25%) was added to the solution under vigorous stirring at 80 °C. The synthesis process was followed by the addition of 12.5 ml of 1 mg/ml TPP solution as cross-linker. Then, the black product was separated by an external magnet and washed three times with distilled water to remove the excess ammonia and finally the product were dried in vacuum conditions at 60 °C for 12 h.

Preparation of glutaraldehyde cross linked magnetic chitosan nanogels (GA/MCNs)

Glutaraldehyde cross-linked magnetic chitosan nanogels (GA/MCNs) were prepared as follows: Typically, ferric chloride hexahydrate (2 mmol) and ferrous sulfate heptahydrate (1 mmol) were dissolved in 50 ml of acetic acid buffer solution of chitosan (1 w/v%) and followed by the addition of 0.5 ml glutaraldehyde solution (25%) under vigorous stirring to obtain a homogeneous solution. Then, the solution was held until the chitosan hydrogel was formed completely due to the cross-linking effect of glutaraldehyde. The product was separated by an external magnet and washed several times with distilled water and lastly dried at 60 °C for 12 h.

Drug loading and release study

The drug loading was accomplished by dissolving 5 mg of doxorubicin in 25mL of deionized water containing 8 mg/mL of nanogels, and stirring for 24 h under dark condition at room temperature. Next, the drug loaded suspended nanoparticles were separated from the dispersion by centrifugation at 12000 rpm and then washed twice with deionized water to remove any loosely adsorbed drug on the surface of nanoparticles. Afterwards, the doxorubicin entrapped MCNs were dried in vacuumed oven at 30 °C for 12 h. To determine the extent of unloaded drug (free doxorubicin), the centrifuged solution was collected and analyzed for drug content by UV–Vis spectrophotometry at wavelength of 480 nm. The Entrapment efficiency was calculated from the following expression:

$$Entrapment\ efficiency(\%) = \frac{total\ amount\ of\ drug - free\ amount\ of\ drug}{total\ amount\ of\ drug} \times 100$$

The drug release behavior of MCNs was studied in physiological pH of 7.4. Typically, 2.0 mg of MCNs were placed into a dialysis bag (cut off 12 kDa) and introduced to 15 mL of phosphate buffered saline (PBS) with desired pH under stirring (100 rpm) at 37 °C. At predetermined time intervals, in order to determine the extent of released drug, and thereby time dependent drug release profile, 1.0 mL of solution was taken out and replaced with 1.0 mL of fresh buffer solution maintained at 37 °C and assayed by UV–Vis spectroscopy at wavelength of 480 nm. To evaluate the pH dependency of drug release from MCNs, the release study was also conducted in acidic media with pH value of 5.3.

Characterization techniques

FT-IR spectra of nanoparticles were taken with a Matson FT–IR spectrophotomer in the range of 400–4000 cm^{-1} as KBr disks at room temperature. The XRD patterns of nanoparticles were recorded on an x-ray diffractometer (Bruker D8 Advence). The samples were irradiated with monochromatized Cu Kα radiation (0.154060 nm). The voltage and current used were 40 kV and 30 mA, respectively. Vibrating sample magnetometer (VSM, Meghnatis Daghigh Kavir Co, Iran) was used to characterize the magnetic properties of MCNs. The hysteresis of the magnetization was obtained by changing H between +8500 Oe and −8500 Oe. The zeta potential and particle size distribution of the prepared

nanoparticles were determined by photon correlation spectroscopy (PCS) using a Nano/zetasizer (Malvern Instruments, Nano ZS, Worcestershire, UK) working on the dynamic light scattering (DLS) platform. Scanning electron microscopy (SEM), Hitachi f 4160 was used to examine the morphology and size distribution of the carriers.

Results and Discussion

FT-IR analysis

The chemical structure and functional groups of TPP/MCNs and GA/MCNs were investigated by FT-IR (Figure 1). As it can be seen, the appearance of characteristic peak of Fe–O stretching band at 577 cm^{-1} could evidence formation of magnetite nanopartcles. In chitosan, the broad band at 3430 cm^{-1} corresponds to the stretching vibration of –NH and –OH groups and the bands at 2920 cm^{-1} and 1600 cm^{-1} represent the presence of sreching and bending vibration of –CH and $–CH_2$ groups, respectively. The bands at 1026 and 1061 cm^{-1} (Figure 1a) were attributed to the ν(C-O) of chitosan and ν(P=O) of TPP/MCNs, respectively. In Figure 1c and d, the peak at 880 cm^{-1} can be clearly attributed to the presence of $=C–O–CH_3$ in the doxorubicin structure, which is indicative of the successful drug entrapment by the nanocarriers.

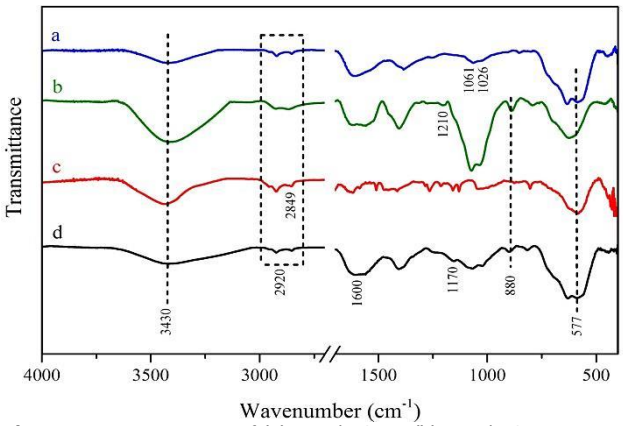

Figure 1. FT–IR spectra of (a) TPP/MCNs, (b) TPP/MCNs -doxorubicin, (c) GA/MCNs, (d) GA/MCNs -doxorubicin nanocomposites

Size and zeta potential measurements

The size of all nanoparticles was analyzed by DLS technique (Table 1). Particle size distribution curves exhibited only one peak with a relatively low polydispersity index. As it is clear, there was no significant difference between the particle size of TPP/MCNs and GA/MCNs. From the results, it can be concluded that type of crosslinking method has no influence on particle size of nanogels. The measurement of zeta potential provides valuable insight into the stability of colloidal aqueous dispersions. Usually, particle aggregation is less likely to occur for charged particles with optimum zeta potential because of electrostatic repulsions. The zeta potential and standard deviation values of nanogels are shown in Table 1. From the data presented in Table 1, higher negative charge of TPP/MCNs compared to the GA/MCNs can be related to the presence of TPP with negative charge in the nanogels structure.

Table 1. Average particle diameter, polydispersity index, and the surface zeta potential of chitosan nanogels, magnetite nanoparticles, and magnetic composites

Composite	Z-Average (nm)	PDI[a]	Zeta potential (mV)
Fe_3O_4 nanoparticles	186.0 ± 2.4	0.100	-31.00
TPP/MCNs	68.7 ± 4.6	0.466	-15.20
GA/MCNs	65.5 ± 2.3	0.250	-7.35

[a]Polydispersity index

Figure 2 shows the typical SEM images and corresponding histograms for TPP/MCNs and GA/MCNs. The histograms shows the average size of nanogels were about 40 and 35 nm for TPP/MCNs and GA/MCNs, respectively. Difference between the results of particle size of nanogels Size of particles from DLS technique was slightly larger than the ones from SEM technique which can be arise from measurement condition. Indeed, DLS analysis measures hydrodynamic diameter, while SEM results corresponds to the particle size in dry condition.

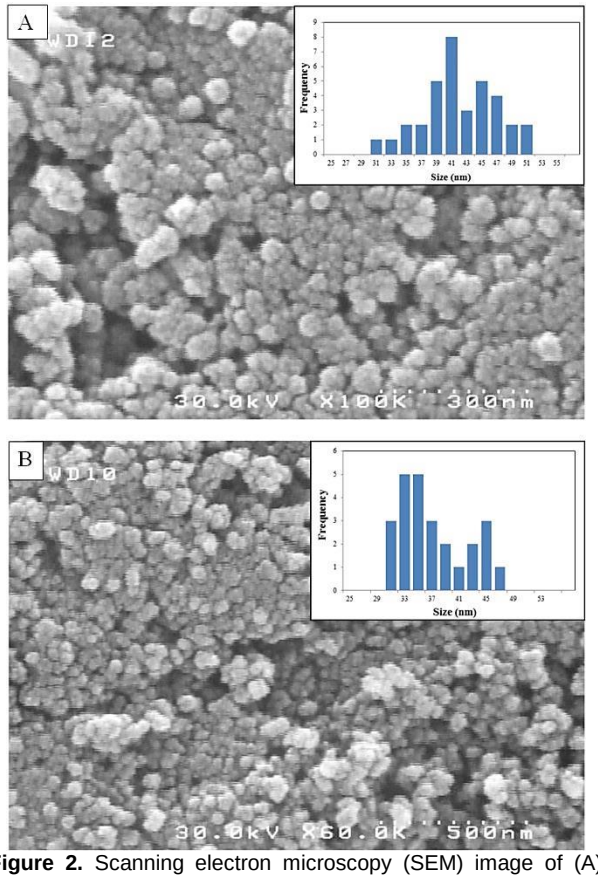

Figure 2. Scanning electron microscopy (SEM) image of (A) TPP/MCNs and (B) GA/MCNs

Magnetic properties of MCNs

High saturation magnetization is required so as to maximize the ability to target the particles using an

external magnetic field. A representative hysteresis curve of the TPP/MCNs and GA/MCNs were studied by vibrating sample magnetometer as illustrated in Figure 3 by plotting of magnetization versus applied magnetic field (M–H loop) at 300 K. The saturation magnetization were 47.62 emu/g and 30.28 emu/g for TPP/MCNs and GA/MCNs, respectively. Giving consideration to the magnetization curve, it is clear that they exhibited neither remanence nor coercivity indicating that both MCNs show superparamagnetism. It was not surprising in the light of the size of nanoparticles, since it has been demonstrated that particles at a size in nanometer scale exhibit superparamagnetism.[11] The saturation magnetization of TPP/MCNs was slightly lesser than that of GA/MCNs probably due to their size.[12] Generally, it can be concluded that both nanoparticles have adequate superparamagnetic properties and could respond well to magnetic fields without any permanent magnetization as a magnetic drug carrier for targeted delivery.

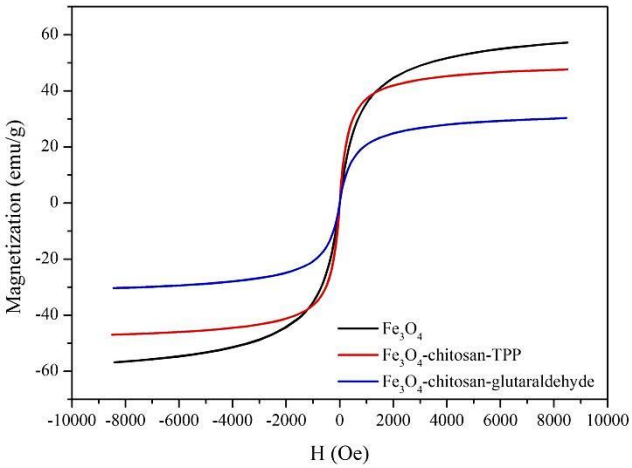

Figure 3. Magnetization curves obtained by VSM at room temperature

Figure 4 shows X-ray diffraction (XRD) patterns of TPP/MCNs and GA/MCNs nanogels. A series of characteristic peaks which correspond to (220), (311), (400), (422), (511) and (440) Bragg reflections of magnetite nanoparticles were observed for nanogels. The low intensity and broaden peaks indicates the presence of chitosan on the surface of Fe_3O_4 nanoparticles.

Drug loading and "In vitro" drug release studies

The results revealed that the drug loading for TPP/MCNs and GA/MCNs nanogels were 71% and 58%, respectively. Low drug loading for GA/MCNs can be related to the reaction of amino functional group of chitosan with carbonyl group of glutaraldehyde which consequently limits their possible interaction with carbonyl group of doxorubicin. Figure 5 illustrates the drug release response of the MCNs in PBS with two different pH values of 5.3 and 7.4 at the physiological temperature of 37 °C. Clearly, the drug release profiles of doxorubicin from both MCNs were approximately steady-linear throughout the time evaluated. Surprisingly in spite of common physically drug loading carrier in which generally there are a high extent of burst drug release, the results demonstrated that both variants showed a considerable sustained drug release behavior with no significant initial burst release in all media. Such behavior can be explained in view of various effective parameters on drug release behavior. Indeed, it is believed that the rate of drug release from the nanogels is theoretically affected by many factors such as rate of polymer degradation, diffusion of the drug through the nanogels particles, and extent of swelling of nanogels in aqueous media. As a results, sustained drug release profile of both MCNs can be presumably originate from high cross linking efficiency of nanogels which in turn restricts corresponding swelling ratio.

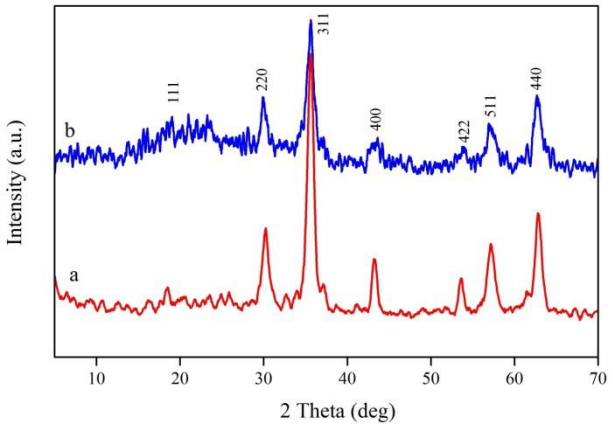

Figure 4. XRD patterns of the TPP/MCNs and GA/MCNs nanogels

From Figure 5, it is obvious that in case of TPP/MCNs about 34.5% of payload drug was released over 72 h, while for GA/MCNs it was close to 26% during the same period of time (pH=7.4). A possible explanation for this behavior could be the high stability of chemical cross linking compared to the physical cross linking. In fact, as the cross linking degrades the structure might become loose which, in turn, lead to high rate of drug release.

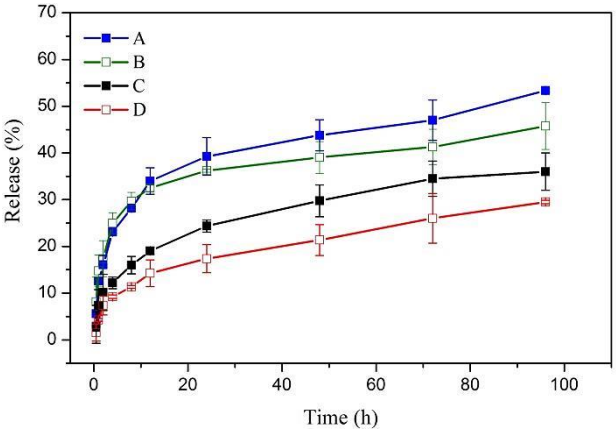

Figure 5. Release profiles of doxorubicin from TPP/MCNs (A in pH=5.3, C in pH=7.4) and GA/MCNs (B in pH=5.3, D in pH=7.4). Each data point represents the mean ±S.D. (n=3).

Additionally, another important and surprising feature of both carriers was pH dependency of drug release where it was high rate of drug release in acidic media (pH, 5.3) compared to the neutral pH (7.4). We believe that there are presumably two impetuses behind such observation. First, formation of pH sensitive imine bonds between amine functional group of chitosan and carbonyl group of doxorubicin in the course of drug loading. This hypothesis is supported by the appearance of imine characteristic peaks at 880 cm-1 in the FT-IR spectrum both MCNs (Figure 1). Secondly, acidic medium may enhance the swelling properties of chitosan and increasing the size of pores, and consequently increase the extent of drug diffusion from chitosan. There were similar reports in the literature to support this explanation.[13]

Drug release kinetic

To understand the factors that control the drug release from MCNs-doxorubicin nanogels, the release data were fitted with mathematical model. Drug release kinetics analyzed by fitting the cumulative release data to an exponential equation using the equation proposed by Korsmeyer-Peppas.

$$M_t/M_\infty = kt^n$$

Where M_t/M_∞ is a fraction of drug release at time t, n is the release exponent and k is the release rate constant. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model. From TPP/MCNs and GA/MCNs (R^2=0.9473 and R^2=0.9694), respectively. A scattering plot of Ln-Ln fit is shown in Figure 6 for our experimental data. Using the least squares procedure, the values of n for TPP/MCNs and GA/MCNs were estimated to be 0.30 and 0.23 which indicate that for both nanogels the majority of drug release study (about 60%), the release mechanism is mainly dominated by the diffusion control mechanism.

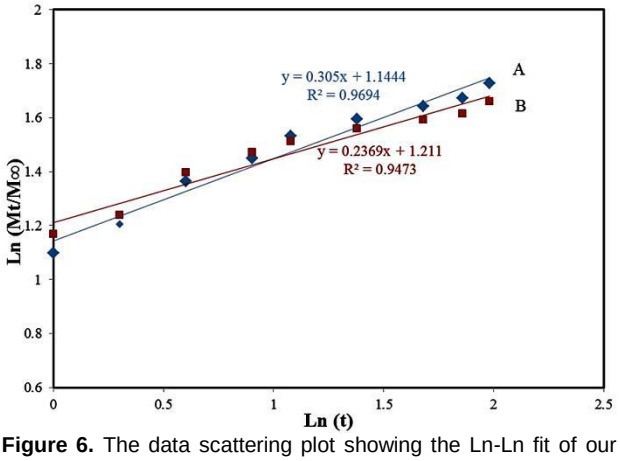

Figure 6. The data scattering plot showing the Ln-Ln fit of our experimental data according to the Korsmeyer and Peppas model (A=. TPP/MCNs-Doxorubicin, B= GA/MCNs-Doxorubicin)

Conclusion

In the present contribution, a novel pH sensitive magnetic chitosan nanogels (MCNs) composed were prepared using two different crosslinkers, i.e. tripolyphosphate (for physical crosslinking) (TPP/MCNs) and glutraldehyde (for chemical crosslinking, GA/MCNs). The FT-IR confirmed the successful synthesis of both MCNs. Scanning electron microscopy (SEM) and particle size analysis (DLS) confirmed the formation of spherical nanoparticles with the final average particle size about 35-40 nm (SEM). The VSM analysis showed saturation magnetization values of 47.62 and 30.28 emu/g for TPP/MCNs and GA/MCNs, respectively. Both MCNs possessed outstanding Entrapment efficiency for doxorubicin (76.6, 65.0%, for TPP/MCNs and GA/MCNs, respectively). The finding revealed that both MCNs provide a sustained release pattern. It was found that the maximum drug release attainable for TPP/MCNs was higher than the one for GA/MCNs, probably due to the high stability of chemically cross linking compared to the physically cross linking method. The release study of doxorubicin also revealed that the extent of drug release at pH=5.3 was promisingly more than drug release at pH=7.4 for both MCNs. Generally, it can be concluded that type of drug cross linking provide an opportunity to tune drug release pattern of MCNs according to the specific drug delivery purpose.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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