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Sericin-Montmorillonite Composite Nanoparticles as Drug Delivery System in Human Liver Cancer: Development, Drug Release, Cellular Uptake and Cytotoxicity

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Keywords
Sericin,
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Doxorubicin,
Drug delivery

Abstract: Composite nanoparticles obtained increasing interest because of their scientific and curative importance. Herein, sericin-montmorillonite composite nanoparticles (Ser-Mt NPs) were synthesized by taking advantage of the biodegradability and functional surface diversity of sericin, and biocompatibility and high adsorption properties of montmorillonite as natural resources. The composite nanoparticle was obtained by the desolvation technique and crosslinked with glutaraldehyde for the first time. Doxorubicin was selected to be used as a model anticancer drug to perform the loading and release studies. After chemical and morphological characterization studies with various methods such as Fourier Transform Infra-Red Spectroscopy and Electron Microscopy, the cytotoxic effect of Ser-Mt composite NPs were quanlitatively and quantitatively evaluated on HepG2 (human liver cancer cell line) cells. The results obviously exhibited that high drug loading capacity, sustainable drug release property and its effect on cancer cells made Ser-Mt composite NPs as a good candidate as a drug delivery system on cancer therapy with monodisperse, small average size and good polydispersity index.

İnsan Karaciğer Kanserinde İlaç Taşıyıcı Sistem Olarak Serisin-Montmorillonit Kompozit Nanopartiküller: Geliştirilmesi, İlaç salımı, Hücresel Alım ve Sitotoksisite

Anahtar Kelimeler
Serisin,
Montmorillonit,
Kompozit nanopartiküller,
Doksorubisin,
İlaç Dağıtım

Özet: Bilimsel ve iyileştiri özelliklerinden dolayı kompozit nanopartiküller artan ilgi görmüşlerdir. Burada, doğal kaynaklardan serisinin biyolojik olarak parçalanabilirliği ve fonksiyonel yüzey çeşitliliği ile montmorillonitin biyolojik uyumluluk ve yüksek adsorpsiyon özelliklerinden yararlanılarak serisin-montmorillonit kompozit nanopartiküller (Ser-Mt NPs) sentezlenmiştir. Kompozit nanopartiküller ilk defa desolvasyon tekniği ile sentezlenmiş ve glutaraldehit ile çapraz bağlanmıştır. Doksorubisin, yükleme ve salım çalışmalarını gerçekleştirmek için model antikanser ilaç olarak seçilmiştir. Zayıflatılmış Toplam Yansıma - Fourier Dönüşümü Kızılötesi Spektroskopisi ve Taramalı Elektron Mikroskobu gibi çeşitli yöntemlerle yapılan kimyasal ve morfolojik karakterizasyon çalışmaları sonrasında Ser-Mt kompozit NP'lerin sitotoksik etkisi, HepG2 (insan karaciğer kanseri hücre hattı) hücreleri üzerinde kalitatif ve kantitatif olarak değerlendirilmiştir. Sonuçlar, yüksek ilaç yükleme kapasitesi, sürdürülebilir ilaç salım özelliği ve kanser hücreleri üzerindeki etkisinin, monodispers, küçük ortalama boyut dağılımı ve iyi çoklu dağılım indeksine sahip Ser-Mt kompozit NP'lerini, kanser terapisi için ilaç dağıtım sistemi olarak iyi bir aday olduğunu açıkça göstermiştir.

1. Introduction

Nature has always offered us new possibilities and resources. Therefore, when looking for solutions to some problems or setting new possibilities, we must first look at our environment, that is, nature. Besides, the development of nanotechnology allows more effective use of resources and of nature. For example, montmorillonite (Mt) is a natural clay mineral with nano-sized structure has many application areas from industry or engineering to medicine [1, 2]. Mt has a high ion exchange, adsorption ability, and

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storage potential due to the regular arrangement in the structure. It belongs to the smectit group of clay minerals which means 2:1 clay that is two tetrahedral sheets of silica and an octahedral sheet of alumina located centrally [3]. Among these layers there are elements such as Mg, Na, K, and Ca which then can exchange with Al inside the octahedral sheet [4-6]. The replacement of lower valence cations causes the adjacent oxygen atoms with a net negative charge which may invite cations [7]. By this exchange, Mt can absorb a high amount of water and swells which gives Mt to attack the mucus layer in the body. These extraordinary properties have enabled the use of Mt in biomedical areas. Mt has been selected as a drug delivery system in cancer treatment by many researchers. The Mt was mixed with different polymer or protein to participate in the production of composite materials [8]. In this way, the unique properties of both Mt and the mixture material used were exploited.

Sericin is a natural macromolecule received from *Bombyx mori,* a silkworm*.* As another source from nature, sericin is a protein that contains 18 amino acids with very significant properties. For example, it is antibacterial and antimicrobial with high UV and oxidation resistance. Also, its functional surface has hydroxyl, carboxyl, and amino groups offer huge modification strategies that may include cross-linking, copolymerization, blending with polymers, etc. which ensure the new materials with improved properties [9,10]. Its molecular weight comes across from 10 to 400 kDa. Sericin with small molecular weight or sericin fragments that are soluble in cold water are used in cosmetics and the medical industry while with high-molecular weight which are soluble in hot water are used as biomaterials and biomembranes, copolymers, fibers and especially fabrics [10,11]. Nowadays, progress in biomaterial science has demonstrated promising potential, especially for protein based nanoparticles. Therefore, having diverse usage areas make sericin a good candidate to be selected as a part of drug delivery systems.

In decades, considerable attention on nanotechnology has affected the strategies on the development of successful theragnostic materials. By these strategies, the requirements for non-toxic and non-antigenic drug delivery agents with no or less side effects and improved stability of drugs can be provided. Therefore, natural and synthetic polymers were used to synthesized nanoparticles or as matrix materials which made them candidates for drug encapsulation.

In this study, sericin-montmorillonite composite nanoparticles (Ser-Mt NP) were synthesized by taking advantage of the unique properties of sericin and montmorillonite with the desolvation method and crosslinked with glutaraldehyde to increase the stability. Doxorubicin (Dox), a chemotherapy drug, interferes with the proliferation of cancer cells by blocking topoisomerase 2 enzyme, was used as a model drug for cancer therapy. To characterize the morphology and chemical structure of the Ser-Mt NP; Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), Dynamic Light Scattering (DLS), Ultraviolet-Vis (UV-Vis) Spectroscopy, Attenuated Total Reflection–Fourier Transform Infra-Red (ATR-FTIR) Spectroscopy, Thermogravimetric Analysis (TGA), the in vitro Dox release profile was performed. The cytotoxic effect of Dox loaded Ser-Mt composite NPs was quantitatively evaluated on HepG2 cells by the microplate reader. Also, cellular uptake of the NPs was evaluated by the Confocal Laser Scanning Microscopy (CLSM).

2. Material and Method

2.1. Materials

For cell culture studies; PBS (pH 7.4), DMEM Ham's F-12 as growth medium with 10% FBS and 1% pen–strep and 1% L-glutamine, trypsin/EDTA, MTT were collected from Serva (Germany) for composite nanoparticle production; sericin, glutaraldehyde, montmorillonite K-10 (Mt), ethanol and Dox·HCl were purchased from Sigma-Aldrich (Germany). HepG2 liver cancer cell line was collected from Hacettepe University Advanced Technologies Application and Research Center in Turkey. All chemicals and materials were used as collected with no treatment.

2.2. Methods

2.2.1. Composite nanoparticle development

Composite nanoparticles were developed with a desolvation method reported previously by [12,13] with brief modification. Desolvation is a self-assembly process especially used with proteins such as sericin or albumin that is managed by desolvation agents and the environmental conditions such as pH of the solution and the amount of protein.

Firstly, a 10 mg/mL sericin solution was prepared with ultrapure water. Then, 1mL of 3 % of Mt was prepared and added to 1mL of the aforementioned sericin solution. Nearly 3 mL of ethanol was added drop wisely as desolvating agents to the sericin-Mt mixture solution under constant stirring (650 rpm). When the color of the mixture turned to cloudy white after the addition of ethanol, 15µL of glutaraldehyde (GA) was used for cross-linking the amine ends of sericin. The prepared solution was kept under the speed of 650 rpm for 4 h at room temperature. Finally, the composite nanoparticle suspension was purified (12.000 rpm, 20 min) three times with ultra-pure water to get rid of untreated materials. The obtained Ser-Mt composite nanomaterials were

suspended in 1 mL ultra-pure water and lyophilized at -80 °C under 0.1mbar atmosphere for 24 h for further experiments.

2.2.2. Characterization studies of Ser-Mt NPs

The morphology of the composite nanoparticles was collected and assessed by Electron Microscope (SEM) with a model of Tescan GAIA 3. Also, Energy-Dispersive X-Ray Spectroscopy (EDX) was collected to demonstrate the incorporation of Mt into composite nanostructure. To perform the SEM and EDX studies, a trace amount of NPs was added on a stab and incubated under vacuum, and then covered with 5 nm of gold by using the Coating System (PECS).

The size and zeta potential of composite nanostructure were recorded by the Zetasizer Nano ZS instrument (Malvern Instruments, UK). The analysis was performed at 25 °C and 173° backscatter angle. The electrophoretic mobility of the composite nanoparticle suspension was quantified and the data was altered to size and zeta potential by the Helmholtz Smoluchowski equation.

Absorbance records were collected to approve Dox, sericin, and montmorillonite in the construction of composite nanoparticles by UV-vis spectroscopy (Shimadzu, Japan). The study was performed at a normal climate condition between 190–800 nm.

The chemical bond identification of nanoparticles with and without drug was managed by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). The records were collected using Nicolet™ İS™ 50 spectrometer (Thermo Fisher Scientific). The FT-IR spectra were analyzed between 4000-600 cm^{-1} wave numbers with 16 scans.

The thermal behavior of composite nanoparticles was evaluated by Thermogravimetric Analysis (TGA) (TA® instruments, DE). The results gathered by operating with a 10°C/min heating rate. The analysis was carried out under a 99.5% nitrogen atmosphere, within 25°C to 800°C.

2.2.3. Quantitative and qualitative determination of the cellular uptake of Ser-Mt NPs

To analyze the cellular uptake of Ser-Mt NPs quantitatively, cells were cultured in a 96-well plate exposure with Dox loaded Ser-Mt NPs (Dox/Ser-Mt NPs) suspension. First, 1.0 x 10^4 cells/well were seeded on 96-well plate. After incubation overnight, the medium was renewed with fresh medium containing Ser-Mt NPs. The plate was conserved for 2 and 4 h. The nanoparticle suspension was placed on each wells. At separate times, the suspension was withdrawn and wells were cleaned with PBS. Triton X-100 (0.5%) was added to wells to rupture the cell membranes. Then the cells were centrifuged and the supernatant was analyzed by a microplate reader [14], [15].

Qualitative cellular uptake of nanoparticles in HepG2 cells was recorded with an FV1000 model CLSM, Olympus, Japan). To do this, the cells were cultured in a 6-well plate including glass coverslip coated with poly-L-lysine (PLL) and stained with Alexa Fluor® 594 WGA that particularly attaches to the cell membrane by the help of manufacturer's protocol. Then, the composite nanoparticles were stained with FITC and added on the cells for 2 and 4 h incubation. Fluorescence responses were determined at 593/614 nm for WGA (red) and 488/530 nm for FITC (green).

2.2.4. Loading and release studies of doxorubicin

To prepare Dox/Ser-Mt composite NPs, a known amount of Ser-Mt blend was suspended in ultrapure water followed by the addition of Dox with 1 mg/mL concentration. Then 3 mL of ethanol was dropped to Dox and Ser-Mt blend and crosslinked with GA (8%), then incubated 4h at room temperature under stable stirring (600 rpm). The mixture was precipitated by centrifugation under 12000 rpm for 30 min to remove free Dox and excess additives and freeze-dried.

Encapsulation Efficiency (EE %) of Dox was estimated by Equation (1). A means the total amount of drug used and B the amount of unloaded drug.

$$EE\ (\%): [(A-B)/A]x\ 100 \quad (1)$$

To conclude the amount of Dox released from the synthesized composite nanoparticles, 10 mg Dox-loaded nanoparticles were incubated into a dialysis bag (MWCO 8000–14000 Da) with 50 mL of PBS at pH 5.0 and 7.4 in a shaking water bath at 37 °C under constant shaking. At different times (1, 12, 24h) an amount of incubation medium was taken and absorbance values of dissolved Dox were quantified by spectrophotometer at 480 nm.

2.2.5. In vitro cytotoxicity assay (MTT)

In this study, cytotoxicity of the Ser-Mt composite nanoparticles with and without Dox on a HepG2 liver cancer cell line was assessed by MTT assay. Cells were placed in an incubator containing 5% CO_2 at 37 °C. DMEM- F 12 with 10% FBS and 1% L-glutamine was employed as a culture medium. The cells were collected after getting the desired confluency and 1.0 x 10^5 cells/ml of the cells were placed to 96-well plate and conserved during the night under the same condition. The composite nanoparticles were prepared with different concentrations (1, 10, 25, 50, and 100 μg/mL) and pipetted to cells and preserved for 24h. After maintenance, the medium that contained composite nanoparticles was removed and

the MTT solution was placed to each well. After exposure for 4 h in the dark at 37 °C, 100 µl DMSO was dropped on cells. The formazan crystals in purple color metabolized from MTT dye by living cells were dissolved in DMSO. The plate was read at 570 nm in a microplate reader (Shimadzu, Japan) to conclude the percentage viability of the cells. All tests were performed in triplicate.

3. Results

3.1. Characterizations of Ser-Mt composite NPs

In this study, spherical and monodisperse composite nanoparticles were synthesized from Sericin (Ser) and montmorillonite (Mt) by self-assembling with desolvation technique. Mt has, mucoadhesive and water-uptake properties which make Mt a good candidate to be used by researchers, especially in drug release studies. Also, having no immunogenicity and excellent biocompatibility and carrying diverse bioactivities are the reason for its use in this study [12], [16]. Here, Mt was successfully incorporate with Sericin and formed a composite structure and then crosslinked with glutaraldehyde (GA) to form stable Ser-Mt composite nanoparticles for the first time in literature. Before, it has been concluded that the incorporation of clay minerals with nano-size into a template causes a noteworthy augmentation of the diverse key properties of these materials. Also, the synthesized composite nanoparticles were crosslinked with GA which reacts with amine ends of sericin protein through Schiff base (–C=N-) construction in which crosslinking stable Schiff bases were managed. The crosslinking of Ser-Mt NPs with GA occurred into the free amine groups of glutamic and aspartic acid (24%) residues of the sericin structure to construct Schiff base intermediates [17], [18].

3.2. SEM and EDX analysis

The synthesized Ser-Mt NPs were monodispersed and spherical shape as demonstrated in the SEM image (Figure 1.A) According to the SEM image, the mean size of composite nanoparticles was nearly 100 nm. The size distributions of composite nanoparticles from DLS measurements demonstrated consistency with those gathered from SEM analysis. Additionally, EDX analysis showed that Mt was intercalated into the Sericin (Figure 1.B). It is well known that Mt contains potassium (K), sodium (Na), aluminum (Al), magnesium (Mg), iron (Fe) and silicon (Si), and sericin contains carbon (C), nitrogen (N), and oxygen (O) in nature [19] which was demonstrated on the EDX spectrum. Therefore it can be concluded that Mt was successfully doped into sericin structure.

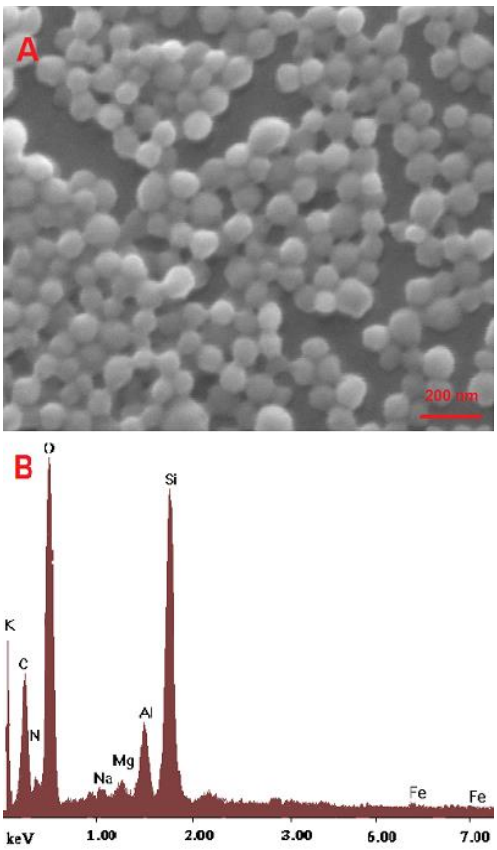

Figure 1. A) SEM image (scale bar: 200 nm), B) EDX spectrum of Ser-Mt composite NPs

3.3. Dynamic light scattering (DLS) analysis

In the study, composite nanoparticles were prepared before and after adding of Mt to sericin solution. The average diameter and zeta potential of Ser NP and Mt doped Ser composite NP with different concentrations were evaluated with DLS analysis. The results were demonstrated in Table 1. According to results the average size of Ser-Mt composite NP was found as 110 ± 2.6 nm with a good polydispersity index. The particle diameter frequency of Ser doped with 3 % (v/w) Mt nanoparticle was also presented in Figure 2. Also, the zeta potential was recorded as an average of −28.1 mV ±-1.9 mV. As it was mentioned before, the size of the nanoparticle and zeta potential of the NP surface are two significant key affecting the duration of the circulation; distribution in the body, and therapeutic profit [20]. The nano-size is known to enhance the nanoparticle deposition in tumor side through an enhanced permeability and retention effect [21]. The negative zeta potential of the Ser-Mt NPs may improve antitumor efficacy because it interferes with the nanoparticle clearance and increases deposition in the target tissue.

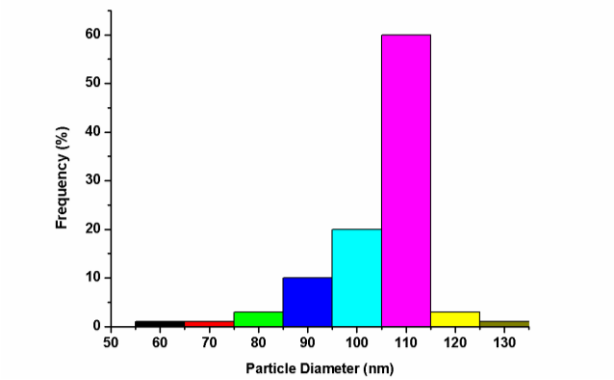

Figure 2. Particle size distribution of Ser-Mt composite NPs

Table 1. The average size, zeta potential and polydispersity index of different nanoparticle formulations

Sample Name	Zeta Pot. (mV)	Average size (nm)	Poly Dispersity Index
Ser NP	-32.0	101.1 ±1.6	0.336
Ser NP +1%Mt NP	-37.9	123 ±3.5	0.193
Ser NP +3%Mt NP	-42.4	174.3 ±2.7	0.083
Ser + 1%Mt NP	-26.7	160.2 ±4.0	0.116
Ser + 3%Mt NP	-28.1	110 ±2.6	0.019
Dox- Ser + 3%Mt NP	-25.4	122 ±3.9	0.109

3.4. UV-Vis spectroscopy analysis

The absorption spectra of Sericin, Mt, Dox, and Dox loaded Ser-Mt composite NPs were demonstrated in Figure 3. Free Sericin showed two absorption peak at 235 and 280 nm which was due to the backbone of Sericin and the aromatic structure, respectively. Mt performed an absorption peak nearly at 260 nm that can be ascribed to Fe3+ ions in the octahedral structure of Mt. The characteristic absorption peak of free Dox was seen at 490 nm on the spectrum. The absorption peaks came from Sericin, Dox and Mt were seen on the spectrum of Dox loaded Ser-Mt composite NPs which demonstrated the inclusion of both Dox and Mt into Sericin structure and formation of composite NPs [22-24].

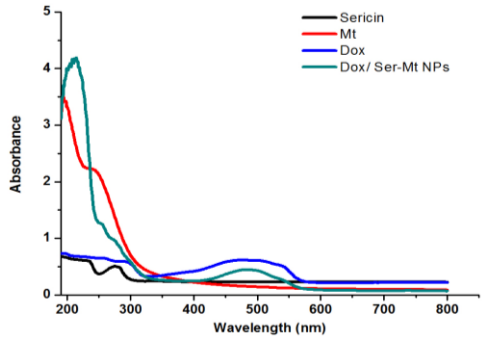

Figure 3. Uv-vis spectrum of Sericin, Mt, Dox and Dox/ Ser-Mt composite NPs

3.5. Infrared analysis

Figure 4 illustrated the FT-IR spectra of Sericin, Mt, Ser-Mt composite NP and Dox loaded Ser-Mt composite NP. In the spectrum, Sericin displayed characteristic bands at 1645 cm^{-1}, 1516 cm^{-1} and 1235 cm^{-1} for C = O stretching, N-H bending and C-N stretching of amide-I, amide-II and amide III, respectively, that belong to proteins [9,25-27]. In addition, the spectrum of Mt demonstrated the Si-O-Si stretching absorption band at 1016 cm^{-1} which is characteristics of Mt and extensively used for its characterization which was also present in the Ser-Mt composite NPs spectrum with a small shift at 1040 cm^{-1}. Also, the peak at 880 cm^{-1} was because of the Si-O-Al stretching and the peak at 1631 cm^{-1} was attributed to OH distortion of the water that is also depicted in Ser-Mt composite NP spectrum together with Dox loaded Ser-Mt NPs with a small move in the absorption band and overlapped by sericin band at 1645 cm^{-1}. On the other hand, after Dox loading Ser-Mt composite NPs in addition to sericin and Mt absorption bands, new peaks were observed at Dox loaded Ser-Mt composite NPs spectrum. For example, the bands at 3624 cm^{-1}, 2947 cm^{-1}, 910 cm^{-1}, and 797 cm^{-1} were attributed to quinone and ketone carbonyl groups which showed the –OH stretching vibration, C–H stretching, primary NH2 vibration and N–H deformation bonds, respectively. Also, the absorption band at 1226 cm^{-1} are due to C–O–C stretching while the band at 1406 cm^{-1} was came from C–C stretching.

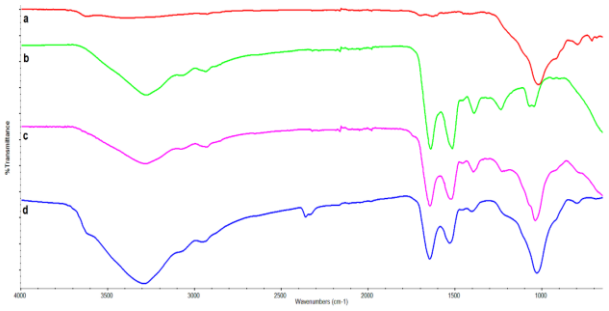

Figure 4. FT-IR spectrum of a) Mt, b) Sericin, c) Ser-Mt composite NP and d) Dox loaded Ser-Mt composite NPs.

The spectrum of Dox loaded Ser-Mt NP revealed the characteristic peaks of Sericin and Mt with a tiny move in the absorption frequency and the extra absorbance bands at the spectrum of Dox loaded Ser-Mt NP confirmed successful loading of Dox into composite nanoparticles. Therefore, it can be concluded that according to ATR-FTIR analysis, both Mt and Dox were successfully combined with Sericin [28-30].

3.6. Thermogravimetric analysis of composite NPs

The stability profiles against heat of Mt, sericin and Ser-Mt composite NPs were performed by TGA. Mt has constancy to high temperatures. In TGA

measurements, the Ser-Mt composite NPs were warmed up to 800 °C. Mt has high stability up to 800ºC but loses some of its mass at about 400 ºC due to the desorption of intercalated crystalline water molecules [31]. Sericin has a degradation temperature of 120ºC. Degradation temperature of the Ser-Mt NPs was 400ºC, while there was a residue of 50% at 800°C. Sericin was demoted and the remainder (28%) was due to it. TGA studies supported the interaction of the Mt with the Sericin doe to mass change against temperature increase in time (Figure 5).

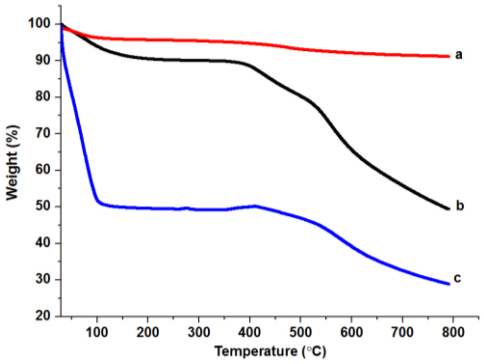

Figure 5. Thermogravimetric analysis of a) Mt, b) Ser-Mt nanoparticles and c) Sericin

3.7. Cellular uptake of Ser-Mt NPs by liver cancer cells (HepG2)

The qualitative cellular uptake of Ser-Mt NPs were recorded using a confocal laser scanning microscope. WGA dye (red fluorescence) and fluorescein isothiocyanate (FITC) (green fluorescence) were chosen to record the image of the membranes of HepG2 cells and Ser-Mt composite NPs, respectively. In this study, the time-dependent uptake of composite nanoparticles by HepG2 cells was performed (Fig. 6A). The cells were treated with Ser-Mt composite NPs for 2 and 4 h at 37 °C. The Ser-Mt composite NPs (green) were intimately placed around the cell membrane (red), marked by WGA, at 2h incubation, and more nanoparticles were observed to settle around the cell membrane and inside the cell at the 4h incubation, revealed that the NPs engulfed by the cells in a time-dependent manner. No auto-fluorescence was observed with HepG2 cells.

The quantitative cellular uptake efficiency of composite NPs were evaluated by a microplate reader that is important for therapeutic effects. Figure 6. B illustrated the uptake of composite NPs by HepG2 cells after 2 h and 4 h exposure with nanoparticles at 37 °C. It was clear the extended exposure time increased the take up of NPs by the cells. Cellular engulfment of nanoparticles could be orientated by particle size [14,32] different cancer cells, compositions of the nanoparticles, and hydrophobic/hydrophilic features of the surface [33].

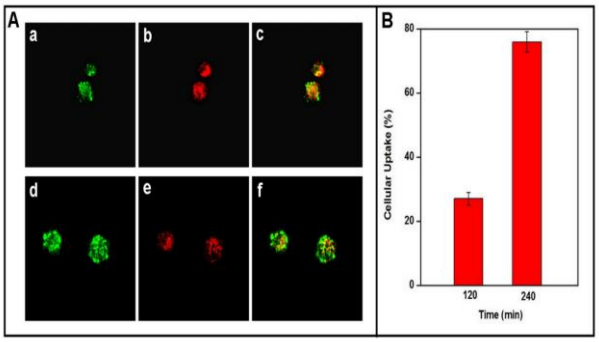

Figure 6. The qualitative (A) and quantitative (B) cellular uptake of Ser-Mt composite NPs. NPs (green) were incubated with HepG2 cells for 2 h (a, b and c) and 4h (d, e and f) and imaged with 40× mag by CLSM. The membranes of the cells were marked with CF594-WGA (red) (b and e). The uptake studies of FITC-blotted Ser-Mt composite NPs were recorded by the FITC section (left), CF594 section (middle), and combined CF594 and FITC sections (right), respectively.

The binding of nanoparticles to the cell membrane is the initial border of the nanoparticle engulfment process by the cell and this process appears to be widely affected by the surface zeta potential of the nanoparticles [34]. In this study, the zeta potential of the NPs are convenient to be chosen as drug carrier systems as aforementioned. Also, the small size of NPs help more of the composite nanoparticles to be taken into the cells. The results were also consistent with the qualitative cellular uptake results.

3.8. DOX Loading efficiency and in-vitro release studies

Drug loading efficiency or encapsulation efficiency (EE) was evaluated by the amount of Dox in the aq phase. The absorbance data of Dox released from composite nanoparticles were analyzed at 480 nm [35]. The mortality rates of cancer cells are affected by EE of NPs. The EE is a requirement for the efficiency of NP used as drug carrier systems [36]. Therefore, EE % of Dox in the Ser-Mt NPs were found to be 79.0 ± 3.3%. Mt has high water uptake and a silica layer containing a high inner area that increases the inclusion of Dox [37]. The FTIR spectra of Dox-loaded Ser-Mt nanoparticles were acquired and proved the loading of Dox.

The drug release studies for Ser- Mt NPs were performed with PBS (pH 7.4 and 5.0) as release medium (Figure 7). Dox release tests demonstrated that after 21 days, 27% and 76% Dox was released from Ser-Mt composite NPs at pH 7.4 and 5.0, respectively. In the study, Mt could adsorb Dox, both at its intercalated in and out layered construction. Dox released from NPs happened by diffusion. As was mentioned before, Mt helps to enhance the crosslinking of NPs due to its layered construction; hence, the internalization of Mt both enhanced the Dox EE and caused a slow and prolonged Dox release [12,38,39].

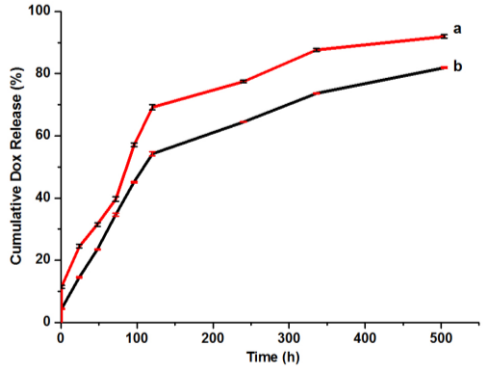

Figure 7. Dox released from Ser-Mt composite NPs a) at pH 5.0 and b) at pH 7.4.

3.9. In vitro cytotoxicity assay (MTT)

The effect of different concentrations of Ser-Mt composite NPs with and without Dox was evaluated on the HepG2 cell line (Figure 8). As known, Dox is a chemotherapeutic drug with side effects such as cardiotoxicity in dose-dependent manner. Therefore, to maximize the effectiveness of Dox in tumor tissue with minimal side effects it should be used with a suitable carrier agent [40]. Here, the fabricated Ser-Mt composite NPs were used as Dox carrier. After 24h exposure of nanoparticle formulations, MTT assay was performed. According to Fig. 8, The Ser-Mt composite NPs demonstrated no toxicity that demonstrated its safety, but the Dox-loaded Ser-Mt composite NPs showed quite toxic side effects on the HepG2 cells. The viability was recorded as 105.3 ± 4.2%, 73.8 ± 3.9 and 41.7 ± 3.2 for 100-µg/mL of Ser-Mt composite NPs, Dox loaded Ser-Mt composite NPs and free Dox, respectively.

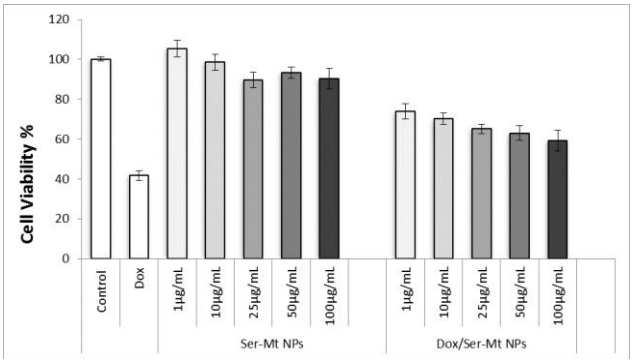

Figure 8. Cytotoxicity of Ser-Mt and Dox loaded Ser-Mt composite NPs. Data was given as percent of mean and standard error (SD) from three independent analysis.

4. Discussion and Conclusion

In conclusion, Ser-Mt composite nanoparticles were synthesized with the desolvation method. Various characterization studies have been conducted and the structure of synthesized composite nanoparticles in accordance with these studies has been revealed. The NPs were crosslinked with glutaraldehyde which reacts with amine ends of sericin protein through Schiff base (–C=N-) construction in which with crosslinking stable Schiff bases were managed. Also, the addition of Mt to the nanoparticle structure provided an extra crosslinking due to its smectite structure that was also increased the drug loading capacity due to high water uptake properties.

Doxorubicin was chosen as model cancer drug and HepG2 human liver cancer cell line was used as a model cancer cell for uptake and cytotoxicity studies. The Dox-loaded Ser-Mt composite NPs performed significantly in vitro cytotoxic effects on HepG2 cells, which resulted in high cytotoxicity with high uptake features. Dox has side effects like many other chemotherapy drugs [41]. Therefore, it is very important to move it to the desired area in the body without damaging healthy cells [42]. To solve these, nanoscale materials were developed with the contribution of nanotechnology and used as a drug delivery system. In the presented study, MTT cytotoxicity test was applied and cellular uptake was evaluated. It is known that when Dox is applied alone, it kills approximately 60% of the cells, and when applied in the body, it may damages healthy cells. With the carrier system prepared, only 27% of the cells died, but it was predicted that healthy cells were not damaged. The results implied that the Ser-Mt composite NPs could have high potentials to be used as a drug delivery agent in cancer therapy. However, the results obtained in this study should be supported with further studies with animal modals.

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