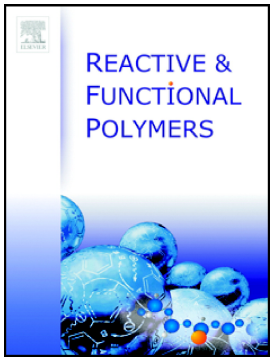

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Preparation and Characterization of Cardamom Extract-Loaded Gelatin Nanoparticles as Effective Targeted Drug Delivery System to Treat Glioblastoma

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ABSTRACT

The purpose of this research study was to prepare and characterize cardamom extract-loaded gelatin nanoparticles (CE-loaded GNPs) with a diameter ≤ 200 nm as a potent drug delivery system (DDS) for treatment of glioblastoma, which is the most common and aggressive type of brain tumor. The BBB poses physical and biological limitations to drug diffusion to reach target tissues. Polymeric nanoparticles, like gelatin, are suitable vehicles for drug delivery into the central nervous system (CNS). They are able to cross the BBB. Hence, we prepared CE-loaded GNPs by a two-step desolvation method. Seizures are detrimental secondary effects of brain tumors. Therefore, we used cardamom extract, which is an herbal anticancer and antiepileptic drug without any side effects, instead of synthetic drugs to load in gelatin nanoparticles during the particles preparation. We also prepared gelatin Type A and Type B nanoparticles for size comparison. Encapsulation efficiency, mean particle size, zeta potential and *in vitro* release profile) were performed, and particle size analysis, dynamic light scattering (DLS), UV-Vis spectrophotometry, differential scanning calorimetry (DSC), X-Ray diffraction (XRD), scanning electron microscopy (SEM) and field emission scanning electron microscopy (FE-SEM) were employed to evaluate structural and physicochemical properties of the samples. CE-loaded GNPs were obtained with diameters of 40-200 nm, zeta potential of −40.1 mV and entrapment efficiency (EE) of 70%. The ratio of extract to polymer, 1:20, was revealed to be more suitable in obtaining smaller nanoparticles without any precipitate or aggregation. We also examined cytotoxic effects of CE and CE-loaded GNPs on human glioblastoma cancer U87MG cells.

Keywords: Cardamom, Gelatin, Glioblastoma, Drug Delivery System, Cytotoxicity

1. INTRODUCTION

Glioblastoma is one of the primary brain tumors that can destroy the brain tissues and cells very rapidly. Tumors produce symptoms mainly by mass effect and destruction of functioning brain tissue. Chemotherapy and radiation therapy cannot treat it effectively. Glioblastoma is a prevalent brain cancer, but common therapeutic efforts to target this aggressive brain tumor act prove to be futile due to several of the following factors: tumor cell resistance to conventional therapies, susceptibility of the brain to damage resulting from conventional therapies, limited capacity of neurons and the affected cerebral white matter for self-repair, inability of many drugs to cross the blood-brain barrier (BBB) to treat the tumor [1]. Various types of therapy have side effects. As mentioned, epilepsy is one of the most important secondary effects of brain tumors [2, 3]. Thus, the patients, in addition to their medication side effects, must tolerate seizures and the side effects of antiepileptic drugs. This problem substantially increases patient fatigue.

The use of colloidal drug delivery systems (DDS) for diagnosis and treatment of brain and central nervous system (CNS) diseases and disorders is a great success for bioengineer. Kreuter [4] reported that by using polymeric nanoparticles with a diameter ≤ 200 nm, they could cross the blood-brain barrier (BBB) without obvious injury, and most peptides and macromolecules can cross the BBB as a result of this drug delivery system. The size and shape of nanoparticles determines the kind of distribution and type of tissue pertinent for therapy [5, 6]. In fact, polymeric nanoparticles, like gelatin nanoparticles (GNPs), with optimal surface modification and engineering are able to enter the brain to release the drugs. Thus, these nanocarriers protect the drugs from any damage until they reach the desired tissues and cells. Therefore, it is possible to employ this great ability of

nanoparticles to diagnose and to treat brain and CNS diseases such as brain tumors and epilepsy [5, 7-9].

Gelatin is a non-toxic natural polymer that has a unique chemical structure with a large number of useful amino acids and functional groups. It is a biocompatible and biodegradable polymer without harmful byproducts [10-15]. It can successfully undergo chemical modification, and by crosslinking, it can create novel opportunities for safe drug delivery nanocarrier synthesis and subsequent drug loading [16]. Thus, the gelatin nanoparticle is a safe nanocarrier to use in brain tissue that is sensitive to any damage and lacks self-repair capability.

The aqueous extract of cardamom gives rise to herbal anticancer and antiepileptic drugs without side effects. Cardamom (Elettaria cardamomum Maton), the queen of all flavors has a history as old as human race. It is one of the extravagant and fascinating flavors in the world. It is the dried fruit of an herbaceous perennial plant belonging to the ginger family, Zingiberaceae. The plant is indigenous to southern India and Sri Lanka. It is also cultivated in Guatemala. Cardamom has more than 17 natural chemical structures that have many useful functional groups, like hydroxyl, carbonyl and amino acids, to increase levels of natural antioxidants, such as glutathione, in the blood and block radicals. They can also control and adjust the diffusion of ions to prevent seizures and inhibit excess ion release [17].

In this research study, we prepared the GNPs by a two-step desolvation method [16, 18, 19]. Then, we loaded aqueous CE during nanoparticle preparation to obtain CE-loaded GNPs to treat glioblastoma without side effects. We also evaluated the cytotoxic effects of CE and CE-loaded GNPs on human glioblastoma cancer U87MG cells.

2. EXPERIMENTAL PROCEDURE

2.1. Materials

Dried fruits of Indian green cardamom were purchased from a local market in Tehran (Iran). Gelatin Type A from porcine skin (300 Bloom) from Sigma-Aldrich (St. Louis, Mo, USA), Gelatin Type B from bovine skin (225 bloom) from Sigma-Aldrich (St. Louis, Mo, USA), Glutaraldehyde solution grade 1 (25% in water) from Sigma-Aldrich, Acetone from Sigma-Aldrich, Tween 80 (Polysorbate 80) 70% from Sigma-Aldrich, NaOH from Merck (Germany), and HCl from Merck (Germany) were purchased.

2.2. Preparation of aqueous extract of green cardamom

After cleaning the adulterant material, the fruits were ground with an electric grinder into a coarse powder. About 150 g of ground material was soaked in hot distilled water and placed in a shaking-incubator (GFL 3031) at 25°C and 40 rpm, overnight. It was filtered through a cloth, and then, we put it in the shaking-incubator again at 25°C and 40 rpm, overnight. We centrifuged (universal 320R hettich zentrifugen) the extract at 4500 rpm for 10 min to separate the large particles. Finally, we freeze-dried the extract, and the cream powder of the extract was obtained.

2.3. Preparation of cardamom extract-loaded gelatin nanoparticles (CE-loaded GNPs)

Gelatin nanoparticles were prepared by a two-step desolvation method. At first, 200-500 mg gelatin was dissolved in 10 mL distilled water, under conditions of heating at 40 ± 1°C and magnetic stirring until a clear solution was obtained. After that, 10 mL acetone was added to the gelatin solution as a desolvating agent to precipitate the high molecular weight gelatin. The white supernatant was discarded, and then, the high molecular weight gelatin

was redissolved by adding 10 mL of distilled water and subsequently stirring. The pH of the resulting solution was adjusted to between 9.2 and 9.4 by adding 0.1 M NaOH for gelatin Type A, but for gelatin Type B, the pH of the resulting solution was adjusted to less than 4.8 by adding 0.1 M HCl. The aqueous extract of cardamom was added drop-by-drop with a syringe under magnetic stirring, followed by drop-wise addition of 30 mL acetone to form GNPs. Acetone was added until a permanent cream turbidity was obtained. Finally, 100 µL glutaraldehyde solution was added as a cross-linking agent, and the solution was stirred at 40 ± 1 °C for 30 min. Polysorbate 80 was added to coat the GNPs and allow them to cross the BBB, and the solution was then stirred. The solution was centrifuged at 15000 RPM for 30 min. The particles were purified by centrifugation and redispersion in water. Prepared nanoparticles were freeze-dried, thus cardamom extract-loaded GNPs were obtained.

The results of phytochemical analysis revealed that cardamom contains alkaloids, flavonoids, saponins, sterols and tannins. It is noticeable that the chemical structures, characteristics and pharmacological activities of saponins and flavonoids are so complex [17].

2.4. Characterization of gelatin nanoparticles

2.4.1. UV-Vis spectrophotometry

We determined the maximum wavelength that has the maximum absorption (λ max) for GNPs without extract and for cardamom extract.

2.4.2. Fourier transform infrared spectroscopy (FTIR)

By using the FTIR test, we can identify chemical compounds and chemical bonds in cardamom extract, gelatin nanoparticles and cardamom extract-loaded gelatin nanoparticles samples. It presents quantitative and qualitative analysis for the cardamom

extract, GNPs and CE-loaded GNPs. We detected functional groups and characterized covalent bonding information in samples.

2.4.3. Particle size analysis

The particle size was determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS (Zeta sizer Nano ZS, Malvern Instruments). It also determined the polydispersity index (PDI).

2.4.4. Zeta potential (ZP)

Zeta potential values were measured using Laser Doppler Anemometry, employing a Zetasizer (Malvern Zetasizer Nano ZS).

2.4.5. Scanning electron microscopy (SEM)

The appearance, shape and size of nanoparticles were visualized by scanning electron microscopy. We also measured nanoparticle size.

2.4.6. Field emission scanning electron microscopy (FE-SEM)

In this technique, we employed very narrow probing beams with high electron energy. Therefore, this method protects susceptible nanoparticles like GNPs against damage, disintegration and charging due to high energy and temperature, and we obtained images with high spatial resolution.

2.4.7. Entrapment efficiency (EE)

The total quantity of CE contained in the GNPs was determined by a 'direct' technique. 5 mg of the nanoparticles were added to 5 ml PBS. After complete dissolution, the drug concentration was determined by UV-Vis spectrophotometer at λ = 302 nm.

$$EE\% = \frac{Total\ of\ entrapped\ extract\ in\ GNPs}{Total\ of\ extrat} * 100 \quad (1)$$

2.4.8. In vitro release of cardamom extract from GNPs

The release of cardamom extract from the gelatin nanoparticles was performed in PBS without enzyme, pH 7.4. The in vitro release experiment was carried out on a magnetic stirrer at room temperature. The CE-loaded GNPs were suspended in PBS and stirred. At predetermined time intervals, 3 mL of supernatant was withdrawn, and then, 3 mL PBS (fresh) was added to the supernatant, and the tube was returned to the stirrer [6].

The release samples were quantitated using a UV spectrophotometer at 302 nm. The percentage cumulative amount of CE released was calculated by using the following equation:

$$Amount\ of\ CEA\ release(\%) = 100 \times \frac{Mt}{\sum_{t-0}^{t-\infty} Mt} \quad (2)$$

Where M_t is the amount of CE released at time *t* [19].

2.4.9. Differential scanning calorimetry (DSC)

The physical state of cardamom extract entrapped in the GNPs was characterized by differential scanning calorimetry. The peak of DSC for GNPs without extract and GNPs with extract was determined. It showed the thermal behavior of GNPs and cardamom extract.

2.4.10. X-Ray diffraction (XRD)

By using the XRD technique, we found crystalline and amorphous regions.

2.4.11. Cytotoxicity of CE-loaded GNPs

In addition to the ideal effects of engineered GNPs, and ideal size and shape, we must consider the toxicity of GNPs [8]. Cytotoxicity tests have been performed to detect the effect of GNPs on the cell viability in the medium. MTT reduction assay was conducted to measure cell viability. Briefly, cells were seeded (5×10^3 cells/well) in a 96-well culture plate and incubated for 24 hours. Cells were given treatment with formulations of CE-loaded GNPs for 24, 48, and 72 h to specify their optimum concentrations and the exposure time [3, 20]. Subsequently, MTT at a concentration of 5 mg/mL in PBS (pH= 7.4) was added to each well (10% v/v), and the cells were further incubated for 4 h at 37°C. After removing the unreacted media, the blue crystals were dissolved in 100 µL isopropyl alcohol, and optical density was determined at 570 nm. Cell viability (%) related to control wells containing cell culture medium without treatment was calculated by $[A]_{test}/[A]_{control} \times 100$, where $[A]_{test}$ is the absorbance of the test sample and $[A]_{control}$ is the absorbance of the control sample. Five replicates were tested for each sample.

2.5. Statistical analysis

Statistical analysis was conducted using SPSS software. One-way analysis of variance (ANOVA) and Tukey test (t-test) were conducted to calculate statistically significant differences between groups. Statistical significance was obtained at $p < 0.05$. Error bars

indicate the standard deviation of the mean value. For each experiment, sample means (n=5 replicates) were tested.

3. RESULTS & DISCUSSION

3.1. UV-Vis spectrophotometry

To inspect the absorption characteristics of the cardamom extract sample, a dilute solution of the sample and GNPs were scanned separately using a spectrophotometer (see Fig. 1). The UV/Vis absorption spectrum of the CE displays strong absorbance at 302 nm (λ_{max} = 302 nm). GNPs displays maximum absorbance at around 200 nm (λ_{max} = 200 nm).

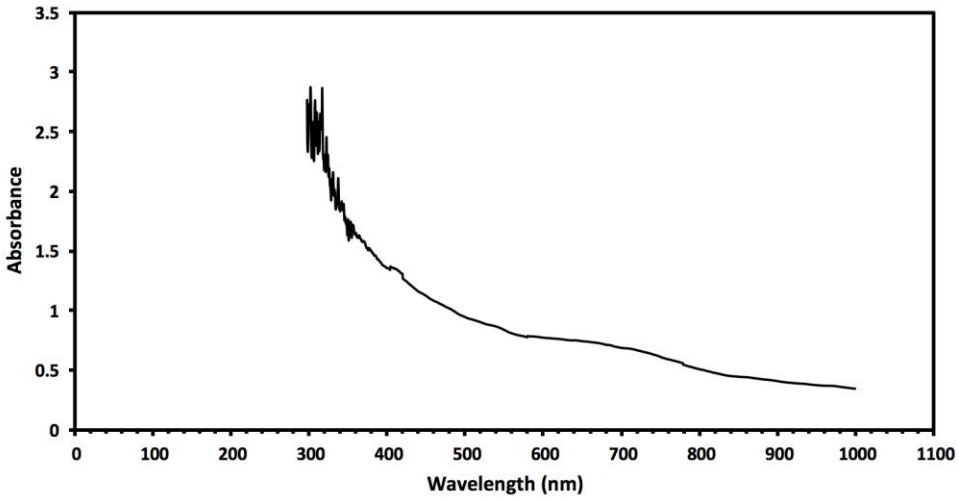

Figure 1. The maximum absorbance of cardamom extract (λ_{max} = 302 nm)

3.2. X-Ray diffraction (XRD)

X-ray diffraction studies are useful for evaluation of the crystallinity of the CE in the cross-linked nanoparticle matrix. The XRD patterns for CE (a) and CE-loaded GNPs (b) are presented in Fig. 2. The CE displayed characteristic peaks at 2θ of 28, 41, 50, 58, 67, and 73° due to its crystalline nature. However, these peaks were not seen in CE-loaded nanoparticles, which ascertained a pattern similar to the nanoparticles. This suggests that

CE in the loaded GNPs was well dispersed and amorphous or of a crystal size too little to possibly be determined by the XRD system.

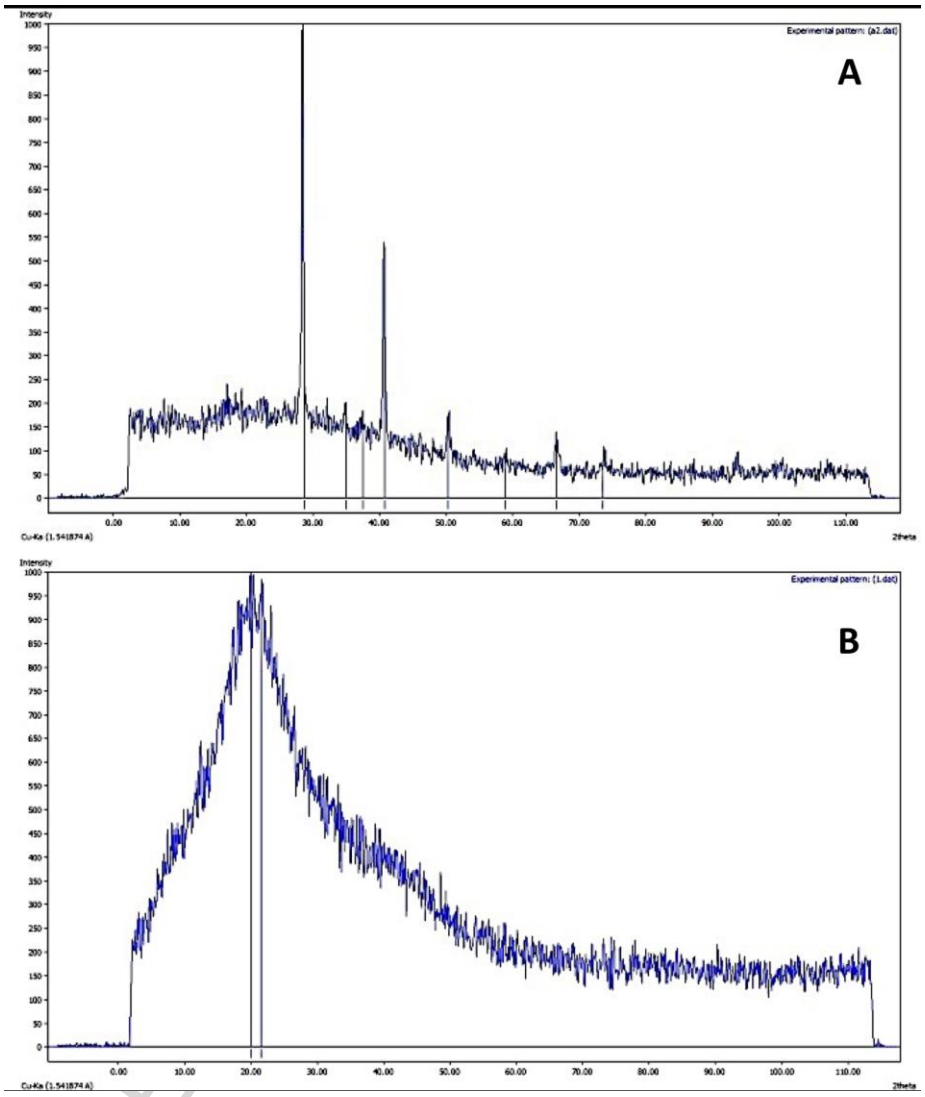

Figure 2. XRD patterns of (a) CE and (b) CE-loaded GNPs

3.3. Fourier transform infrared spectroscopy (FTIR)

By using FTIR technique, we determined functional groups in aqueous extract of cardamom, GNPs without extract and cardamom extract-loaded GNPs. Fig. 3 shows the FTIR spectra of pure GNPs and CE-loaded GNPs. In both cases, the GNPs displays all the characteristics of amide I, amide II and amide III bands at about 1653, 1543 and 1244 cm^{-1}, respectively, which corresponds to C=O stretching, N–H in-plane bending and C–H stretching

and C–N and N–H in-plane stretching, respectively. The absorption band at 2939 and 3082 cm^{-1} correlate to –CH anti symmetric and symmetric stretching of $–CH_3$, CH_2 and =C-H, respectively. The bands close to the region of 3381 cm^{-1} correlate to NH_2 antisymmetric stretch of the primary amide of gelatin and also correspond to O–H stretching, which is indicative of physically adsorbed water [21-23]. The changes in the intensity of the band and slight shifts in the IR bands after the loading of CE confirm the loading of the drug in the gelatin nanoparticles. Also, comparison between of Fig. 3b and c demonstrated that drug (CE) has encapsulated into nanoparticles and hasn't been attached onto their surface.

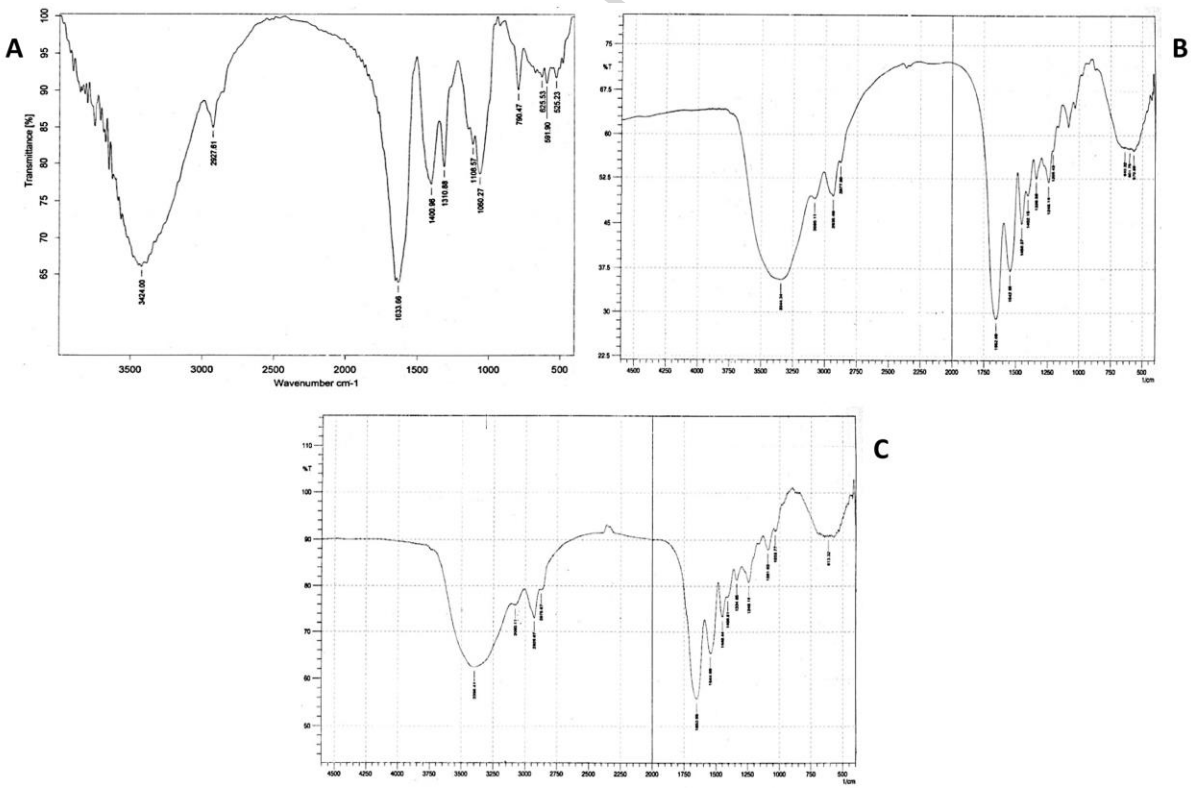

Figure 3. FTIR spectra of (a) CE, (b) GNPs and (c) CE-loaded GNPs

3.4. Differential scanning calorimetry (DSC)

DSC was employed to study the glass transition (T_g) of the maximally freeze-concentrated solution of GNPs formulations, and depending on the applied freeze-drying method, glass transition or melting temperature of the prepared samples was determined.

The DSC graph of CE, GNPs and CE-loaded GNPs is displayed in Fig. 4. Various peaks in the diagram show the presence of different compounds in the cardamom extract. The peak of the GNPs without extract was obtained at 36.16 °C, but the peak of CE-loaded GNPs was obtained at 45.96 °C. The stability and strength of herbal anticancer drugs, like the aqueous extract of cardamom, against fever condition are very important. Therefore, we can synthesize permanent and stable nanoparticles in this research study.

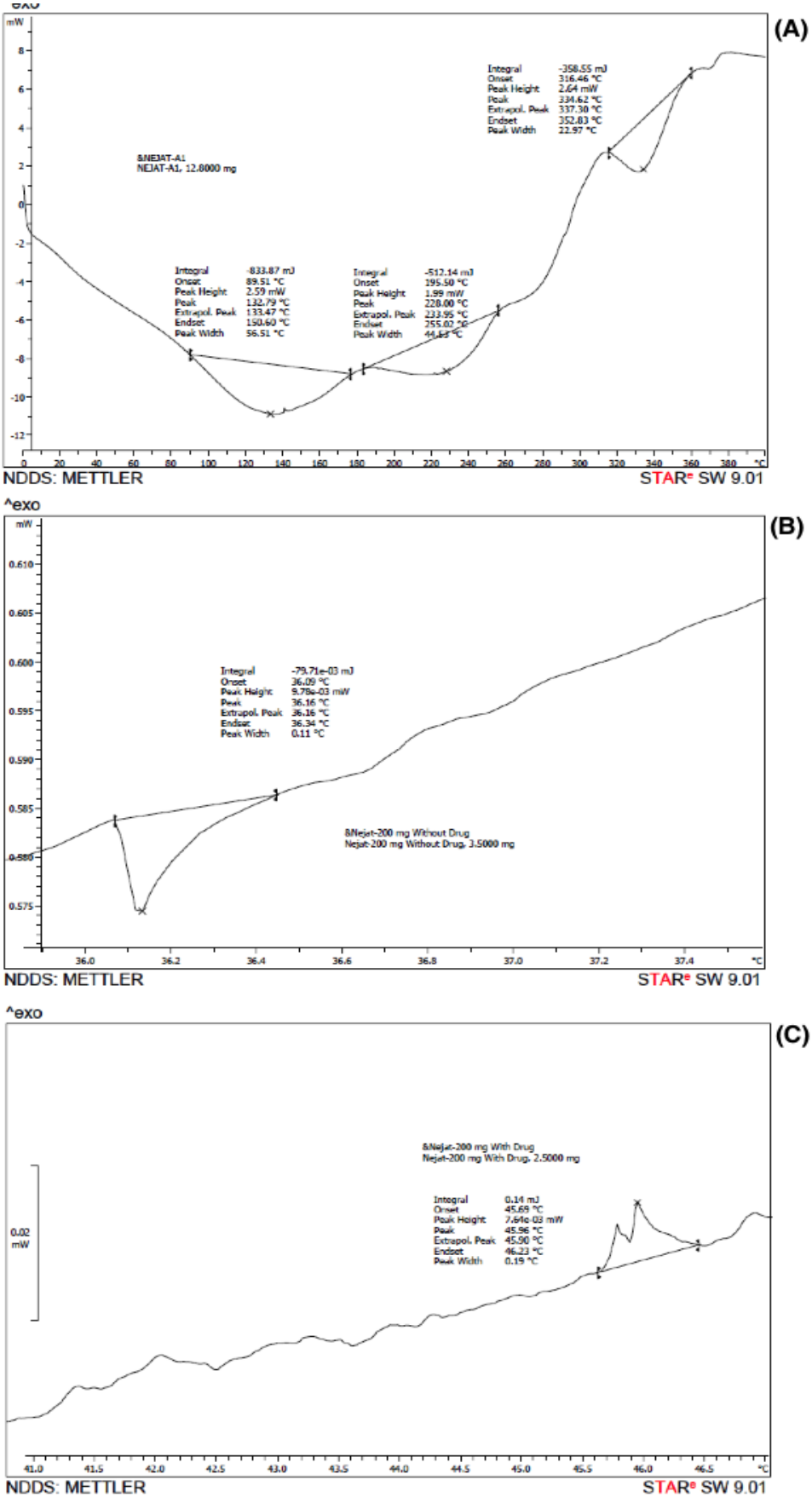

Figure 4. DSC diagram of (a) CE, (b) GNPs and (c) CE-loaded GNPs

3.5. SEM observations

SEM micrographs of GNPs and CE-loaded GNPs have been shown in Fig. 5. The GNPs have round surface morphology of diameter 100-350 nm seen from the SEM micrographs because of the agglomeration of GNPs. Most of the GNPs were individually dispersed, and some of them were present in cluster form.

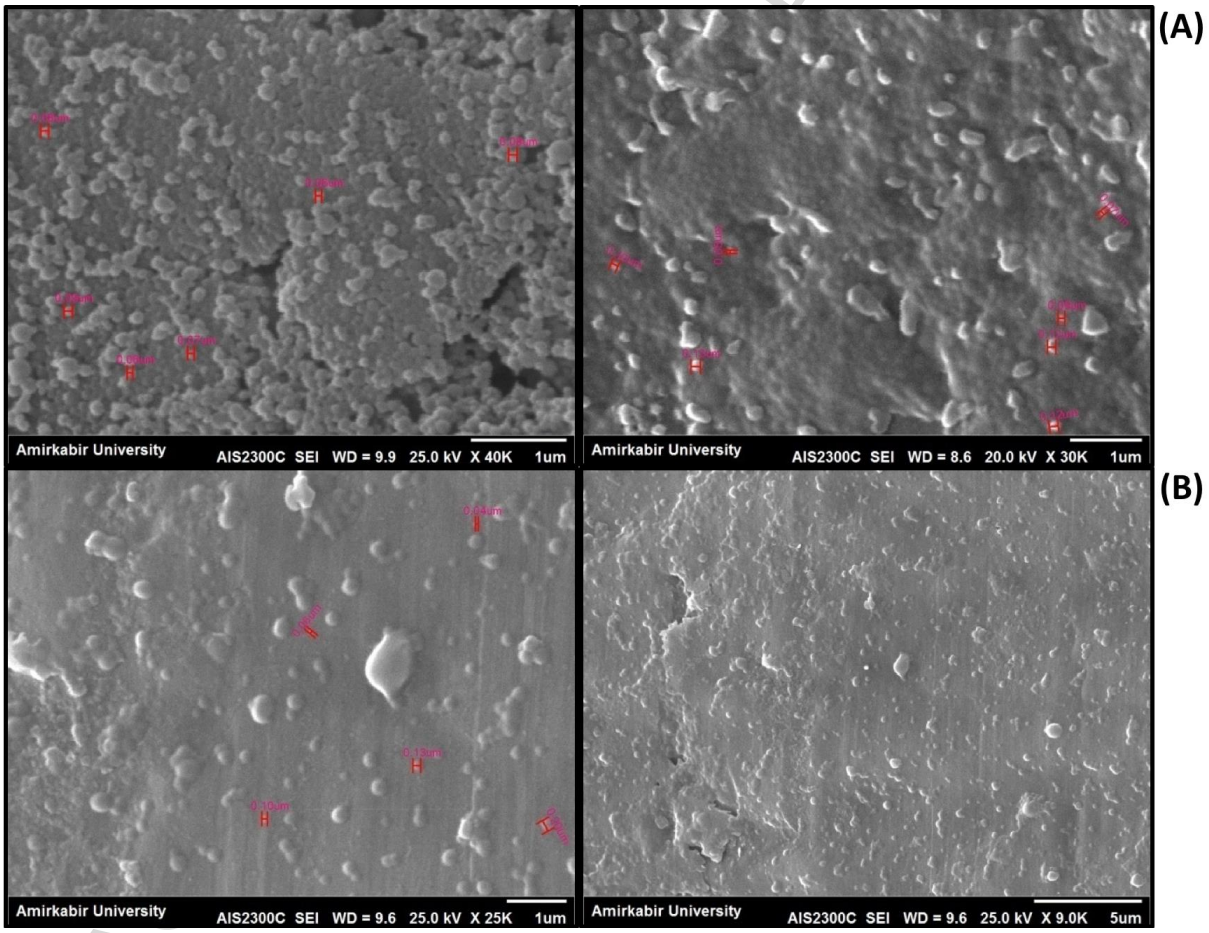

Figure 5. SEM micrographs of (a) GNPs and (b) CE-loaded GNPs

3.6. FE-SEM observations

FE-SEM images of GNPs and cardamom extract-loaded GNPs are shown in Fig. 6.

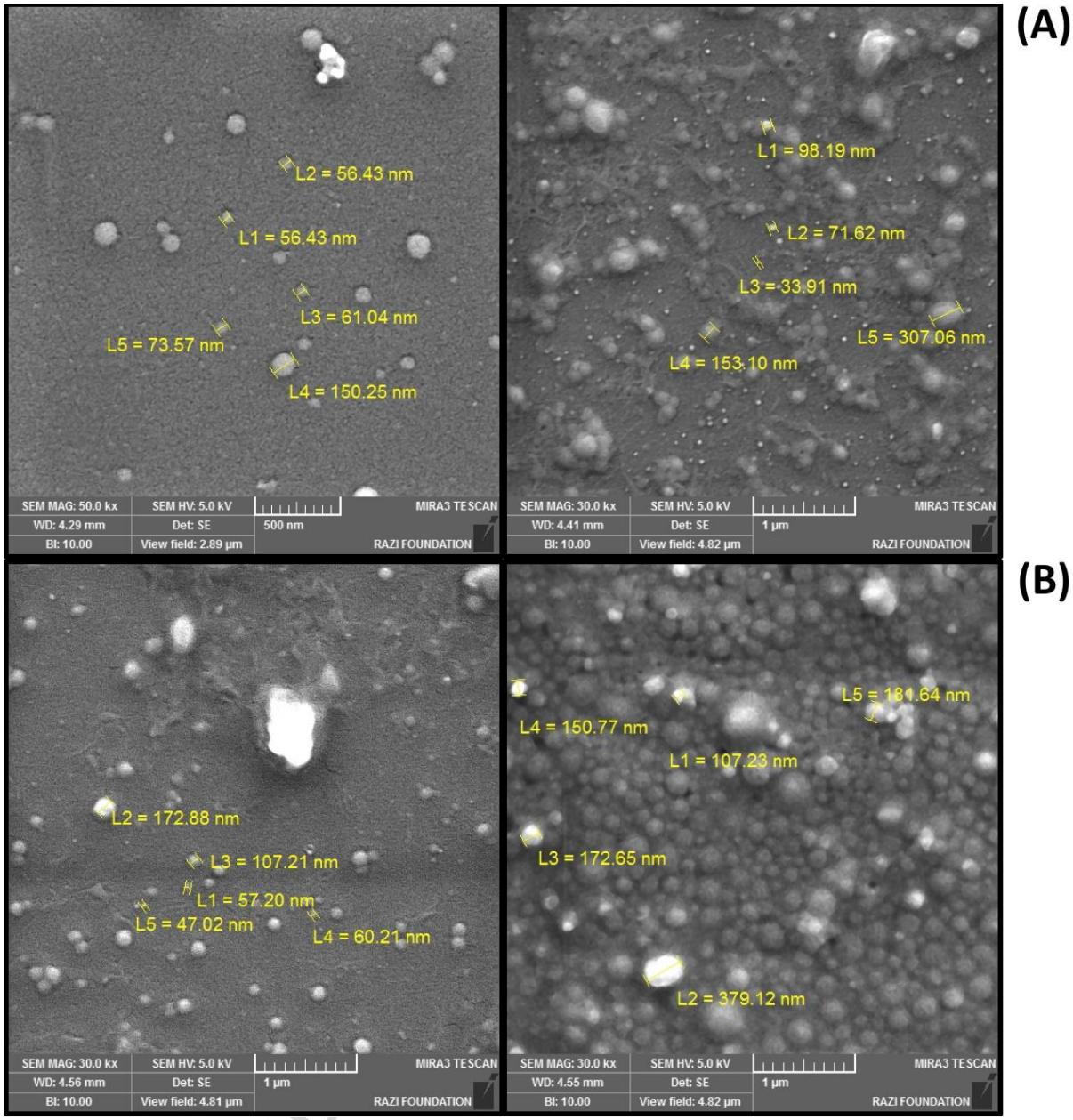

Figure 6. FE-SEM micrographs of (a) GNPs and (b) CE-loaded GNPs

It is worth mentioning that the cross-linking process causes the delivery system to have uniformly spherical morphology of the CE-loaded particles by self-aggregation of GNPs. SEM observations confirmed the smooth surface and spherical morphology of GNPs and CE-loaded GNPs. The particle size of CE-loaded GNPs was found to be 40-200 nm, which is

much higher than the GNPs, which was determined to be 33-153 nm. This phenomenon could be due to the anchoring of drug molecules at the surface of the nanoparticles.

3.7. Particle size analysis

A method of preparing GNPs by two-step desolvation method was described by Coester et al [18]. In our experiments, we studied the influence of varying fabrication parameters (nature of gelatin, amount of gelatin and amount of CE) on the nanoparticle properties. The aim was to produce small nanoparticles with a narrow size distribution. It has been displayed that the particle size is an important characteristic of a nanoencapsulation product employed for drug delivery due to the dependence of drug release rate on the size and size distribution. Desai and colleagues [24] divulged that 100 nm size nanoparticles had a 2.5-fold greater uptake compared to 1 µm and 6-fold higher uptake compared to 10 µm microparticles in a Caco-2 cell line. The results of other researchers also revealed that particle size remarkably affects cellular and tissue uptake, and, in some cell lines, only the submicron size particles are taken up effectively in lieu of the larger size microparticles [25-27].

We evaluated the effect of these different parameters on the particle size and the polydispersity index, where the polydispersity index calculates the second moment of the size distribution of the nanoparticle population. A lower polydispersity index explains a narrower size distribution. The results are shown in Table 1.

During these evaluations, it was demonstrated that the production of nanoparticles at room temperature (25°C) was impossible due to the fact that gelatin formed a highly viscous gel at this temperature. The results at 40 °C displayed that temperature has a crucial effect on the particle size. The smallest nanoparticles Enhancing the temperature to 50 and

60°C increased the particle size were made at 40 °C with gelatin A or B. (data not shown). This could be described by the gelling characteristics of the gelatin. In solution, the triple helix structure starts to uncoil when the temperature enhances. The viscosity simultaneously decreases. At 40°C, the chains appear to be adequately uncoiled and the incorporation of the desolvating agent results in a better-controlled precipitation of the macromolecules compared to higher temperatures.

The nature of gelatin also had an influence on the particle properties. At 40°C, nanoparticles prepared from gelatin B were predominantly larger compared to nanoparticles made from gelatin A (see Table 1). In addition, the polydispersity of the particle size distribution was narrower for gelatin B compared to gelatin A. Whereas, Azarmi et al., revealed that the particle size and polydispersity distribution was narrower for gelatin A compared to gelatin B [25].

The isoelectric points of gelatin A and B are approximately 6.1 and 4.5, respectively. To form nanoparticles in the second desolvation stage, the pH of the gelatin solution must be balanced far from their isoelectric points to either pH 2.5 or 12. The incorporation of the desolvating agent decreases the water available to retain the gelatin in solution, causing shrinkage of the hydrated gelatin chains. At a certain point, the hydration is very low and the protein chains precipitate as nanoparticles. The results using gelatin A and B demonstrated that pH 2.5 was the optimum pH for fabrication of the nanoparticles. Increasing the pH to 4 or higher resulted in early agglomeration of the gelatin when the desolvating agent was incorporated. A possible description of this phenomenon is that at pH 2.5 or 12 protein chains are highly positively or negatively charged. The electrostatic repulsion inhibits the polymer chains from uncontrolled agglomeration. After the

nanoparticles are made, their surface has an adequate zeta potential to inhibit additional agglomeration of the particles.

Table 1. Size (average) and PDI of the GNPs and CE-loaded GNPs (prepared at 40°C) by using DLS technique

Gelatin type	Gelatin (mg)	Distilled water (ml)	Cardamom extract (mg/ml)	Size-average (d nm)	PDI
A	500	10	0	231	0.102
A	300	10	0	189	0.05
A	200	10	0	187	0.186
B	200	10	0	328	0.142
A	300	10	5	251	0.234
A	200	10	5	212	0.225

3.8. Zeta potential (ZP)

Zeta potential of the best sample of cardamom extract-loaded gelatin nanoparticles (CE-loaded GNPs) by using DLS technique is shown in Table 2. The results revealed that zeta potential value of the samples measured is negative.

ZP is an indicator of the stability of NP suspensions. A higher electric charge on the surface of the NPs will prevent aggregation of the NPs in buffer solution because of the strong repellent forces among particles [28, 29].

The surface charge of the particles and binding type between the drug and nanoparticles are the important parameters that determine the rate of desorption of the drug in the nanoparticles and drug loading efficiency. The zeta potential also can be used to determine whether a charged active material is encapsulated within the center of the nanoparticle or on the surface [30, 31]

By measuring the ZP of the drug or substrate, one can determine if the drug is shielded or not by the nanoparticles because if the drug is shielded by the nanoparticles, ZP will be close to nanoparticle ZP or zero ZP [32].

Table 2. Zeta potential of the CE-loaded GNPs

Gelatin concentration (mg/ml)	Cardamom Extract (mg/ml)	Size-average (d. nm)	PDI	ZP (mV)
20	5	212	0.225	– 40.1±6.2

3.9. Entrapment efficiency (EE)

The entrapment efficiency (EE) is one of the very important physicochemical characteristics of drug-loaded polymeric nanoparticles. We calculated mass of extract in the supernatant by using a standard curve and then calculating entrapment efficiency: EE% = 70%.

A standout amongst the most fancied characteristics of effective nanoparticles is its high stacking limit of medication. The loading of the drug into nanoparticles is obtained by the two following methods:

(1) by addition of the drug into the matrix of nanoparticles during fabrication or, (2) by immersion or adsorbing the drug onto the surface of the preformed nanoparticles [33]. An addition technique is thought to display efficient loading compared to immersion technique [34-36]. Different mechanisms could be involved in drug loading into GNPs containing electrostatic attraction, physical entrapment, or chemical bonding [6]. Hydrophilic drugs could be effectively loaded into GNPs by incubating the drug in aqueous gelatin solution for adequate time before nanoparticle development by physical entrapment. Doxorubicin (DXR) can be added into magnetic GNPs through electrostatic interactions between positively charged DXR and the negatively charged FeO^- and COO^- ions as coating layers after permitting GNPs to swell in a newly produced drug solution [37]. Entrapment of hydrophobic drugs in GNPs could be described on the premise of particular localization of the drug inside the nanoparticulate core, which is less hydrophilic than the outer aqueous environment [38]. The amount of drug loading and entrapment efficiency of GNPs relies upon the molecular weight and also on the nature of the substance integrated. Truong-Le et al. previously reported that the entrapment efficiency of GNPs is enhanced by increasing molecular weight [39].

3.10. In vitro release of cardamom extract from GNPs

CE-loaded GNPs prepared by two-step desolvation method released the drug in a sustained fashion, and the drug release at prefixed time intervals was determined and plotted in terms of percentage entrapment (see Fig. 7).

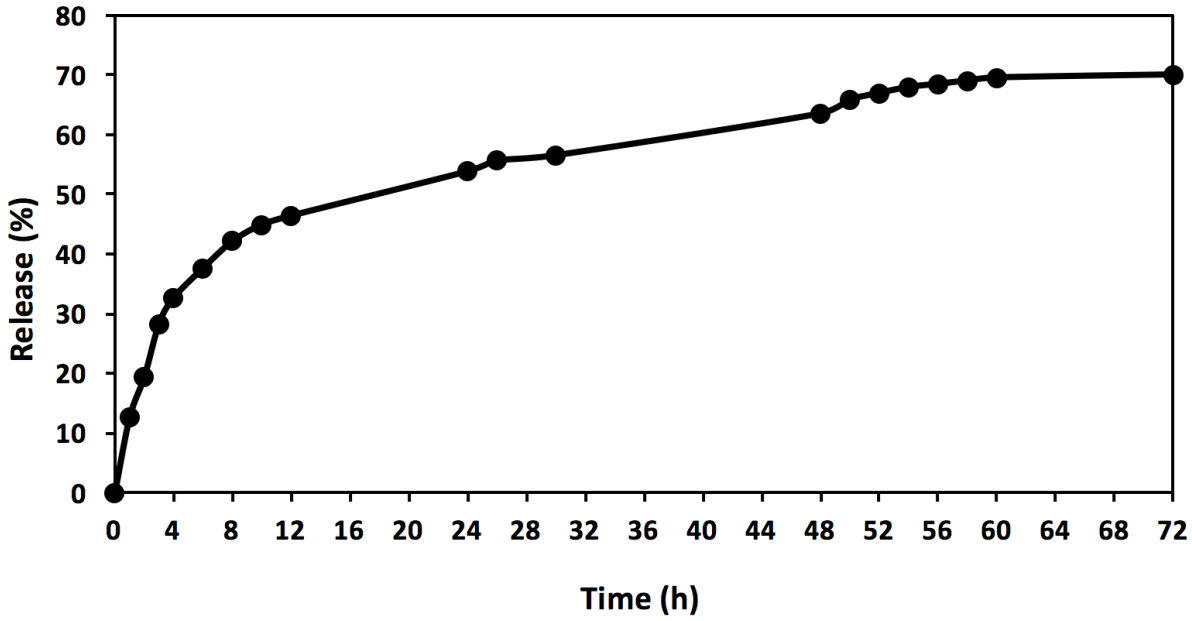

Figure 7. Release profile of CE-loaded GNPs in PBS without enzymes

Drug release from biodegradable polymeric nanoparticles takes place via a combination of several mechanisms. It generally takes place via desorption of a surface-bound drug, diffusion of the drug through the polymer matrix, and erosion of the polymeric nanoparticles [40].

As it can be observed in Fig. 7, the nanoparticles formulations displayed a biphasic pattern of drug release, an initial burst effect because of the immediate release of the surface-connected drug and prolonged release in the second stage because of the slow diffusion of the drug from the nanoparticle matrix. The fraction released during the first 12 h could attribute to the fractions of the free drug that were released without control from the GNPs. However, the drug that was covalently bound to the nanoparticles extended release in a sustained and prolonged way and the total amount of drug in terms of percentage entrapment was 70% at the end of 72 h. The drug release from GNPs could be

due to three leading mechanisms containing desorption, diffusion and biodegradation of GNPs [41, 42]. Many factors were found to affect the rate of drug release from GNPs. It was found that the crosslinking density of gelatin have a remarkable impact on the drug release rate from nanoparticulate matrix. Bajpai and Choubey [43] revealed that both the fractional release of cytarabine and the swelling ratio are enhanced with increasing glutaraldehyde concentration up to 10.6 mM, whereas beyond this threshold, the release and swelling decreased. Since glutaraldehyde is a hydrophilic crosslinker, its increasing number of linkages in the nanoparticles increases their hydrophilicity, which will permit an increase in the number of water molecules to penetrate into the nanoparticle and subsequently increase the swelling ratio and the release of cytarabine. Another factor is the presence of proteolytic enzymes that promotes the biodegradation of GNPs and causes more rapid drug release [16]. Leo et al. [33] have worked on the release of DOX from GNPs by dynamic dialysis both with and without trypsin. Only a small fraction of the drug was released without the enzyme attributing to the free drug fraction. Incorporation of trypsin resulted in the release of a higher amount of the drug probably because of a fraction of the DOX–peptide conjugates made by nanoparticle digestion and evaluated by a molecular weight lower than membrane cut-off.

Our study demonstrated that CE loaded GNPs could be suitable for targeted drug delivery and they are capable of releasing the drug in a slowly sustained manner over days.

3.11. Cytotoxicity of CE-loaded GNPs

We investigated the effect of CE-loaded GNPs and free CE, to determine the ability to induce cytotoxicity on human glioblastoma U87MG cell lines (see Figs. 8-12). The cell viability percent has been shown after 24, 48 and 72 h at different concentrations of CE-

loaded GNPs. We also examined a traditional concentration of free cardamom extract as an herbal anticancer drug of about 5 mg/ml that was 36 times more diluted.

Our results showed that the level of apoptosis induced by CE-loaded GNPs on the human glioblastoma U87MG cells was statistically higher than that of corresponding amounts of free CE (p value <0.05), while the opposite mentioned phenomenon was true for the noncancerous cells, which displayed no noticeable influence at all for noncancerous cells (over the control). These results give further support to the applicability of CE-carrier systems for targeted glioblastoma treatment. Therefore, we can employ CE-loaded GNPs as an effective anti-glioblastoma cancer therapeutic agent that could also act as an antiepileptic drug to reduce the side effects of glioblastoma pathology and synthetic chemical drugs.

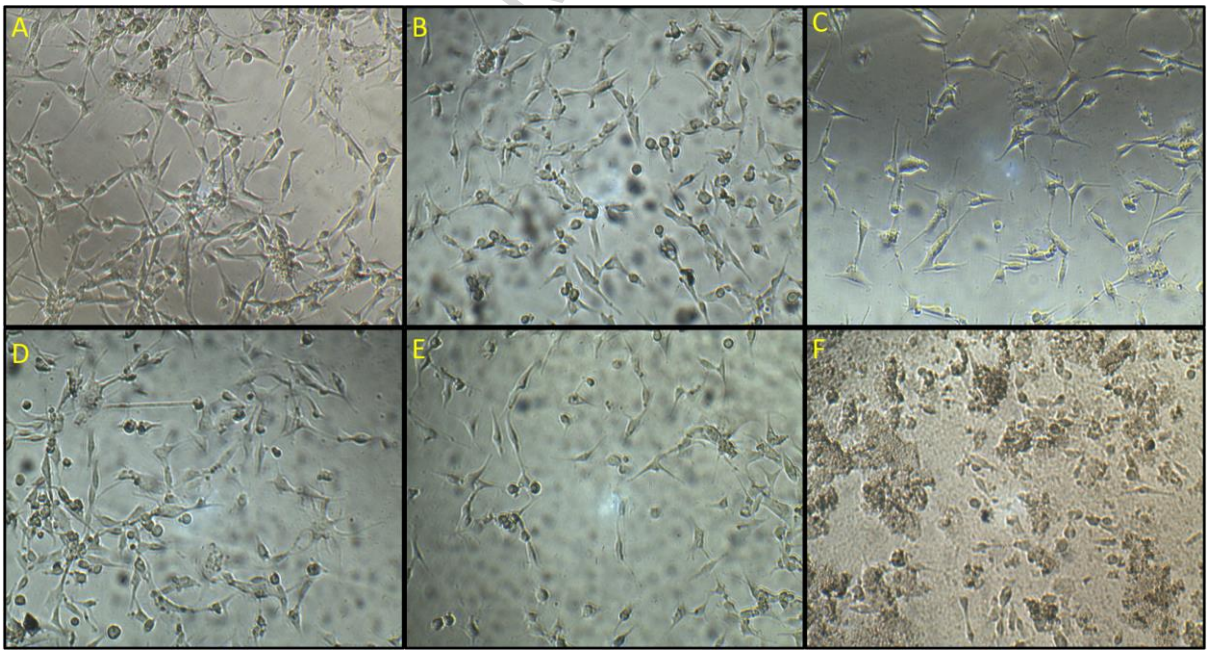

Figure 8. Cell viability images after 24 h in different concentrations of CE-loaded GNPs: (a) Control, (b) 1 µg/ml, (c) 5 µg/ml, (d) 10 µg/ml, (e) 50 µg/ml and (f) 5 mg/ml

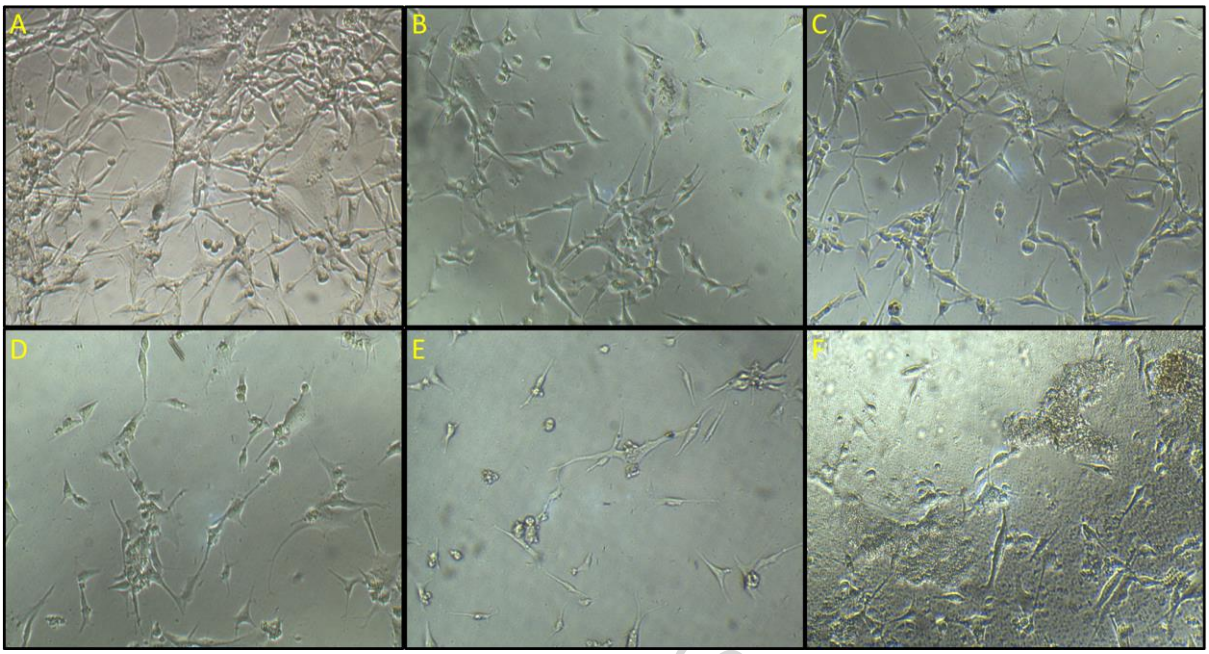

Figure 9. Cell viability images after 48 h in different concentrations of CE-loaded GNPs: (a) Control, (b) 1 µg/ml, (c) 5 µg/ml, (d) 10 µg/ml, (e) 50 µg/ml and (f) 5 mg/ml

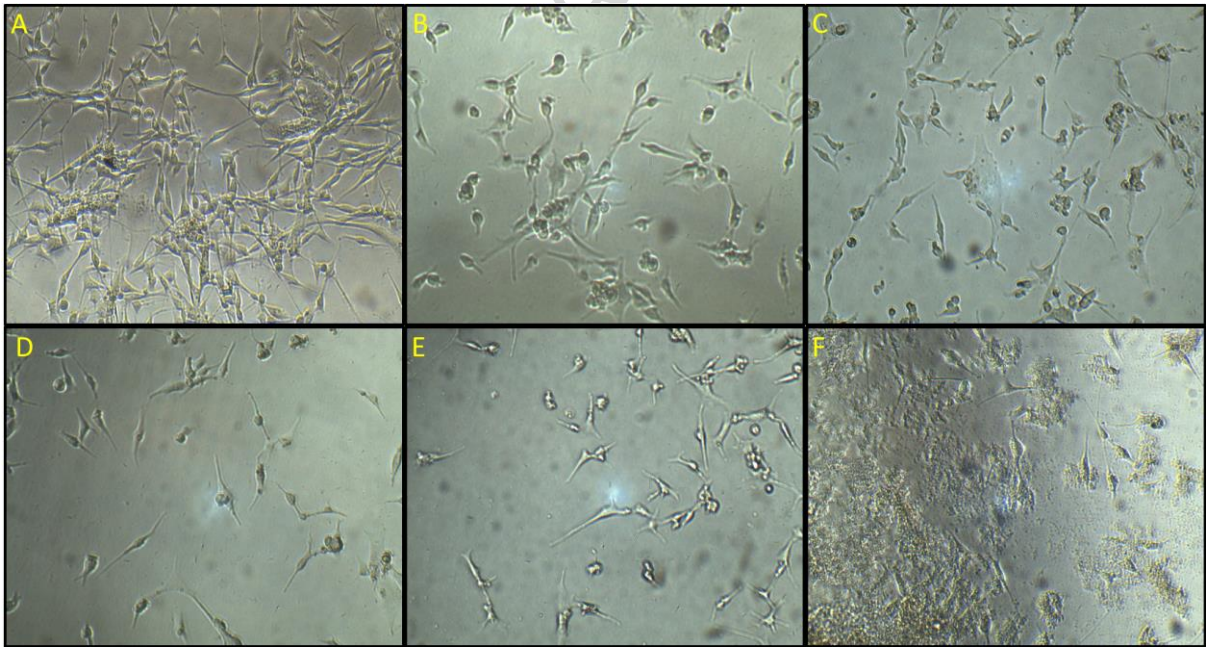

Figure 10. Cell viability images after 72h in different concentrations of CE-loaded GNPs: (a) Control, (b) 1 µg/ml, (c) 5 µg/ml, (d) 10 µg/ml, (e) 50 µg/ml and (f) 5 mg/ml

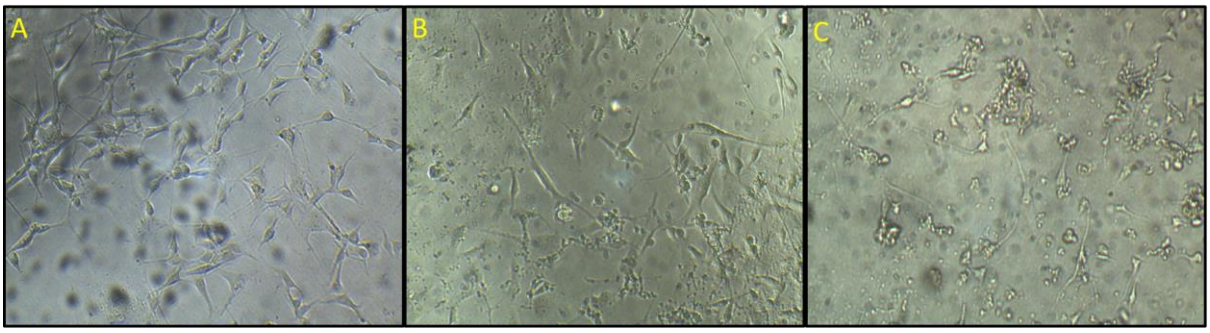

Figure 11. Cell viability images in 5 mg/ml concentration of free cardamom extract after: (a) 24 h (b) 48 h and (c) 72 h

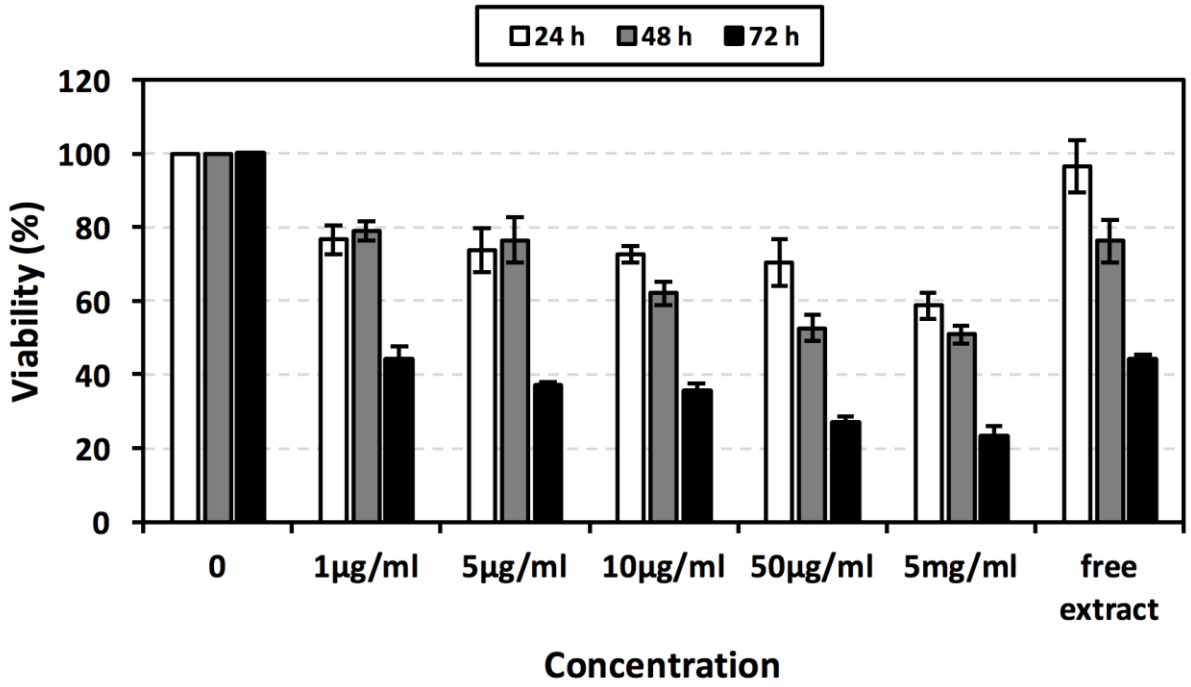

Figure 12. Cell viability in different concentrations of CE-loaded GNPs and 5 mg/ml of free extract after 24, 48 and 72h

4. CONCLUSIONS

In conclusion, we successfully prepared CE-loaded GNPs as an effective targeted drug delivery system to treat glioblastoma. We prepared the CE-loaded GNPs by two-step desolvation method using gelatin Type A due to the fact that GNPs made of gelatin Type B do not have the proper mean diameters for extract loading in order to cross the BBB. The

best ratio of extract to polymer was 1:20, and the entrapment efficiency was calculated to be 70%. We chose a human glioblastoma U87MG cell line to test the cytotoxic effects of CE-loaded GNPs and the free cardamom extract. Free cardamom extract fully and effectively eradicated glioblastoma cells. Therefore, we prepared safe gelatin nanoparticles containing an effective herbal anticancer in a novel and pioneering fashion to potentially serve as a future treatment option for glioblastoma. We employed whole extract without any separation of natural chemical structures to utilize all of the therapeutic benefits of the extract. By using this drug delivery system, we optimized cardamom extract properties, such as stability, safety and solubility in the body. Of course, the cardamom extract half-life plays a vital role in the therapeutic effect of nanoparticles. Thus, we prepared CE-loaded GNPs to improve therapeutic properties of natural polymers, like gelatin, and herbal substances, like cardamom extract, for the safe and effective treatment of the hard-to-treat debilitating neurological abnormality of glioblastoma.

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