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Ellagic acid encapsulated chitosan nanoparticles as anti-hemorrhagic agent

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ABSTRACT

Ellagic acid, a naturally occurring polyphenol was encapsulated in chitosan particles prepared by ionotropic gelation and characterized for its physicochemical properties. A maximum encapsulation efficiency of 49% was achieved. The blood clotting time and clot retraction time were calculated for different concentrations of ellagic acid, chitosan and ellagic acid-encapsulated chitosan. A reduction of 34% in the clot time and 16.4% in the retraction time was observed in ellagic acid-encapsulated chitosan when compared with free ellagic acid at concentrations as low as 0.1 mg/mL. The physical blend in comparison to free ellagic acid displayed a reduction of 13.8% and 4.6% in the clotting time and retraction time respectively under similar conditions. This suggests that the encapsulation of ellagic acid favors thrombosis due to synergistic action of chitosan and ellagic acid on same molecular targets. This study demonstrates the potential of ellagic acid–chitosan system as an effective anti-hemorrhagic system.

1. Introduction

Excessive blood loss is one of the major reasons for high mortality rates in case of accidents and in war field. Most fatalities occur due to excessive blood loss prior to reaching the hospitals or receiving medical attention (Dorros et al., 1983). The chances of survival of trauma patients depend on the ability to stem hemorrhage rapidly. There also exists a need to control bleeding during surgery to minimize complications and improve chances of survival (Wada et al., 2013). Coagulation of blood involving activation and aggregation of platelets through a complex interplay of numerous factors forms an integral part of the hemostasis process (Tocantins, 1936). The key player in coagulation is the platelet, which was first described in 1862 by Giulio Bizzozero (Ribatti & Crivellato, 2007). In the natural process of hemostasis, the damaged blood vessel is covered by the thrombus or blood clot comprising platelets and fibrin there by arresting bleeding followed by initiation of repair mechanisms (Ellis-Behnke et al., 2006; Gorbet & Sefton, 2004). Hemostatic or anti-hemorrhagic agents have been found to improve hemostasis either through initiation of primary hemostasis or thrombus formation (Levi, Vink, & de Jonge, 2002). The formation of an insoluble fibrin clot comprises a complex series of proteolytic reactions involving cellular and molecular components in the blood (Neun & Dobrovolskaia, 2011; Slauson & Cooper, 2002).

The factors in the blood, which are involved in the formation of the thrombus, exist in an inactive form until they are activated through tissue factors or surface-mediated reactions (Furie & Furie, 2008). Another key player in hemostasis is the platelet that serves to preserve the blood vessel integrity through formation of aggregates known as the 'platelet plug'. Platelet activation and blood coagulation are both required hemostasis. It has been found that the fibrin formed during coagulation stabilizes the platelet plug formed during hemostasis (Niewiarowski, Rogoeczi, Steward, Senyi, & Mustard, 1972). Thus, platelet aggregation and blood coagulation are two mutually dependent processes (Heemskerk, Bevers, & Lindhout, 2002).

Conventionally, hemostasis had been achieved through many methods like application of pressure or ligation, cauterization and induced vasoconstriction (Ellis-Behnke et al., 2006). Modern techniques have employed chemical agents that induce hemostasis by activation of coagulation factors thereby minimizing bleeding and promoting clotting. Anti-hemorrhagic properties of zeolite, potassium alum, anhydrous aluminum sulfate, lidocaine,

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benzethonium chloride, titanium dioxide nanotubes, etc., have been investigated (Doyle, Hunter-rinderle, & Singer Jr, 2008). Organic polymers of biological origin like collagen and chitosan have also been reported for their potential anti-hemorrhagic effect (Alam, Burris, DaCorta, & Rhee, 2005; McBee & Koerner, 2005). However, these molecules possess numerous side effects and the search for an efficient and instantaneously acting anti-hemorrhagic agent still continues.

Powder forms of anti-hemorrhagic agents are not effective as they are unable to flow to the site of injury and hence formulations that could be sprayed are preferred (Özmen & Özmen, 2012). In addition to the adverse effects, most of the currently employed anti-hemorrhagic agents possess very short shelf life and hence are not very effective on application (Klotoé et al., 2013). Due to the lacuna in the current materials, there arises the necessity for the development of a better system that enhances thrombosis by reducing the blood clot time without causing any adverse effects.

Ellagic acid is a naturally occurring phenol found in many fruits like pomegranates, raspberries, strawberries, pecans, blackberries and in several vegetables (Ascacio-Valdés, Aguilera-Carbó, Martínez-Hernández, Rodríguez-Herrera, & Aguilar, 2010; Ascacio-Valdés et al., 2011; Damas, Adam, Remacle-Volon, & Grek, 1987). It has been reported to possess anti-oxidant, anti-proliferative and wound healing properties (Govindarajan, Vijayakumar, Rao, Shirwaikar, Mehrotra, & Pushpangadan, 2004). It is naturally abundant, biocompatible and has high chemical stability (Girolami, Agostino, & Cliffton, 1966). Ellagic acid has been reported to promote coagulation of blood through the activation of the Hageman factor (Factor XII) of the intrinsic cascade to an active serine protease (McKay, Müller-Berghaus, & Cruse, 1969). The intrinsic blood coagulation system is initiated when the plasma contacts the negatively charged ellagic acid surface (Becker et al., 1985). However, the use of ellagic acid as an anti-hemorrhagic agent is limited by its poor solubility and poor bioavailability owing to its rapid elimination and poor adsorption (Behl, Sharma, Dahiya, Chhikara, & Chopra, 2011; Lei et al., 2003). Hence the desired therapeutic effects could not be achieved.

The use of a suitable nanocarrier could enhance the therapeutic efficacy of ellagic acid. Chitosan, a polysaccharide prepared by the partial deacetylation of chitin, a naturally occurring polysaccharide has been reported to possess the ability to rapidly clot blood and promote wound healing (Muzzarelli, Mattioli-Belmonte, Pugnaloni, & Biagini, 1999). This property is being exploited to use chitosan in wound dressings and sutures (Peter, 1995). It has been reported that chitosan could activate both blood coagulation and complement systems by recruiting erythrocytes and platelets to its positively charged surface (Brandenberg, Leibrock, Shuman, Malette, & Quigley, 1984; Minami, Suzuki, Okamoto, Fujinaga, & Shigemasa, 1998). Recently, it has been identified that chitosan enhances the release of platelet derived growth factor AB (PDGF-AB) and transforming growth factor β1 (TGF-β1) from platelets (Shen et al., 2006). Chitosan-based nanocarriers have elicited interest for delivery of therapeutics in recent years due to its biocompatibility, biodegradability and pH responsive swelling (Goycoolea et al., 2007). Chitosan possesses mucoadhesive properties and has been reported to attract platelets to its surface (Busilacchi, Gigante, Mattioli-Belmonte, Manzotti, & Muzzarelli, 2013). Chitosan has been demonstrated to regenerate epithelial cells and hence shows promise for wound healing, bone regeneration and corneal regeneration applications (Muzzarelli, 2009). Chitosan-based wound dressings have been shown to retain the functional stability of the platelet growth factors (Jayakumar, Prabaharan, Sudheesh Kumar, Nair, & Tamura, 2011). This property has been explored for wound healing applications by employing chitosan-based systems for the delivery of platelet-rich plasma and platelet lysates (Ong, Wu, Moochhala, Tan, & Lu, 2008). Ellagic acid-loaded chitosan films have been reported to inhibit the proliferation of glioblastoma. The films exhibit a slow and sustained release of ellagic acid. However, the release was accelerated in the presence of lysozyme that served to cleave the glycosidic linkages in chitosan (Kim, Gaber, et al., 2009; Kim, Liu, et al., 2009). Ellagic acid-loaded chitosan films were reported to be effective against the proliferation of melanoma cells (Kim, Liu, et al., 2009) and colorectal cancer cells (Santos, Ponte, Boonme, Silva, & Souto, 2013). The use of chitosan nanocarriers can impart better anti-tumour properties to the formulation owing to its inherent pro-apoptotic and membrane disrupting properties (Santos et al., 2013). A recent study has employed ellagic acid-loaded chitosan nanoparticles against oral cancer cell lines. It was reported that the chitosan-ellagic acid combination was able to promote apoptosis and DNA fragmentation unlike free ellagic acid or chitosan (Arulmozhi, Pandian, & Mirunalini, 2013). The anti-oxidant properties of ellagic acid have been enhanced through encapsulation in chitosan-glycerol phosphate nanocarriers and were successfully employed to counter the nephrotoxic effects of cyclosporine using both *in vitro* and *in vivo* models (Sharma, Italia, Sonaje, Tikoo, & Ravi Kumar, 2007). In the present context, encapsulation of ellagic acid in a chitosan matrix could enhance the retention time and bioavailability of ellagic acid as well as integrate the anti-hemorrhagic properties of both constituents for a synergistic effect to promote coagulation and control bleeding. Moreover, the inherent anti-microbial properties of chitosan could further aid in retarding infection-related complications on application (Katas, Hussain, & Ling, 2012). Therefore, ellagic acid encapsulated into chitosan nanoparticles were developed to explore their potential as anti-hemorrhagic agents in the present work.

2. Materials and methods

2.1. Materials

Ellagic acid (Sigma–Aldrich, USA), dimethyl sulfoxide (DMSO) (Merck, India), sodium tripolyphosphate (Lobachemie, India, India) were used in the study. Chitosan (85% deacetylated, 140 kDa) was a kind gift from India Sea Foods, Cochin.

2.2. Preparation of ellagic acid encapsulated chitosan nanoparticles

Chitosan nanoparticles were prepared using ionotropic gelation (Pan et al., 2002) using sodium tripolyphosphate (TPP). The anionic phosphate groups in TPP interacts with the positively charged amine groups of chitosan thereby stabilizing the nanoparticle. Chitosan dissolved in 0.1% acetic acid was stirred continuously to obtain a gel-like consistency. Sodium tripolyphosphate (TPP) (1%, w/v) was added to the chitosan solution and the mixture was stirred for two hours without heating to obtain a uniform textured solution. The solution was centrifuged at 18,000 rpm for half an hour to pelletize chitosan nanoparticles. For preparation of ellagic acid loaded chitosan nanoparticles, specific quantities of ellagic acid in DMSO was added along with 1 mL of 1% (w/v) sodium tripolyphosphate (TPP)to the chitosan solution. After stirring for 2 h at ambient conditions, the solution was centrifuged at 18,000 rpm for half an hour and the amount of unencapsulated ellagic acid was determined from the supernatant by measuring its absorbance at 340 nm using UV–visible spectrophotometry (Lambda 25, PerkinElmer, USA). The concentration of ellagic acid was determined using a standard plot and the percentage encapsulation was calculated from the following equation:

$$\text{Encapsulation Efficiency} = \frac{\text{Total concentraion of ellagic acid} - \text{unencapsulated ellagic acid}}{\text{Total concentraion of ellagic acid}} \times 100$$

The pellet containing the ellagic acid loaded chitosan nanoparticles was redispersed in water, lyophilized (Martin Christ, Germany) and subjected to further experiments.

2.3. Characterization of chitosan nanoparticles

The morphology of the chitosan nanoparticles was studied using cold field emission scanning electron microscopy [JSM 670IF, JEOL, Japan].The lyophilized sample was sprinkled over a double-sided conducting carbon tape and then sputter coated with gold.The samples were examined at an accelerating voltage of 3 kV. Fourier Transform Infrared Spectroscopy (FTIR, Spectrum 100, PerkinElmer, USA) was used to elicit information about the presence of ellagic acid in the chitosan matrix. The lyophilized samples were pelletized with potassium bromide (IR grade, Merck, India) and the spectra were recorded by scanning between 400 and 4000 cm^{-1} averaging 20 scans at a resolution of 4 cm^{-1}.The glass transition temperature of the samples was recorded using a differential scanning calorimeter (DSC, Q20, TA Instruments, USA). Approximately 5 mg of the samples were loaded into aluminum pans with lid that also served as the reference. Analysis was performed in a nitrogen atmosphere by controlling the heating rate at 10 °C/min from 0 °C to 200 °C.

2.4. Drug release studies

The ellagic acid encapsulated chitosan nanoparticles were suspended in 1 mLof phosphate buffered saline (PBS, pH 7.4) and placed in a dialysis bag (MW cut-off 22 kDa, HiMedia, India). The bag was then immersed in 3 mL of PBS with mild stirring and the release studies were carried at 37 °C. The buffer was collected at regular time intervals time and the absorbance was measured at 340 nm to construct the release profile. The amount of ellagic acid released from the chitosan nanoparticles was found using the standard plot and Beer–Lamberts law. The experiments were carried out in triplicate to obtain statistical significance.

2.5. Blood clotting time analysis

The whole blood clotting time, a measure of the intrinsic clotting factors in the absence of tissue factors was determined for different concentrations of ellagic acid, blank chitosan nanoparticles and ellagic acid encapsulated in chitosan nanoparticles using the Lee–White method (Lee & White, 1913). One milliliter of fresh rat blood was introduced in a clean glass tube placed in a water bath maintained at 37 °C and the stopwatch was started immediately. The test tube was gently tilted every 30 s until the blood clotted and the clotting time was recorded. The experiments were repeated with test tubes containing different concentrations of the samples.

2.6. Blood retraction time analysis

The clot retraction time was measured for various concentrations of each sample. Clot retraction is the retraction of the blood clots from the walls of the test tube until the red cell mass occupies 50% of total volume of blood. This test gives a measure of the platelet function in the presence of the different samples. The normal retraction time for blood is about two to four hours. The test was carried out after the blood clotting time test with no anticoagulant addition. The test tube after the clotting time test was kept at 37 °C and inspected at regular intervals to observe the clot retraction. Further, the morphology of the blood clots was observed using a scanning electron microscopy. The blood clots were fixed using 5% (v/v) glutaraldehyde and incubated for 20 h at room temperature prior to imaging.

3. Results and discussion

3.1. Encapsulation of ellagic acid in chitosan

Fig. 1 shows the encapsulation efficiencies obtained for various ratios of ellagic acid to chitosan. The maximum encapsulation of about 50% was obtained for the ellagic acid to chitosan ratio 1:12. This implies that there is a balance between the associative and repulsive forces between ellagic acid and chitosan chains at this ratio. Beyond this ratio, a decrease in the encapsulation efficiency was observed. This may be attributed to the difference in hydrophilicities of the polymer and drug. Chitosan with its hydroxyl groups and amino groups is extremely hydrophilic. Ellagic acid, on the other hand, possesses considerable amount of hydrophobicity inspite of its phenolic groups, owing to its aromatic rings and hence will exhibit only a limited tendency to associate with the highly polar chitosan matrix. This is reflected in the decrease in the encapsulation efficiency as the polymer content is increased. The retention of the drug within chitosan is expected to be predominantly through hydrogen bonding associations.

3.2. Morphological characterization

Fig. 2 shows the scanning electron micrographs of the blank and drug encapsulated chitosan nanoparticles. It was observed that the blank chitosan particles had an average size of 67 nm and appeared as spherical clusters (Fig. 2A). This may be due to the tendency of chitosan particles to fuse to one another through extensive hydrogen bonding. The ellagic acid encapsulated chitosan nanoparticles also appear as clusters with an average particle size of about 80 nm (Fig. 2B). The slight increase in the size of the nanoparticles could be due to the incorporation of the drug between the polymeric network.

3.3. Spectroscopic characterization

Fourier transform infrared spectroscopy (FTIR) was used to identify the presence of the drug in the polymer matrix. Fig. 3 shows the FTIR spectra of ellagic acid, blank chitosan and ellagic acid

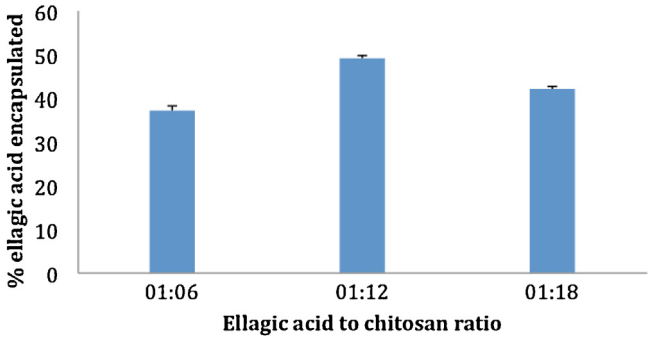

Fig. 1. Percentage of encapsulated ellagic acid for varied ratios of drug to chitosan. Values are a mean of three trials.

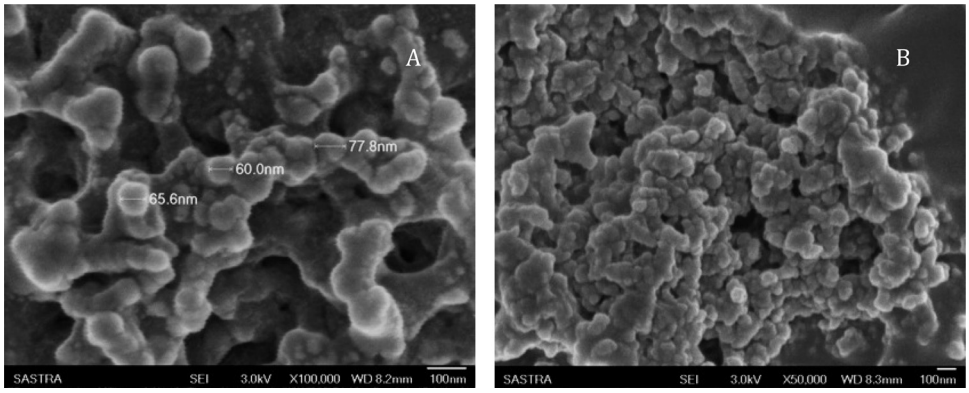

Fig. 2. Scanning electron micrographs of (A) blank chitosan nanoparticles and (B) ellagic acid encapsulated chitosan nanoparticles.

loaded chitosan nanoparticles. In the FTIR spectrum of blank chitosan, the –NH and –OH stretching vibrations of chitosan appear at 3434 cm^{-1}. The weak band at 2929 cm^{-1} may be attributed to the –CH– stretching in chitosan. Further, the FTIR spectrum of blank chitosan reveals vibration bands at 1640 cm^{-1} and 1555 cm^{-1} that arise due to the amide carbonyl stretch and –NH– bend of the amine groups respectively. The band at 1099 cm^{-1} corresponds to the C–O stretching. The FTIR spectrum of the ellagic acid loaded chitosan exhibits bands at 1099 and 1065 cm^{-1} characteristic of C–O stretching. The additional bands at 810 and 675 cm^{-1} indicate the presence of phenyl ring due to the presence of ellagic acid. The slight shift in the band from 3434.87 cm^{-1} to 3412.70 cm^{-1} in the FTIR spectrum of drug loaded chitosan indicates the interaction of the –OH group of ellagic acid with –NH group of chitosan through hydrogen bonding. The shift in the bands in the range 1260–1000 cm^{-1} could be attributed to the interaction ellagic acid with the –C–O– groups of chitosan.

3.4. Thermal analysis

Fig. 4 shows the glass transition temperature of blank and ellagic acid loaded chitosan nanoparticles. The glass transition temperature of chitosan is about 77 °C while the glass transition temperature exhibits a positive shift to about 93 °C in the case of ellagic acid encapsulated chitosan nanoparticles. This suggests the existence of additional associative forces between ellagic acid and the chitosan matrix resulting in the increase in the glass transition temperature. It may be possible that ellagic acid interacts with the chitosan chains through hydrogen bonding resulting in stiffening of the matrix. This observation may have an implication on the anti-hemorrhagic property of ellagic acid-loaded chitosan.

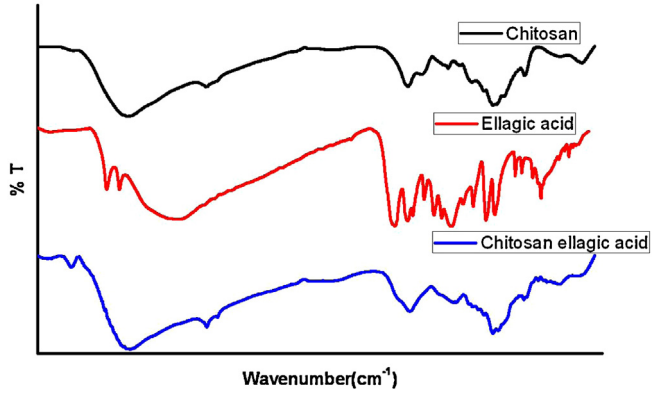

Fig. 3. FTIR spectra of blank chitosan, ellagic acid and ellagic acid encapsulated chitosan.

Vournakis et al., have reported that poly(N-acetyl glucosamine) exhibits superior activation of platelets due to its high affinity for GPIIbIIIa and GPIb that are present on the surface of platelets. This interaction has been reported to result in release of thromboxane and serotonin leading to the triggering of the intrinsic coagulation cascade (Vournakis, Demcheva, Whitson, Guirca, & Pariser, 2004). The authors had attributed the activation of platelets to the highly oriented arrangement of the glycosidic residues in poly(N-acetyl glucosamine) resulting in stiffer chains. However, chitosan does not exhibit a high degree of crystallinity and tends to exist in a more flexible random coil configuration. As a result, its hemostatic effect is lesser than that of poly(N-acetyl glucosamine) (Vournakis et al., 2004). Our results show that encapsulation of ellagic acid increases the stiffness of chitosan and hence may augment its ability to interact with the surface receptors of platelets leading to their activation.

3.5. Release profile of ellagic acid from the chitosan matrix

Fig. 5 shows the drug release profile for ellagic acid encapsulated chitosan nanoparticles at 37 °C and pH 7.4. It was observed that nearly 84% of ellagic acid was released within the first twelve hours beyond which a gradual release occurred upto 108 h. The very

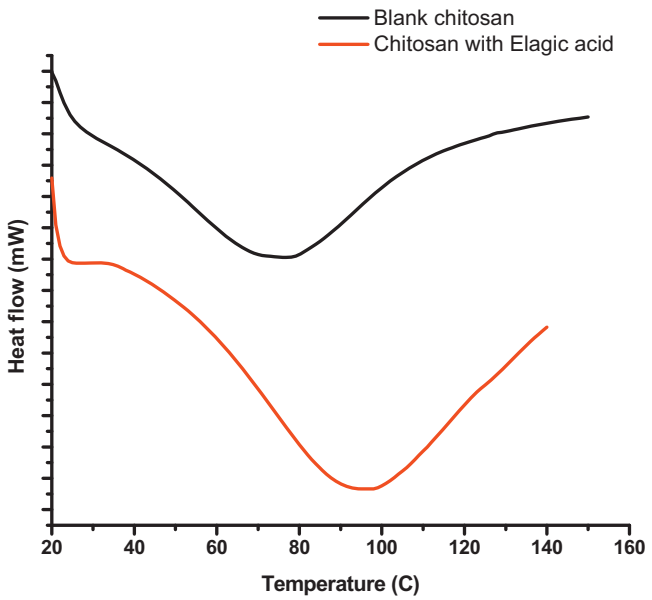

Fig. 4. Glass transition temperature of blank chitosan (black line) and ellagic acid loaded chitosan (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

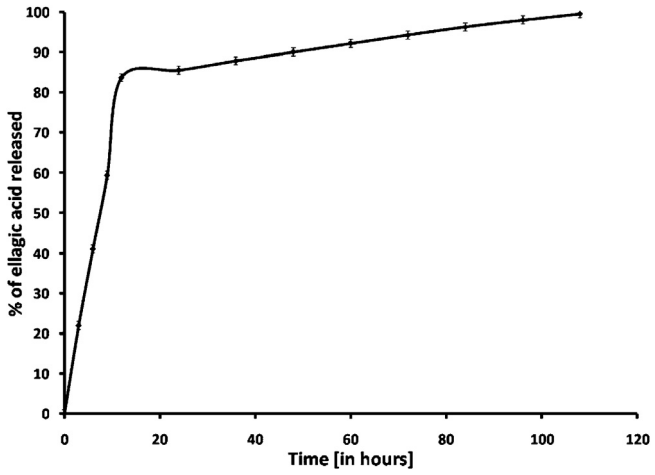

Fig. 5. Release profile of ellagic acid from chitosan matrix.

slow release seen after 12 h may be due to the tight association of ellagic acid within the chitosan matrix. The initial burst release was due to the loosely associated and surface-bound drug molecules. Similar burst release profiles have been reported for ellagic acid-loaded chitosan nanoparticles for oral cancer therapy (Arulmozhi et al., 2013). In the present study, the rapid release of ellagic acid from chitosan matrix in the initial phases is desirable for clotting applications.

3.6. Blood clotting time and blood retraction time of ellagic acid loaded nanoparticles

The normal whole blood clotting time is 5–8 min and the normal clot retraction time is about 2–4 h. The whole blood clotting time (WBCT) by the Lee–White method and the clot retraction time (CRT) measured for different concentrations of ellagic acid, blank chitosan andellagic acid loaded chitosan nanoparticles are shown in Fig. 6. It was observed that ellagic acid loaded chitosan nanoparticles exhibited the shortest WBCT and CRT when compared with equivalent amounts of free ellagic acid. The WBCT of blood in the absence of any additive was 360 s. Blank chitosan nanoparticles did not exhibit any significant decrease in the WBCT except at the highest concentration employed in the present study (7.44 mg/mL). Free ellagic acid exhibited only a slight reduction in the WBCT with a maximum reduction of about 19% at the highest concentration observed at the highest concentration employed in the study (0.6 mg/mL). In contrast, the ellagic acid encapsulated chitosan nanoparticles exhibited a significantly faster clotting time when compared with free ellagic acid. A similar trend was observed in the case of clot retraction time where blank chitosan nanoparticles produced a small reduction in the clot retraction time when compared with the control samples that exhibited a clot retraction time of about 180 min.

Ellagic acid exhibited better CRT when compared with blank chitosan, while the ellagic acid encapsulated chitosan nanoparticles exhibited the best clot retraction times. In order to understand whether the observed improvement in the clotting parameters when compared with the individual components arises due to the encapsulation phenomenon or because of the synergy of two anti-hemorrhagic components in the system, the WBCT and CRT values obtained for the ellagic acid loaded chitosan nanoparticles were compared with those obtained for a physical blend of ellagic acid and blank chitosan nanoparticles and the results are shown in Table 1. It was observed that the WBCT as well as CRT values obtained for the ellagic acid loaded chitosan nanoparticles is slightly superior to those observed for the physical blend. The improvement in the thrombus formation in the case of ellagic acid loaded chitosan nanoparticles may be due to the synergistic effect of chitosan on the function of ellagic acid.

Recently, it has been recognized that TGF-β1 activates Factor XII (Hageman Factor) through the Smad 3 and JNK signaling pathways (Jablonska, Markart, Zakrzewicz, Preissner, & Wygrecka, 2010). Chitosan increases the production of TGF-β1 from platelets while ellagic acid has also been demonstrated to activate the factor XII through a surface binding process (Ratnoff, 1981). In the case of

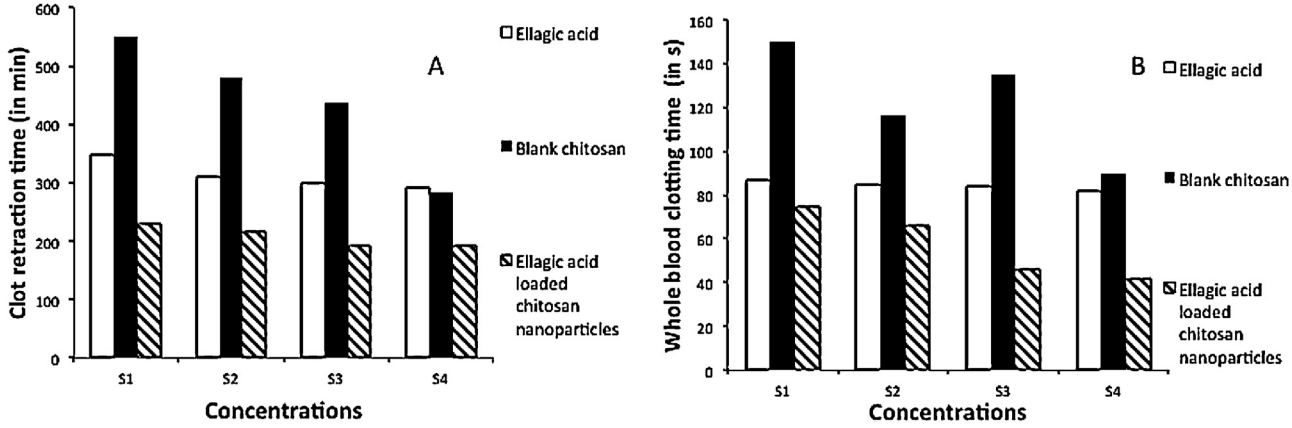

Fig. 6. (A) Whole blood clotting time and (B) clot retraction time for different concentrations of ellagic acid, blank chitosan nanoparticles and ellagic acid loaded chitosan nanoparticles. The values shown are an average of two independent trials.

Table 1
Whole blood clotting time and clot retraction time obtained for different concentrations of ellagic acid loaded chitosan nanoparticles and a physical blend of ellagic acid and blank chitosan nanoparticles.

Concentration of ellagic acid (mg/mL)	Whole blood clotting time (s)		Clot retraction time (min)	
	Ellagic acid loaded chitosan nanoparticles	Physical blend	Ellagic acid loaded chitosan nanoparticles	Physical blend
0.1	230	290	75	83
0.2	215	278	66	73
0.4	192	207	46	57
0.6	193	186	42	53

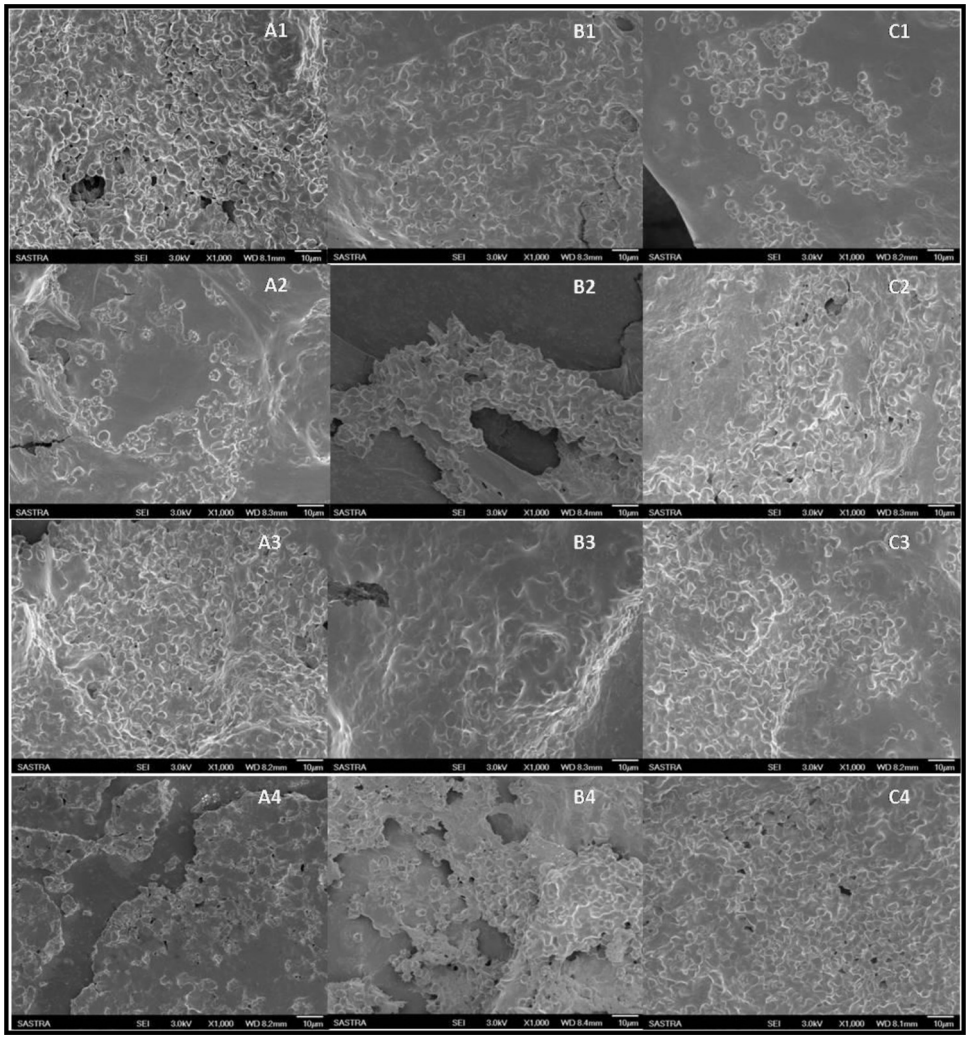

Fig. 7. Scanning electron micrographs of the clots obtained on treatment of whole blood withdifferent concentrations of (A1) 1.22mgof blank chitosannanoparticles; (A2) 2.44 mg of blank chitosan nanoparticles; (A3) 4.88mgof blank chitosan nanoparticles; (A4) 7.44 mg of blank chitosan nanoparticles; (B1) 0.1 mg of free ellagicaci; (B2) 0.2 mg of free ellagic acid; (B3) 0.4 mg of free ellagic acid; (B4) 0.6 mg of free ellagic acid; (C1) 0.1 mg of ellagic acid encapsulated chitosan nanoparticles; (C2) 0.2 mg of ellagic acid encapsulated chitosan nanoparticles; (C3) 0.4 mg of ellagic acid encapsulated chitosan nanoparticles and (C4) 0.6 mg of ellagic acid encapsulated chitosan nanoparticles.

the ellagic acid loaded chitosan nanoparticles, chitosan promotes the recruitment of platelets and activates the Smad3 and JNK pathways leading to the activation of factor XII while presence of ellagic acid induces the surface binding activation of factor XII. This synergy will result inan amplification of the coagulation cascade. In the case of the physical blend, these effects will be additive but the magnitude of amplification will be reduced as ellagic acid and blank chitosan nanoparticles are not present as a single entity and hence a slightly reduced efficiency was observed in the case of the physical blends when compared with the ellagic acid encapsulated chitosan nanoparticles.

Fig. 7 shows the scanning electron micrographs of the blood clots obtained on addition of different concentrations of ellagic acid, blank chitosan and ellagic acid loaded chitosan nanoparticles. In all cases, extensive aggregation of erythrocytes were discernible along with a layer of fibrin network was observed indicating activation of the coagulation pathway.

4. Conclusions

Ellagic acid has been effectively loaded into chitosan nanoparticles and its efficiency in promoting blood coagulation has been reported here for the first time. Ellagic acid loaded chitosan nanoparticles exhibited rapid blood coagulation as well as clot retraction due to the synergistic effects of chitosan and ellagic acid. The results of the present study indicate the potential of ellagic acid encapsulated chitosan system as an anti-hemorrhagic system that could be further explored for commercial applications.

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