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In-vitro drug release studies of Chitosan-Alginate nanoparticles loaded with non-volatile extracts of *Cymbopogon species*

Md Shamim Rahman[1], Sooriamoorthi Sri Murugan[1], Amni Nik Adnan[1], Raihana Edros[1,2*], Siti Umairah Mokhtar[3], Abd Almonem Doolanea[4]

[1]Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang Al Sultan Abdullah, 26300 Gambang, Kuantan, Pahang, Malaysia.

[2]Centre of Excellence for Advanced Research in Fluid Flow, Universiti Malaysia Pahang Al Sultan Abdullah, Lebuhraya Tun Razak, 23600 Kuantan, Pahang, Malaysia.

[3]Faculty of Industrial Science and Technology, Universiti Malaysia Pahang Al Sultan Abdullah, 26300 Gambang, Kuantan, Pahang, Malaysia.

[4]Research and Development, Sabrena Experience, 1500 Dragon Street, Suite 160 Dallas, Tx 75207, USA.

Abstract

The conventional topical dosage forms have plenty of drawbacks which cause patient inconveniences as well as treatment failure. To overcome these complications, drug design engineering is emphasizing on nanoparticle technology for its better drug carrier activity and targeted delivery in desired site of body. The objective of this study was to develop and evaluate topical formulation containing chitosan-alginate nanoparticles loaded with non-volatile extracts of *Cymbopogon species*. Among all of the test samples, nanoparticles loaded with 6 mg of extraction exhibited best entrapment capacity. This formulation successfully captured around 36.56% drug in nanoparticles. Thermo Gravimetric Analysis also showed thermal stability of the drug loaded nanoparticles. Furthermore, the formulation of nano cream that contained 6 mg of *Cymbopogon sp*. nanoparticles yielded the smallest nano-cream, with a particle size of 157.3 ± 20.80 nm. Using the basket dialysis approach, nanoparticle formulations loaded with 12 mg and 24 mg extracts showed a release rate of roughly 25%. On the other hand, the dialysis bag approach revealed that the drug released from the 24 mg of Cymbopogon sp. loaded in nanoparticles was about 37%. Lastly, the antioxidant study's findings indicate that cream-loaded nanoparticles containing 6 mg might be capable of scavenging radicals, with an IC50 value of 19.411g/ml. Chitosan-alginate nanoparticles loaded with Cymbopogon species exhibited significant drug release and antioxidant activity, indicating that this method could be useful for the formulation development of nanoparticles for skincare products.

Keywords: Chitosan, Alginate, Cymbopogon, Nanoparticle, Cream.

Full length article **Corresponding Author*, e-mail: *rzahirah@umpsa.edu.my*

1. Introduction

Nowadays, nanoparticles are widely used in modern medicines and cosmetic products. Materials or particles known as nanoparticles which have overall diameters of less than 100 nanometers (nm), it can be up to 500 nm in some cases. In recent years, nanoparticles are essential in producing medications and cosmetics with topical delivery. Researchers are enthusiastic to formulate nanosized drug delivery systems because of the novelty and better treatment outcome. Additionally, nanoparticles have the potential for passive targeting due to their greater permeability and retention. Moreover, a surfactant or co-surfactant with a size range of 100 nm to 500 nm that serves as a stabilizer and two immiscible liquids, oil and water, make the potential drug delivery known as a nano-emulsion [1]. It can be classified as either single or double emulsions which are based on the dispersed phases and continuous phases. A single emulsion can occur in the form of oil-in-water (O/W) or water-in-oil (W/O) in which the main particle is composed of either water or oil respectively.

Meanwhile, double emulsions or known as multiple emulsions (W/O/W or O/W/O) are the type of emulsions within an emulsion wherein the primary oil and water phases

are dispersed in the system [2]. As nano-emulsions have a large surface area and low surface tension because of the small droplet size, the nanoparticles are able to allow effective delivery of the active compounds through and into the skin [3]. Moreover, the nano-size also helps to enhance the stability of antioxidant properties and aqueous solubility, thus increase the therapeutic effect [4]. On the other hand, to enhance the delivery of drug to the targeted site, drug release from nanoparticles must be slow enough to avoid loss of drug and to reduce toxicity because of the carrier material. After reaching at the target site, it is essential to optimize the efficacy of the rate of drug release. Thus, in-vitro drug release kinetics is an important aspect in monitoring nanoparticle formulation quality control and the establishment of product behavior. Moreover, in-vitro testing provides critical data and information regarding pharmacokinetics of dosage form. Besides, establishing in vitro-in vivo correlations and characterizing formulation efficacy require accurate in-vitro release kinetics assessment [5]. Conversely, nanoparticles used for commercial purposes have specific surface functionalization. For instance, complex nanoparticles have a core-shell around the nanoparticle, functionalized for medical uses. To comprehend its potential as a commercial product, analytical characterization is conducted on the core, coatings, and any other aspects affecting the nanoparticle to evaluate the effects of its release for medicinal usage [6]. In this study, a barrier cream for topical use is applied. The topical application was formulated by encapsulating the extraction of *Cymbopogon species* into chitosan-alginate nanoparticles using ionic gelation method. Later, studies were done on synthesized chitosan-alginate nanoparticles loaded with *Cymbopogon species* extraction. Therefore, the synthesized nanoparticles were formulated for topical application, and further investigations were done of the product. However, nanoparticles are not always amenable to the technologies available for bulk-scale material analysis [6]. Thus, characterization and in-vitro studies were conducted on prepared samples. The characterization studies include entrapment efficiency, particle size analysis and thermal analysis, which will determine the efficiency of the encapsulation process, nanoparticles' size and stability and the thermal effect of nanoparticles, respectively. On the other hand, the in-vitro studies consist of basket dialysis and dialysis bag methods. These methods show the presence of the drug in the synthesized nanoparticles and formulated nano creams. Finally, antioxidant study determines the biological activity of the *Cymbopogon sp.* extracts as its syntheses.

2. Materials and methods

2.1. Materials

The primary materials used in this method were *Cymbopogon species* which were selected according to their mature state and extracted using the Soxhlet extraction process. Polymers those were used to prepare nanoparticles were chitosan low molecular weight (448869, Sigma-Aldrich) and sodium alginate medium viscosity (W201502, Sigma-Aldrich). The chemicals used were acetic acid (CH_3CO_2H, Sigma-Aldrich); sodium hydroxide (Sigma-Aldrich) and hydrochloric acid (Fisher). Other materials involved in this experiment were Titan3 0.2 µm syringe filter with nylon membrane, Nipro 27 g x ½ in the sterile needle (0.4x12 mm) and TERUMO® 5 mL sterile syringe. All the solvents used in this study were analytical grade. The samples were prepared using ultra-purified water by the Milli-Q® Plus Filtration system.

2.2. Sample Preparation

The leaves of *Cymbopogon species* were dried and cut into small pieces. Then, it was grounded into coarse using a mixer. Later, the coarse powder was extracted using the Soxhlet extractor apparatus. The subsequent sections illustrate the extraction and formulation of nanoparticle cream for topical delivery loaded with *Cymbopogon species.*

2.3. Extraction

The powdered leaves of *Cymbopogon species* were mixed with water at room temperature for 8 hours to obtain the supernatant. Later, the supernatant was collected by filtering the extract using a Soxhlet extractor [7]. While water flown in and out of condensers, the extraction device was clamped to a heating mantle. The sample was stored in the thimble before being placed in the Soxhlet tube. Water was poured into the flask with a circular bottom. Then, the solvent evaporated and entered into the Soxhlet extractor through the tube. Due to gravity, the vapor condensed and flowed. Therefore, it percolated through the sample beds to extract the oil. At the end of the extraction, Rotary Evaporator RV8 (IKA) was used to evaporate the solvent to lower the volume of the aqueous solution. Lastly, the aqueous extraction solution was freeze-dried for 48 hours under -55˚C at 0.5 mBar to obtain the solid extraction.

2.4. Preparation of Unloaded Chitosan-Alginate Nanoparticles

The Ionotropic gelation technique was used to prepare chitosan-alginate nanoparticles, technically known as unloaded nanoparticles using the technique described by Algharib et al., [8]. 0.08% w/v of chitosan solution was added to 0.10% w/v of sodium alginate solution using a syringe with a 0.2 µm filter. Parameters such as the stirring condition were maintained under 1000 rpm for one hour [9-10]. In contrast, the pH of the chitosan solution and sodium alginate solution was maintained at a range of 4.7 to 4.9 [10]. The resultant suspensions were treated using a high-intensity ultrasonic processor (probe-type sonicator, Misonix Sonicator).

2.5. Preparation of Loaded Chitosan-Alginate Nanoparticles

The Ionotropic gelation technique was used to prepare chitosan-alginate nanoparticles of aqueous extracts by the method described by Algharib et al., (2022). 6 mg of freeze-dried *Cymbopogon species* extracts were added into the chitosan solution in gentle stirring at room temperature. Then, the chitosan solution containing freeze-dried extraction of *Cymbopogon species* was added to sodium alginate using a filter of 0.2 µm while kept under the stirring condition at 1000 rpm for one hour [10]. The pH was maintained at 4.7 to 4.9 [10].

The resultant suspensions were treated using a high-intensity ultrasonic processor (probe-type sonicator, Misonix Sonicator). The experiment was repeated with different concentrations of freeze-dried *Cymbopogon species* extraction.

2.6. Lyophilization

Nanoparticles were lyophilized without cryoprotectants. The nanoparticles samples were dispersed in 5 mL to 10 mL of ultrapure water and then lyophilized at -55˚C at 0.5 mBar for 24 hours.

2.7. Cream Formulation

The experiment was conducted by heating the mixture of Phase A (Oil phase) and Phase B (Aqueous phase). The composition of this cream formulation consisted of 10.0% stearic acid, 5.3% sunflower oil, 6.5% glycerol, 70% distilled water, 0.2% zinc oxide, 3.0% xanthan gum and 5.0% nanoparticle (unloaded and loaded with different concentration). The oil phase consisted of sunflower oil and stearic acid was then heated into Beaker A. Then, the aqueous phase consisting of distilled water, xanthan gum, glycerol, zinc oxide and nanoparticle were measured using measuring balance and placed into Beaker B. Both Beakers A and B were covered with aluminium foil and placed into a water bath at a predetermined temperature. The beakers were taken out once the powder of stearic acid melted. Phase A was continuously homogenized with a homogenizer, and the oil phase was slowly introduced. The mixture was continuously homogenized until the texture of the mixture became creamy. The experiment was repeated with the maximum value of sunflower oil and distilled water. The mixing time and mixing speed were also in minimum and maximum values. For mixing speed, the value was set at an approximation of 8000 rpm.

2.8. Characterization of Nanoparticles and Cream

2.8.1. Entrapment Efficiency

The experiment was conducted using a modified USP dissolution apparatus I to determine the percentage of drug release from three different concentrations of nanoparticles. Before the experiment, the dialysis membrane was soaked in a release medium overnight. The soaked dialysis membrane was attached to the lower end of the basket, and 5 mL of the prepared sample was placed on it with 3.8 cm^2 available, the total surface area for medication release as a replacement to the original basket. Rubber was used to tie up the dialysis membrane attached with the basket to prevent any leaking. Then the basket was immersed in a dissolution vessel containing a release medium, Sorenson phosphate buffer, with a volume of 100 mL at pH 7.4. The temperature was maintained at 37ºC. The shaft was adjusted and positioned such that the basket was ensured to be evenly dipped to a depth of 3 mm in the dissolution media. All the tests used MWCO 12-14 KDa regenerated cellulose dialysis membrane, and the glass basket was rotated approximately at 300 rpm. The aliquots of the release medium were collected and analyzed after 240 minutes using UV-Vis Spectroscopy at 365 nm. Then, the entrapment efficiency was calculated from Equation 1 [11].

$$Entrapment\ efficiency\ (\%) = \frac{weight\ of\ drug\ in\ nanoparticles\ (mg)}{weight\ of\ initially\ used\ drug\ (mg)} \times 100 \quad (1)$$

2.8.2. Differential Scanning Calorimetry (DSC) Analysis

Differential scanning calorimetry (DSC) analysis was done by using TA Instruments Q1000 DSC. The DSC measurement was carried out on the following samples: Unloaded nanoparticles (chitosan-alginate nanoparticles) and chitosan-alginate nanoparticles loaded with non-volatile *Cymbopogon species* extracts. Lyophilized samples were used to analyze the changes in material properties at exact temperatures, revealing important transition ranges and deterioration points. Approximately 1-3 mg of each sample was placed in an aluminium pan. The pan was sealed, and the samples were allowed to equilibrate overnight at room temperature. The samples were scanned from 35°C to 350 °C at a heating rate of 10˚C/min.

2.8.3. Thermo Gravimetric Analysis (TGA)

STA7200 TGA was used to conduct Thermo gravimetric analysis (TGA). The TGA measurement was carried out on the following samples: Unloaded nanoparticles (chitosan-alginate nanoparticles) and chitosan-alginate nanoparticles loaded with non-volatile *Cymbopogon species* extracts. Lyophilized samples were used to conduct the analysis. Approximately 6-7 mg of each sample was placed in an aluminium pan. The pan was sealed, and the samples were allowed to equilibrate overnight at room temperature. The samples were scanned from 30°C to 500°C. The nanoparticle dispersion thermogram containing nanoparticles was investigated using TGA scans at a heating rate of 10˚C/min and a constant nitrogen flow rate of 20 mL/min.

2.8.4. Particle Size Analysis

To determine the diameter of particles and zeta potential, DelsaMax Pro was used. This analysis made a comparison between the cream of unloaded chitosan-alginate nanoparticles and the cream of chitosan-alginate nanoparticles loaded with different concentrations of non-volatile *Cymbopogon species* extracts. Before testing, an approximation of 0.2 mL of each sample was diluted in 100 mL ultrapure water. The analysis was repeated five times.

2.9. In-vitro Drug Release Studies

2.9.1. Basket Dialysis Method

The experiment was conducted using a USP dissolution apparatus I. The objective of this study was to calculate the percentage of drug release from nanoparticles at three different concentrations: 6 mg, 12 mg, and 24 mg. The basket contained Sorenson phosphate buffer dissolution medium, with a volume of 100 mL at pH 7.4. The temperature was 37±0.05 ºC. The aliquots of the release medium were collected and absorbance were taken at predefined time intervals of 15, 30,45,60,120,180 and 240 minutes using UV-Vis Spectroscopy at 365 nm.

2.9.2. Dialysis Bag Method

The dialysis bag method was used to determine the in-vitro drug release of *Cymbopogon* cream from various concentrations. Before the experiment, the dialysis bag was soaked in the buffer solution for 24 hours. 1 g of cream sample was diluted in 100 mL of phosphate buffer and 5 mL of the diluted cream was placed in a dialysis bag tied on both ends with a thread. Then the dialysis bag was placed in a beaker filled with the same buffer solution at a temperature of 37°C and was agitated by using a magnetic stirrer

controller at a speed of 300 rpm. At set time intervals, the surrounding medium was collected for 3 mL each and sample absorptions were taken by UV-Vis Spectroscopy at 365 nm. The analysis was repeated three times to calculate mean values.

2.10. Antioxidant Study

The antioxidant activity of all extracts was determined according to Khalaf et al. [12] with slight modification. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is widely used, fast, easy and cost-effective method to determine antioxidant capability of any substance by using free radicals to determine whether it has the ability to produce hydrogen or act as free radical scavengers.

3. Results and Discussions

3.1. Characterization of Nanoparticles and Nano-Cream

3.1.1. Entrapment Efficiency

Three samples were tested for entrapment efficiency: Sample 1 (S1), which contained nanoparticles loaded with 6 mg of *Cymbopogon sp*., Sample 2 (S2), which contained nanoparticles loaded with 12 mg of *Cymbopogon sp*.; and Sample 3 (S3), which contained nanoparticles loaded with 24 mg of *Cymbopogon sp*. Additionally, Table 1 shows the sizes of nanoparticles at various concentrations. The entrapment of non-volatile *Cymbopogon sp.* in chitosan-alginate nanoparticles was evaluated through the determination of entrapment efficiency. From Table 1, the values of entrapment efficiency obtained were 36.56%, 22.77% and 10.64% for S1, S2 and S3 respectively. In contrast, Soltanzadeh et al., 2021 [13] presented the values of entrapment efficiency for the encapsulation of *Cymbopogon sp*. The results obtained were between 19% and 30%. Thus, it indicates that the presented values show better entrapment efficiency. On the other hand, other studies have shown higher values of entrapment efficiency but with different bioactive compounds. This table also illustrates that the size of nanoparticles increases with increasing amount of active substance (*Cymbopogon sp.*). However, the increased concentration resulted in reduction in entrapment efficiency. This is due to the saturation of *Cymbopogon sp.* extracts loading into chitosan-alginate nanoparticles. The finding agrees with a previous study of Hosseini et al., 2013 [14].

3.1.2. Differential Scanning Calorimetry (DSC) Analysis

Thermal analysis is necessary to determine the thermal changes in compounds and the maximum temperature to which they can be exposed without losing their properties. DSC thermograms are presented in Figure 1a. Unloaded chitosan-alginate nanoparticles, which served as the study's negative control, showed two distinct peaks: an exothermic one at 93˚C and an endothermic one at 237˚C. S1 exhibited two peaks: one endothermic at 255˚C and one exothermic at 110˚C. Moreover, S2 showed two peaks: an exothermic one at 95˚C and an endothermic one at 232˚C. Finally, S3 showed endothermic peak at 238 ˚C and exothermic peak at 97˚C. Differential scanning calorimetry (DSC), which determines the temperature and heat flow of transitions to create exothermic and endothermic peaks, is then used to determine the thermal stability of nanoparticles [15]. In DSC, dehydration, depolymerization, decomposition of acetylated and deacetylated units of polymers, and decarboxylation of carboxylic groups of polymers produce exothermic peaks [16]. In contrast, S1 and S2 exhibited a broader exothermic peak at 110˚C and 97˚C, respectively. This is probably due to the coalescence of the exothermic peaks of chitosan and alginate polymers. However, S2 formed a narrower exothermic peak, which might be due to the complexation between chitosan and alginate polymers and the formation of new bonds [17]. The loss of water linked with the hydrophilic groups of polymers causes endothermic peaks [16]. In addition, S1, S2 and S3 are broader at 255˚C, 232˚C, and 238˚C, respectively. This refers to chitosan's interaction with alginate, developing a more stable polyelectrolyte complex [18]. The synthesized chitosan-alginate nanoparticles loaded with *Cymbopogon sp*. extraction coating's barrier have been shown to efficiently retain the structure's integrity and the heat stability of the loaded *Cymbopogon sp.* extraction. Most products undergo heat treatments during processing, such as pasteurization and sterilization. Thus, the coating's durability at high temperatures is great [15].

3.1.3. Thermo Gravimetric Analysis (TGA)

TGA studies on unloaded and loaded nanoparticles with different concentrations of *Cymbopogon sp.* are shown in Fig. 1b. From this analysis it is evident that the unloaded nanoparticles and S1, S2 and S3 exhibited weight loss of 91.94%, 87.51%, 95.52%, and 94.24%, respectively. TGA is a technique for investigating a sample's thermal stability and determining the weight change of a sample due to parameters like temperature [19]. Moreover, TGA Analysis is also used to examine the weight changes due to the thermal effect. Unloaded nanoparticles exhibited three transitions of weight loss. The first transition of weight loss was due to the moisture evaporation that may occur because of nanoparticles adhering to the surfaces of chitosan and alginate polymer. Secondly, the second transition with the highest weight loss indicates the thermal degradation of chitosan-alginate nanoparticles like dehydration of saccharide rings and depolymerization of the acetylated deacetylated units of chitosan and alginate polymer [14]. The third and final transition indicates the weight loss of the decomposition of the residue of chitosan-alginate nanoparticles. Furthermore, the loaded nanoparticles with different concentrations of *Cymbopogon sp*. also exhibits three-step weight loss but coincided with the temperature ranges compared to unloaded nanoparticles. The first transition of weight loss for the loaded nanoparticles with different concentrations shows the result of moisture produced from the evaporation of the nanoparticle's surface.

However, as shown in Figure 1b, loaded nanoparticles' first weight loss transition occurred earlier than unloaded nanoparticles. This is due to *Cymbopogon sp.* extraction traces that adheres to the surface of nanoparticles. The second weight loss transition shows massive changes compared to the first and third transitions. The mass reduction is due to the thermal degradation of chitosan and alginate polymer. The third transition of the weight-loss event is attributed to the encapsulated *Cymbopogon sp.* extraction decomposition [13]. On the other hand, unloaded nanoparticles and loaded nanoparticles with different concentrations of extracts exhibited weight loss under 100˚C due to the sample's water evaporation [20].

3.1.4. Particle Size Analysis

Particle size in nano formulations significantly affects the release of the drugs. Delivery of drugs occurs quickly because the surface area of nanoparticles increases with their size. A significant quantity of the drug is released when a drug-loaded nanocarrier comes into contact with the body's surface area. The minute size of the nanoparticle permits the nanoparticles to escape renal exclusion and the reticuloendothelial system, resulting in increased permeability and retention capacity through tumor vasculature [21]. Particle size analyses were done on five different samples in the experiments. Later, the particle size studies were focused on two parameters: particle size and zeta potential. Figure 2a and Figure 2b show results for the diameter of the samples. A comparison between negative controls of the study, which are cream loaded with unloaded nanoparticles and cream loaded crude extracts without encapsulation are presented in Figure 2 a. Moreover, figure 2 b depicts the study's positive controls: cream loaded with 6mg, 12mg and 24mg of extracts. The average size is critical for characterizing stable and functional nanoparticles because it affects compound loading, release, and stability inside the nano tool. As a result, in the characterization of nanoparticles, the particle size analysis determines the loaded nanoparticles' repeatability and homogeneity [15]. As shown in Figure 2a, the cream loaded with unloaded chitosan-alginate nanoparticles was detected to have a particle size of 234.1 nm. In contrast, the cream loaded with extraction only produced an average diameter of 1740.6 nm. Thus, this is evident that nanoparticles execute smaller particle sizes with greater surface area for rapid drug delivery. On the other hand, figure 2 b illustrates that the particle size increases significantly ($P<0.005$) with the increasing concentration of *Cymbopogon sp.* extraction. The extraction, which has been loaded into the chitosan alginate nanoparticle, produced a result of $157.3 \pm 20.80\ nm$, $222.4 \pm 58.10\ nm$, *and* $236.2 \pm 12.80\ nm$ for cream loaded with 6mg, 12mg and 24mg of extracts respectively. This result agreed with previous results with prepared chitosan-alginate nanoparticles with an average size range from 216.0 nm to 441.8 nm [22]. However, from particle size analysis, it can be observed that the particle size of unloaded nanoparticles is greater than S1. The obtained results are below the diameter of 5 to 6 μm. Hence, transporting nanoparticles loaded with *Cymbopogon sp.* through capillaries is quite evident [23].

3.1.5. Zeta Potential

The zeta potential determines the difference between surface charges, while opposite-sign charges are derived from the medium around the particle. The zeta potential is a proxy for the surface charge of the particle. When the zeta potential increases positively or negatively, the colloidal stability of the nanoparticles increases too. Zeta potential with the range of ± 40–50 mV indicates optimal colloidal stability [24]. Figure 3 a and 3 b show results for the zeta potential of samples. A comparison between negative controls of the study, which are cream loaded with unloaded nanoparticles and cream loaded crude extracts without encapsulation, is exhibited in Figure 3a. On the other hand, figure 3b depicts the study's positive controls that involve cream loaded with different concentrations of *Cymbopogon sp.* extracts. Figure 3a illustrates the cream loaded with unloaded chitosan-alginate nanoparticles presented a zeta potential of -42.08 mV, whereas the cream loaded with extraction only produced a zeta potential of -38.27 mV. In contrast, this indicates that nano-cream has higher colloidal stability compared to cream with extractions only. On the other hand, as the loading of *Cymbopogon sp.* increased from 6 mg to 24 mg into the chitosan-alginate nanoparticles, the zeta potential value decreased ($P<0.005$) from -48.19 ± 0.52 mV to -42.70 ± 0.53 mV as shown in Figure 3b, which might be influenced by the concentration of free ammonia absorbed by *Cymbopogon sp.* on the surface [25]. A similar finding was shown by Cai et al., who observed a decrease in the zeta potential value when more *Ocimum basilicum* L. essential oil was encapsulated into chitosan nanoparticles [25]. Another study demonstrated the addition of *Cinnamomum zeylanicum* essential oil reduced the zeta potential from 49.9 mV to 38.7 mV, confirming the findings studied before [26]. On the other hand, negative zeta potential can arise based on the composition of nano-formulation, which could be owing to the carboxylic acid group in stearic acid [24]. Thus, it can be concluded that the zeta potential of cream loaded with 6mg, 12mg, and 24mg of *Cymbopogon sp.* resulted from optimal colloidal stability as the obtained zeta potential is within the range of ± 40 mV–50 mV [24].

3.2. Drug release studies

3.2.1. In vitro drug release studies: Basket Dialysis Method

Evaluation of drug release experiments of S1, S2 and S3 is performed using the basket dialysis method. The evaluation of drug release is the higher the absorbance of nanoparticles into the release medium, the higher the drug release from nanoparticles [27]. This statement is aligned with the results obtained for the drug release profile, as revealed in Figure 4a. S2 and S3 showed around 25% drug release, which is higher than S1. Meanwhile, S1 showed around 21% drug release over time. After one hour, drugs released in all samples showed a constant rate indicating that the drugs had reached maximum freedom [28]. The results suggested that the lag phase present in the *Cymbopogon sp.* extracts were released from the loaded 6 mg nanoparticles due to the incomplete release of extracts where a portion of extracts remained in the nanoparticles. This may be due to the strong interactions between the chitosan and *Cymbopogon sp.* extracts, attributed to the incomplete release of extracts [11].

Moreover, the release of S3 was consistent with the previous research. The higher the concentration of extracts loaded into the nanoparticles, the smaller the size of the nanoparticles, which showed an interaction between the chitosan and the composition of the extract. The plant extracts with lower concentrations have contributed to higher particle stability [29-30]. Due to the smaller nanoparticles having greater surface area, the drug release was also prolonged, as more drugs were diffused out of the dialysis membrane.

3.2.2. Dialysis Bag Method

When characterizing the effectiveness of a nano formulation, the drug release rate is crucial. The active moiety was released from the nanocarrier as it came into contact with the epidermal surface following the application of the cream to the skin. Thus, it is readily available to penetrate the

stratum corneum through the dermis [31]. The ability of the nanoparticles to release the active substance through the membrane surface was analyzed by calculating the UV absorbance of samples [32]. The measurements were taken for a total period of 240 minutes at set time intervals. The results of the dialysis bag method are plotted as seen in Figure 4 b. On the other hand, the dialysis bag method determines the drug release rate which describes the efficacy of nano cream formulation. Generally, the drug release profile for nano cream formulation possessed higher release when compared to the cream with *Cymbopogon sp.* crude extracts only, due to the small particle sizes and greater surface area that enhanced the permeability and solubility in which the drug release was extended [33]. Meanwhile, the formulation of cream with crude extracts of *Cymbopogon sp.* showed lower drug release as it had poor solubility and uneven particle shape and size [28,31]. These statements can be proven in Figure 4 b, where the cream formulation containing nanoparticles showed higher drug release with a range from 15% to 50%. In contrast, the cream formulation containing *Cymbopogon sp*. crude extracts showed only about 19% drug release. Among the formulations of nano-cream samples, cream with 24mg loaded nanoparticles showed 37% drug release, which was the highest, followed by 12 mg and 6mg loaded nano cream formulations. Consequently, as the concentration of the drug in the buffer solution increased with time, the gradient of the graph became less steep. Initially, the buffer solution in the external reservoir was hypotonic to the dialysis bag content, and hence, the drug was likely to diffuse out to the buffer solution. As time passed, the equilibrium and the isotonicity of diffusion generated in the system, which led to the less steep gradient, indicated the drug release rate was getting slower, or the drug was fully released.

Table 1: Size of nanoparticles and entrapment efficiency of nanoparticles loaded with different concentrations of Cymbopogon sp. Extraction.

Sample #	Weight of initially used drug (mg)	Weight of drug in nanoparticles after 240 minutes (mg)	Size of nanoparticles (nm)	Entrapment efficiency (%)	Average of Entrapment efficiency (%)
S1	6.00	2.1948	252.6±0.033	36.58	36.56
	6.00	2.1925	252.5±0.033	36.54	
	6.01	2.1975	252.6±0.033	36.56	
S2	12.00	2.7323	295.1±0.067	22.77	22.77
	12.01	2.7339	295.1±0.067	22.76	
	12.00	2.7319	294.9±0.067	22.77	
S3	24.00	2.5531	355.4±0.033	10.64	10.64
	24.00	2.5564	355.4±0.033	10.65	
	23.99	2.5532	355.3±0.033	10.64	

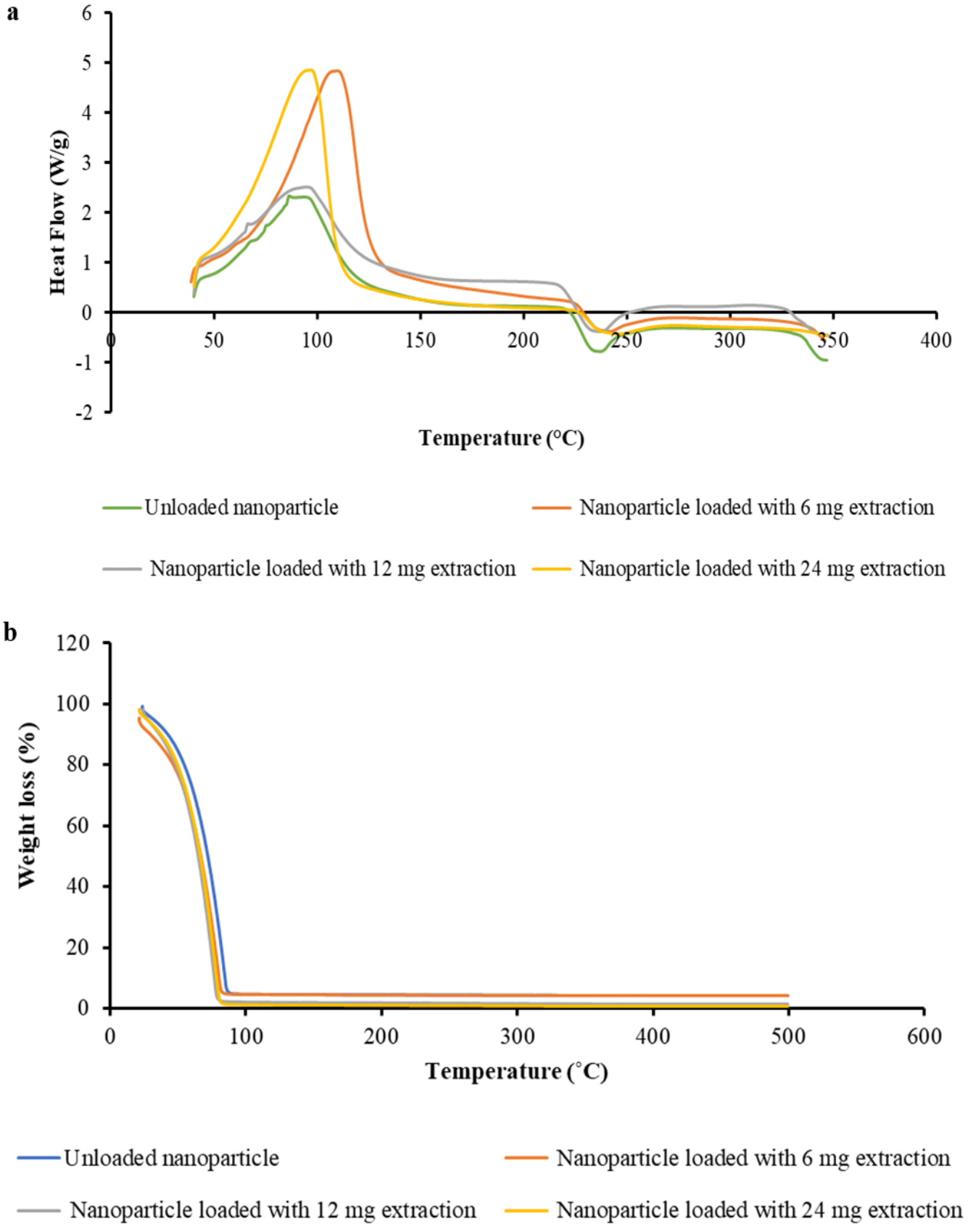


Figure 1: Thermal analysis on the prepared nanoparticles samples of different concentrations and controlled samples. **a** DSC thermograms of unloaded nanoparticles and nanoparticles of different concentrations of *Cymbopogon sp.* extracts; **b** TGA Analysis on unloaded nanoparticles and nanoparticles loaded with different concentrations of *Cymbopogon sp.* extracts.

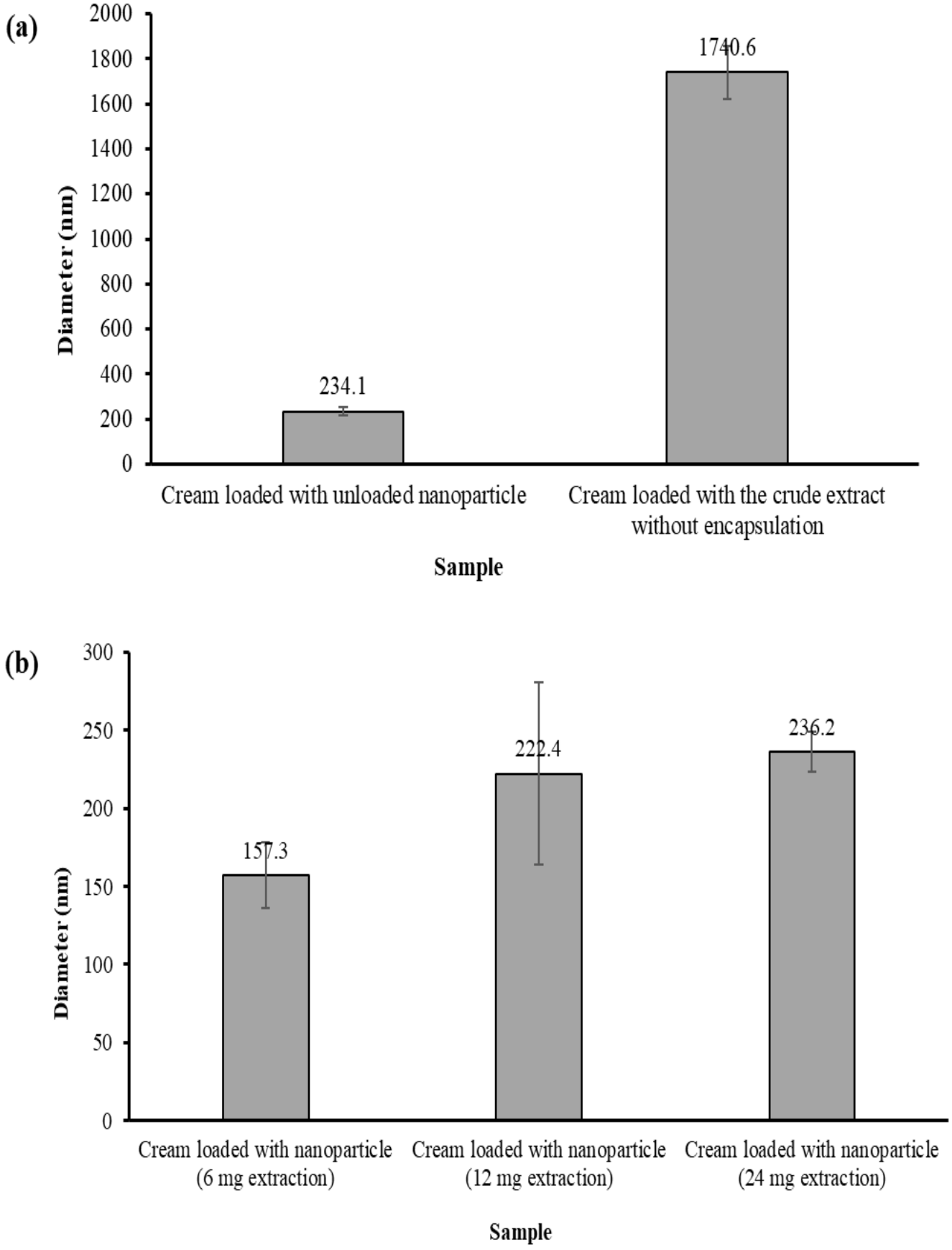


Figure 2: The particle size of cream loaded with (a) unloaded nanoparticles and crude extracts without encapsulation (Mean $\pm SD$, n=5); (b) nanoparticles of different concentrations of *Cymbopogon sp*. extracts (Mean $\pm SD$, n=5).

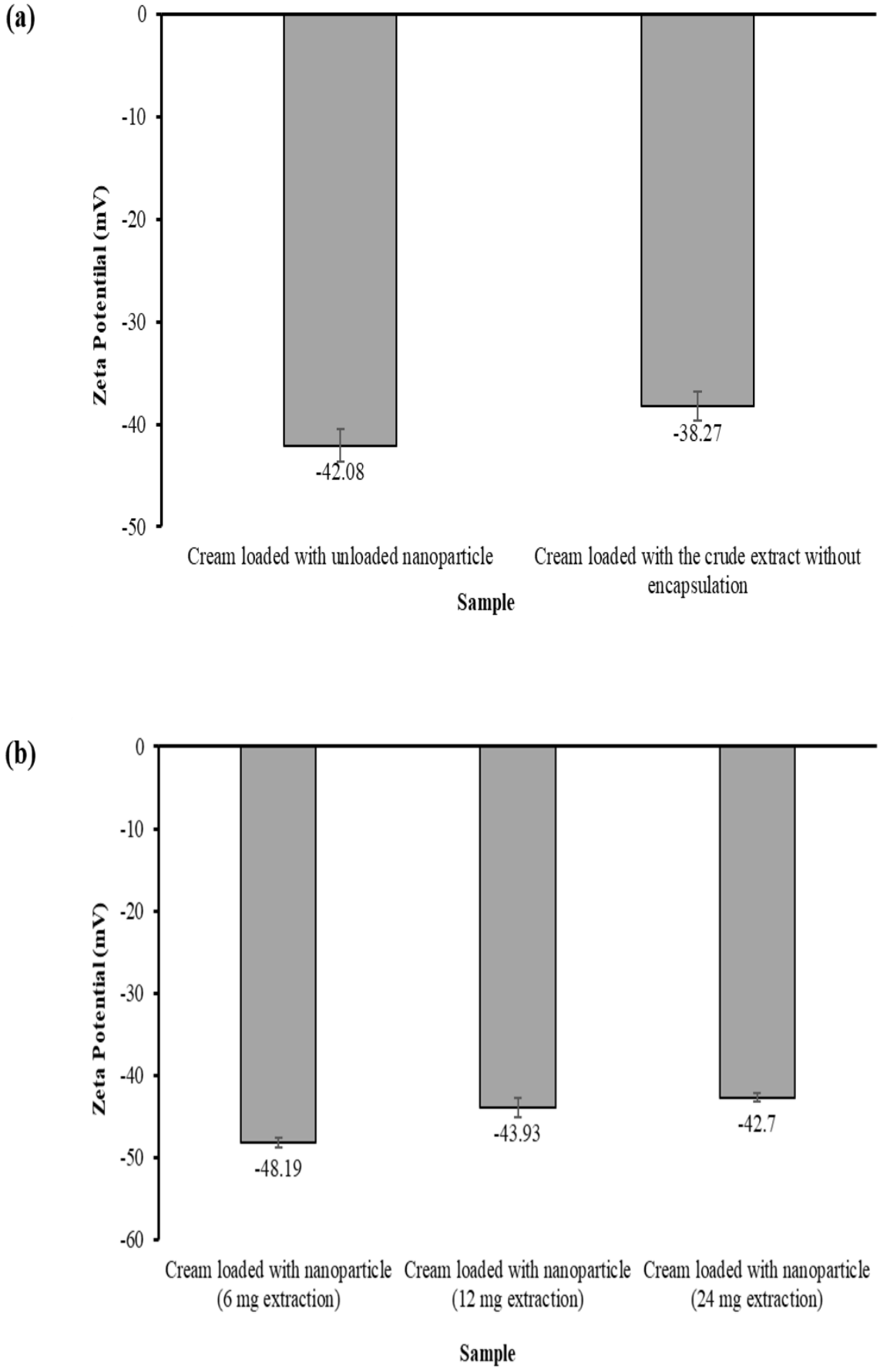
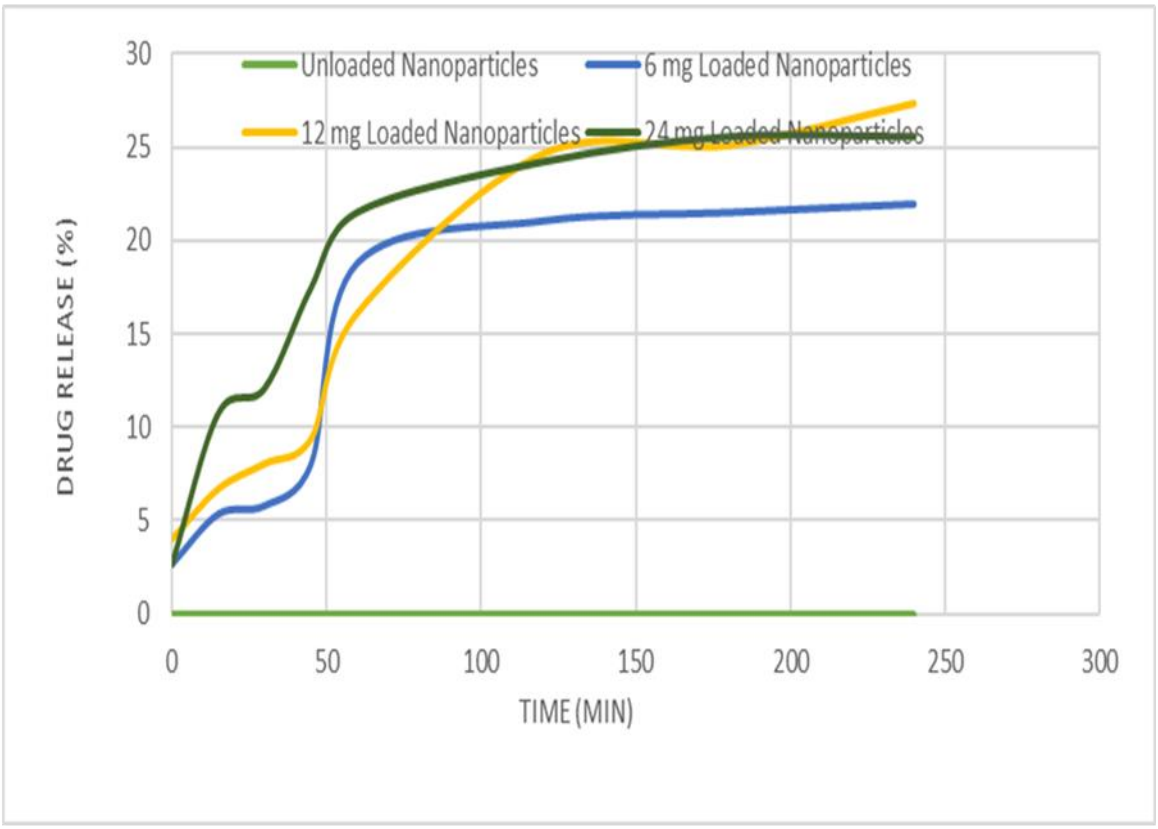


Figure 3: Zeta potential of cream loaded with (**a**) unloaded nanoparticles and crude extracts without encapsulation (Mean ± *SD*, n=5); **b**) nanoparticles of different concentrations of *Cymbopogon sp.* extracts (Mean ± *SD*, n=5).

a



b

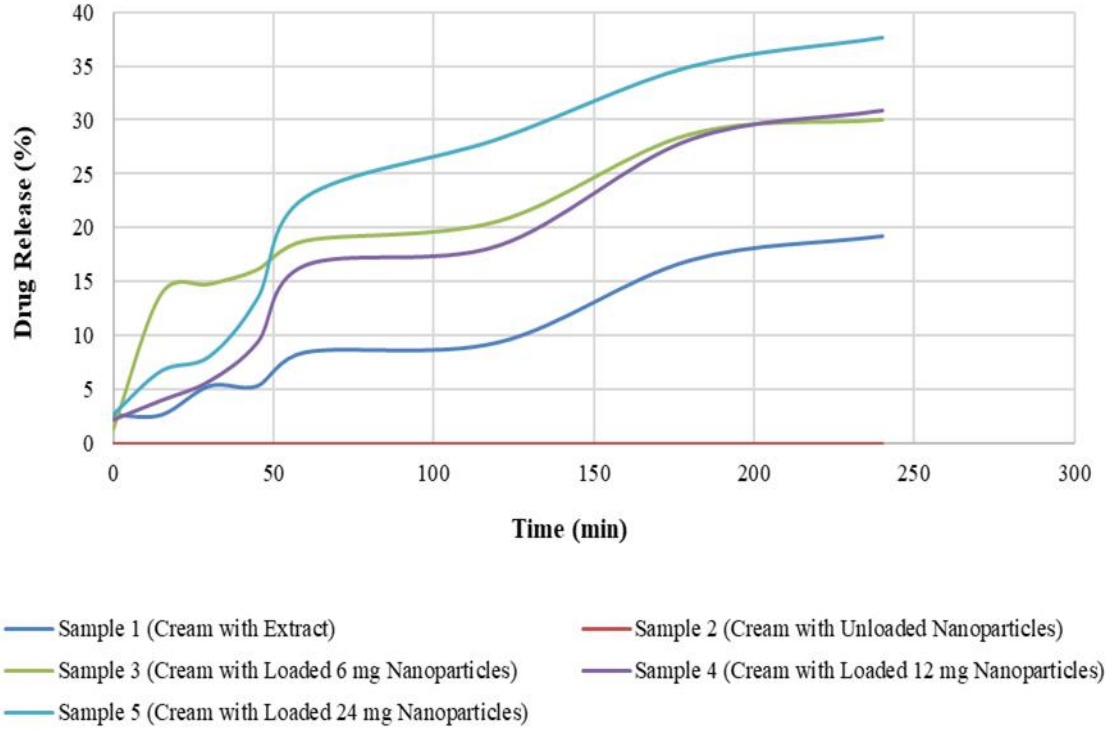


Figure 4: The drug release profile of (**a**) unloaded nanoparticles and nanoparticles loaded with different concentrations of *Cymbopogon sp.* extracts; (**b**) Nano-Cream of *Cymbopogon sp.* crude extracts, cream with unloaded nanoparticles and Nano-Cream loaded with different concentrations of *Cymbopogon sp.* extracts.

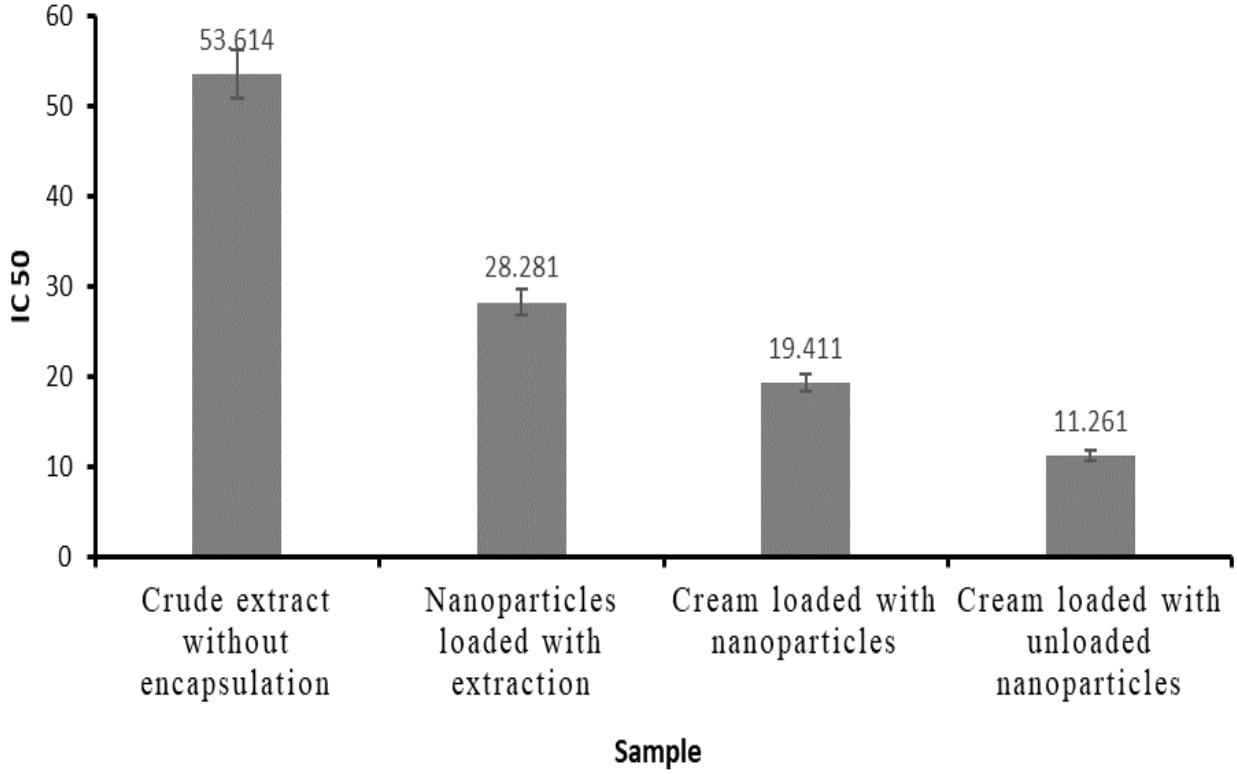


Figure 5: Comparison of antioxidant study on the crude extract of *Cymbopogon sp*., nanoparticles loaded with the extraction of *Cymbopogon sp.*, cream loaded with nanoparticles and cream loaded with unloaded nanoparticles.

3.3. Antioxidant Study

IC_{50} (µg/ml) is used to express the measurement of antioxidant activity. The concentration of test samples those can inhibit free radicals by up to 50% is known as the IC_{50} value. The lower IC50 value means higher level of free radical reduction activity [34]. When an IC_{50} value falls within a certain range, it is classified as potent if it is less than 10 µg/ml, strong if it is between 10 and 50 µg/ml, mild if it is between 50 and 100 µg/ml, weak if it is between 100 and 250 µg/ml and not active if it is greater than 250 µg/ml [35]. The scavenging activities of *Cymbopogon* crude extracts, extracts loaded with nanoparticles, cream loaded with nanoparticle and cream loaded with unloaded nanoparticles showed clear difference in ability and given IC_{50} values, as shown in Figure 5. Figure 5 depicts that the value of IC_{50} decreases as well as drug potency increases as the *Cymbopogon sp.* extract is encapsulated into nanoparticles and forms into Nano-Cream. The desired preparation of cream loaded with nanoparticles shows value of 19.411 µg/ml, which confirms nano cream has strong antioxidant activity. On the other hand, crude extract without encapsulation exhibits highest IC_{50} value of 53.614 µg/ml. In addition, this scenario occurs as all the phenolics in the extracts were consumed during the synthesis. Thus, the synthesis of chitosan alginate nanoparticles requires a higher concentration of *Cymbopogon sp.* extracts to sustain the optimal inhibitory of the nanoparticle. Some authors proposed that a smaller particle size is associated with a larger surface area, which offers a greater number of active sites where free radical scavenging and oxidation reaction inhibition can occur. In addition to the presence of antioxidant capping biomolecules, the distinct size to volume ratio of the nanoparticles might also play a significant role in the antioxidant activity of the samples [36]. The values of IC_{50} among all the samples were above the value of cream loaded with unloaded nanoparticles, the controlled samples, IC50 of 11.261 µg/ml.

4. Conclusions

This study has proven the potential of Chitosan-alginate nanoparticles' compatibility in dermal delivery with different concentrations of *Cymbopogon species.* Improved thermal stability, excellent entrapment efficiency and small nanoparticles (252.6nm) of 6 mg loaded *Cymbopogon sp.* are produced in the nano formulation. Ultimately, nano cream comprising 24 mg loaded *Cymbopogon sp.* achieved about 37% drug delivery. Thus, despite being developed in a topical formulation, chitosan-alginate nanoparticles successfully held the non-volatile *Cymbopogon sp.* extracts and kept its bioactivity, according to characterisation and in-vitro drug release studies. The prepared formulation also showed free radical scavenging antioxidant activity. To put it briefly, this novel method has demonstrated that the formulation of nano cream can be utilised for the production of nanoparticles in both pharmaceutical and skincare products. It further supports the use of nano formulation in skincare manufacturing technology in the years to come.

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