Encapsulation of lemongrass essential oil in chitosan nanoparticles: Characterization and in vitro release study

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ABSTRACT

Lemongrass (*Cymbopogon citratus*) essential oil (LEO) as a bioactive component source has a notable role in pharmaceutical, agricultural and food additives, preservative agents, medicine and nutritional supplements. The aim of the study is to produce a high quality and safe nano-formulation for human needs and an ecofriendly nano-capsule for potential applications on different natural matrices. A Gas Chromatography mass spectrophotometry analytical study of (LEO) revealed that citral was the major compound representing (64.02%) of total essential oil composition followed by myrcene, geranyl acetate and cis-verbenol. LEO was encapsulated in chitosan nanoparticles (CN) using an ionic gelation with sodium tripolyphosphate (TPP) as a cross-linking agent, and validated by attenuated total reflectance-Fourier transform infra-red ATR-FTIR spectroscopy. Most of the LEO nanocapsules particles were nearly spherical in shape with smooth surface and particle size of 205 - 210 nm and 249 nm as observed by SEM and TEM micrographs, respectively. However the Dynamic Light Scattering (DLS) studies showed that average particle size was 206.9-370.9 nm. Loading LEO onto chitosan increased the particle size and reduced the surface positive charge. This reduction in zeta potential values had a positive correlation with the initial content of LEO. By increasing the amount of LEO loaded into chitosan nanocapsule, the loading capacity (LC) increased, while the encapsulation efficiency (EE) decreased. The oil from LEO/chitosan nanoparticles demonstrated an initial rapid release profile of up to 35% after the first 10 h, followed by subsequent slower release.

Keywords: lemongrass oil; nanocapsule; nanochitosan.

INTRODUCTION

Essential oils play an important role in pharmaceutical, agricultural, cosmetic, and food additives. However, the effectiveness of essential oils is determined by stability and bioactivity (Lammari et al., 2020). Essential oils (EOs) are phytocomplex mixture of volatile compounds such as terpenes, ketones, alcohols,phenolics, esters, amines, amides, etc. Their chemical structures are affected by the geographical location, environmental condition, phase of maturation and extraction method. Moreover, these compounds are easily oxidized resulting in less biologically active products (Kapustová et al., 2021).

In spite of their composition and importance, EOs have several limitations in their use in novel preparations mostly due to their high volatility, hydrophobicity, oxidation susceptibility, high sensitivity to light, temperature, and moisture, low stability, and low solubility in aqueous media (Kumar et al., 2020). Encapsulation technology has the potential to alleviate these issues because the capsule can hold an active, sensitive substance that allows the EO to be protected from thermal and light decomposition. Thus, increasing the solubility in aqueous media, decreasing the degradation or evaporation of volatile components, controlling the rate of oil release protecting bioavailability as well as protecting odor and flavor (Gupta and Variyar, 2016 ;Kapustová et al., 2021).

Lemongrass [*Cymbopogon citratus* (DC) Stapf.] family Poaceae, is one of the most important herbs that produce EOs commonly used in the treatment of anxiety and digestive disorders, as well as antispasmodic, anti-inflammatory, anti-pyretic, diuretic, and has a relaxing effects (Santin et al., 2009). Moreover, lemongrass extracts have proved other strong therapeutic effects such as anti-cancer, antihypertensive and anti-mutagenicity (Kiani etal., 2022). Others reports include non-toxic, anti-diabetic and anti-oxidant properties of lemongrass extract (Shah et al. 2011). Research conducted on extracts from *C. citratus* leaves confirmed has that it possesses anti-bacterial and anti-fungal activities (Wifek et al., 2016).

In the present study, lemongrass oil in chitosan nanoparticles (CN) was prepared in order to preserve the benefits of the EOs and enhance their antimicrobial action. This include improved water solubility, effective degradation protection, prevention of the

evaporation of volatile components, and controlled targeted release.

Chitosan is a natural cationic linear polysaccharide consisting of copolymers of D-glucosamine and N-acetyl-D-glucosamine units, with a higher ratio of nitrogen (6.89%) (Abdelkader et al., 2018). As a result, it is one of the best polymeric shell components for oily nanocapsules. It has beneficial biological properties, such as biocompatibility, biodegradability, antimicrobial activity, non-toxicity, permeability enhancement, cheap and high efficiency (Hosseinnejad and Jafari, 2016; Xiao et al., 2017).

The present work concerns the preparation of lemongrass oil-loaded chitosan nanoparticles. The oil was analysed into its main components by gas chromatography-mass spectrometry (GC-MS), then the characterization of the successful encapsulation was recorded by attenuated total reflectance-Fourier transform infra-red (ATR-FTIR) spectroscopy. Zeta sizer and zeta potential were measured and the shape and size of the particles was determined by transmission electron microscope (TEM) and the morphology was clarified by scanning electron microscope (SEM). In addition, the encapsulation efficiency, loading capacity and the rate of release of oil from chitosan nanoparticles were investigated.

The goal of this research is to produce an ecofriendly, safe and effective lemongrass oil nanocapsule for potential applications on different natural matrices.

MATERIALS AND METHODS

Materials

Chitosan with a medium molecular weight (190–310 kDa) derived with deacetylation degree not less than 85% was purchased from Starchemie, Pratap Chemical Industries, Pvt. Ltd, Nagpur, India. Lemongrass (*Cymbopogon citratus*) essential oil (LEO) was prepared from the air- dried aerial parts, which were processed by the hydro-distillation method in adapted Clevenger equipment for extraction of essential oils (Meyer-Warnod etal., 1984). The oil was stored in amber-colored glass recipients at 4ºC until use.

Identification of the lemongrass essential oil (LEO) chemical components.

The chemical composition of LEO was accomplished by GC-MS analysis using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a column TG–5MS. The process was started with adjusted temperature at 50°C and then raised by 5°C /min to 230°C, and held for two min. After that, it was raised by 30 °C/min to 290 °C and held for another two min. Temperatures were kept constant at 250 °C for the injector and 260 °C for the MS transfer line.

Helium as a carrier gas was adjusted at a constant flow rate of 1 ml/min. Three min delaying time of the solvent. AS1300 attached with GC in the split mode. Electron impact mass spectra were collected at 70 eV ionization voltages. The ion source temperature was set at 200°C. The components were defined by comparing their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database (Adams, 2017).

Preparation of LEO encapsulated in chitosan nanoparticles (CN).

Ionic gelation was used to produce encapsulated chitosan nanoparticles according to Hosseini etal., (2013) and Yoskan, etal., (2010) with certain modifications. Chitosan (0.5 g) was dissolved in (1%) aqueous glacial acetic acid and incubated overnight at 25°C until completely dissolved. The mixture was centrifuged for 60 min at 4500 rpm at room temperature to remove any aggregation or solid traces and then the supernatant was divided into 5 portions (nearly 20 ml for each). According to Do etal., (2020), tween 80 (as a surfactant) was added with different volumes corresponding to the volume used from the oil to give 1:1 (v/v) oil to surfactant ratio and stirred for 2 h. Various amounts of oil (0.04, 0.08, 0.16, 0.32 and 0.64 ml) were added to form weight ratios of chitosan:oil 1: 0.08, 1:0.16, 1:0.32, 1:0.64, and 1:1.28, respectively. An amount of oil was dissolved in 1 ml dichloromethane and then during homogenization at 13000 rpm for 10 min under an ice bath (4°C) the oil was gradually dropped in the prepared chitosan solution. Sodium tripolyphosphate (TPP) (0.17 g) was dissolved in 20 ml purified water and then 5 ml were added drop wise into each solution with magnetic stirring for 45 min. The whole formula was homogenized using a high-shear homogenizer for 2 min at 25000 rpm for 10 homogenization cycles and sonicated for 30 min in a water bath sonicator. The nanoformulations were stored in a dry condition at 6°C until use.

Characterization of LEO-loaded chitosan nanoparticles.

Fourier-transform infrared (FTIR) spectroscopy analysis

The spectrum in the infrared region of the nanocapsules and the presence of functional group was characterized by ATR-FTIR spectroscopy, THERMO NICOLT, 50.

Scanning electron microscope (SEM)

Surface images of nanocapsules were described using Quanta FEG 250 scanning electron microscope (SEM) (FEI Company, Hillsboro, Oregon- USA). The sample was freeze dried and was mounted onto SEM stubs. SEM conditions were: a 10.1mm working distance, with an in-lens detector with an excitation voltage of 20 Kv.

Transmission electron microscope (TEM)

The morphology of the nanocapsules was observed using a Transmission Electron Microscope (TEM) (JEOL-JEM 1230), Work at magnifications from 1200x to 600,000x. With a digital camera 1024x1024 pixels. Average particle size was calculated from TEM images using the Image program.

Dynamic Light Scattering (DLS) and Zeta Potential

The zeta potential records the potential difference between the dispersion medium and the stationary layer of the fluid associated to the dispersed particle, which aids in determining the stability of the nanoformulations. The zeta potential, mean particle size and polydispersity of the nanoparticles were identified by Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, UK prepared with a He–Ne laser operating at 4.0mW and 633nm with a fixed scattering angle of 90◦). Analysis took place using nanoparticles dispersed in deionized distilled water at a temperature of 25°C. Particle size distribution is determined as a polydispersity index (PDI). Results were expressed as mean ± standard deviation (SD).

Determination of loading capacity (LC) and encapsulation efficiency (EE) of lemongrass essential oil.

UV-Vis spectrophotometry was used to measure the amount of lemongrass essential oil (LEO) in chitosan nanoparticles. LEO-loaded nanoparticles (10 mg) was taken into aqueous HCL acid solution (2 M, 4 mL) and boiled at 95°C for 30 min. After cooling down, ethanol (2 mL) was added to the mixture. The mixture was centrifuged at 9000 rpm at 25^{O}C for 5 min (Keawchaoon and Yoksan, 2011). The supernatant was collected and the content of LEO was measured using UV-vis spectrophotometer (Thermo Ultraviolet visible Spectroscopy (UV-Vis)-USA- supplied with thermo scientific vision pro software) at a UV wavelength of 275 nm. The calibration curve of free LEO in ethanol was used to calculate the amount of LEO. Chitosan nanoparticles without loaded LEO were prepared as blank sample but treated alike as the loaded samples. Each sample was measured three times. Each sample was measured three times. The encapsulation efficiency (EE) and loading capacity (LC) of LEO were calculated from Eqs. (1) and (2) respectively

$$EE\% = \left(\frac{\text{Total weight of loaded LEO}}{\text{Initial weight of LEO}}\right) \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

$$LC\% = \left(\frac{\text{Total weight of loaded LEO}}{\text{Weight of sample}}\right) \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

Rate of in vitro release of LEO from nanocapsule .

Rate of release of LEO was studied as described by Hosseini *et al.* (2013) and Yoksan *et al.* (2010). Twenty mg of freeze-dried LEO-loaded CN with ratio chitosan:oil 1:0.32 were placed in 5 mL of 40% ethanol + 60% phosphate buffer saline (pH 7.4). The samples were centrifuged at 9000 rpm for 5 min; then a definite proportion of supernatant was taken out for analysis, and replaced with an equal volume of fresh media. Then, at regular time intervals release rate was recorded by a UV–Vis spectrophotometer at 275 nm. Accumulative (%) of LEO released was calculated by dividing the total amount of LEO released at each sampling time (Mt) by the starting weight of the LEO-loaded (M0), i.e.:

$$\text{Cumulative release percentage} = \sum_{t=0}^{t} \frac{Mt}{M0} \times 100$$

Statistical analysis:

One way ANOVA was used to evaluate the effect of LEO concentration on particle size, LC and EE. Significant differences were determined using Duncan's test ($P \leq 0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of lemongrass essential oil.

The EO is a complex component consisting of a mixture of different aromatic derivatives. Data in Table (1) and Fig (1) demonstrated the GC-MS analysis of lemongrass essential oil, which identified main 16 components. The major component was citral as α-citral (27.42%) and β- citral (36.60%) with total ratio

(64.02%), followed by myrcene, which was (11.61%). Other main components were geranyl acetate (9.99%) and cis-verbenol (5.04%), neryl acetal (4.06%) verbenol (1.97%) and, citronellol(1.62%), linalool , caryophyllene, and *α*-farnesene each in percentage lower than one. These results are coordinated with other studies in LEO. The chemical composition of LEO differs depending on genetic variability, habitat, agronomic techniques, intensity of light, agricultural practices and geo-climatic factors (Ranitha, 2014). Soliman et al., (2017) found that Lemongrass leaves have a pale- yellow essential oil with an average concentration of 0.25-0.90% and that 79.69% of total LEO composition was citral, other main compounds including myrcene (8.05%), geraniol (3.22%), and cis-verbanol (1.84%). Pino, et al. (2018) reported that the main component was citral (73%), which consisting of geranial (39.8%) and neral (33.2%). Other components were myrcene (9.6%) and geraniol (4.2%). Masamba et al. (2003) showed that lemongrass oil consisted of 82% citral (41.67% geranial and 40.33% neral), and 10% myrcene.

FTIR results

The ATR-FTIR technique is used to learn about the formation and interaction of main functional groups engaged in essential oil encapsulation. (Fig.1). This data aids in approving the presence of oil in our nanocapsules.

Chitosan powder (Fig.2.a) showed characteristic peaks at 3317cm^{-1} (OH and NH_2 stretching), 2924 cm^{-1} (C-H stretching), 1650 cm^{-1} (amide I), 1100 cm^{-1} (C-O- C stretching) and 582 cm^{-1} (pyranoside ring stretching vibration). In chitosan nanoparticles (CN) (Fig.2.b) the peak at 3317 cm^{-1} is shifted to 3295 cm^{-1} and becomes broader, representing an improvment in hydrogen bond due to hydrostatic interaction between amino and phosphoric groups in TPP. These findings agree with Hosseini et al. (2013) who recorded that chitosan powder showed significant peaks at 3433 (OH and NH2 stretching), 2920 (CH stretching), 1647 (amide I), 1088 (C- O- C stretching) and 591 cm^{-1} (pyranoside ring stretching vibration), for chitosan nanoparticles, also reported the peak of amide I (NH_2bending) shifted from 1647 to 1651 cm^{-1},and new peaks developed at 1238 (C- O- C stretch) and 1555 cm^{-1} (amide II). Similar results were observed by (Oluoch etal., 2021) who reported that wide peak of chitosan at 3408 cm^{-1} due to hydroxyl group and primary amine stretching, however, in chitosan nanoparticles this band shifted to 3423 cm^{-1}.

This interaction is also supported by the decrease of amide band I intensity from 1650 cm^{-1}in chitosan compared to 1647cm^{-1} in CN. The other notable band for CN was detected at 1403 cm^{-1}owing to $–CH_2$ wagging. Furthermore, the peak at 1075 cm^{-1}which appears in the FTIR spectra of CN represents characteristic of P=O stretching vibration from phosphate groups. Similar data of CN treated TPP formation were reported in previous study (Lustriane etal., 2018).

The spectra of LEO-loaded CN (Fig2.c) shows the peak at 1732 cm^{-1}, confirming the presence of citral. Such results are in harmony with Natrajan etal., (2015). Hosseini et al., (2013) found that the peak at 3483 cm^{-1} indicating NH_2 ,OH stretching and sharp characteristic peaks at 2925 cm^{-1} (C-H stretching), 1454 cm^{-1} (CH_2 bending), 1247 cm^{-1} (C-O-C stretching), 1093 cm^{-1} (CH-OH) and 941 cm^{-1}(C-H bending).

According to FTIR spectra, there was no new peaks in the LEO/CN nanocapsules, indicating that the LEO/CN are physically linked to one other and do not interact chemically. Each substance preserves its components and therefore its effectiveness. It is notable that there is a shifting and flattening in the peaks linked to O-H and N-H stretching. These changes could be attributed to interactions between molecules without covalent bonds formation e.g. hydrogen bonds and electrostatic interactions. These results are in agreement with Hosseini et al. 2013, and (Hasani etal.2018) who observed no chemical interaction in FT-IR spectra of lemongrass oil loaded chitosan nanoparticles.

Shape, size, and surface charge of LEO - loaded chitosan nanoparticles.

Shape and diameters of LEO-loaded chitosan nanoparticles were determined by SEM, TEM and DLS technique.

SEM studies

SEM provides information about the external morphology, shape, mean diameter and surface appearance. SEM micrograph of the LEO/CN nanocapsules is shown in Fig. (3). It shows that most particles were aggregated with nearlyspherical shape structures and particle size ranged between 205 and 210 nm. The encapsulated LEOs appeared to be without cracks, and the core was surrounded by a denser material membrane. This lack of surface deformations could be clarified by the

high chitosan concentration of wall matrices. These results were in the same trend as those obtained by OH et al. (2019) and Antonioli et al. (2020) and is coordinated with Hadidi et al. (2020) results which demonstrated that all particle diameters were < 500 nm with regular distribution and spherical shape.

TEM studies

From Fig. (4) It was obvious that the morphological properties of nanocapsules were nearly spherical shape, smooth surface with size about 249 nm. These results confirm those obtained from the SEM studies.

DLS and zeta potential studies

Polydispersity Index (PDI) is a parameter that defines the particle size distribution, it is described as dimensionless number deduced from autocorrelation function in photon correlation spectroscopy. The value of PDI may vary from 0.01 to 0.5-0.7, while PDI values > 0.7 indicate a wide particle size distribution of the formulation and is possibly not suitable for the dynamic light scattering (DLS) technique. The PDI values of LEO loaded CN were ranged between 0.102 and 0.474 which mean that these formulations were in the nano size with uniform particle distribution which is suitable for DLS determination.

Dynamic light scattering (DLS) is a simple, well-established technique for detecting the size and distribution of particles typically in the submicron region in suspension. As shown in Table (2) and Fig(5), CN loaded with LEO were found to have a mean diameter of 206.9-370.9 nm and that diameter was increased as a function of loading different initial ratio of LEO. These diameters are in accordance with those obtained by SEM (205 - 210 nm) and TEM (249 nm) however it had a slightly greater particle size limit (370.9 nm). This may be due to that the size measured by the DLS technique might be the hydrodynamic diameters of aggregate particles and/or hydrated single particles (Lee and Kim, 2014). These results agree with those obtained by Keawchaoon and Yoksan (2011) who stated that average particle diameter obtained from DLS studies was larger than that observed by TEM technique. They also found that carvacrol-loaded CN showed an average hydrodynamic diameter in the range of 532.5–716.6 nm and the diameter increased as a function of initial carvacrol content.

Zeta potential is a measurement of the magnitude for the electrostatic charge between particles which is a important factor for evaluating the stability of distributions. It also indicate the importance of electrostatic repulsive forces in the stability of nanocapsules and avoid accumulation throughout time (Dickinson, 2009). Chitosan nanoparticles gave a zeta potential value of +42.1mV (Keawchaoon and Yoksan 2011). Loading LEO on chitosan nanoparticles increased particle size and reduced the surface positive charge. This reduction in zeta potential value increased with increasing initial content of LEO to values ranging from +40.7 to +31.2 mV (Table 2)& Fig(6). A relatively high zeta potential value, considered as |30 mV|, reflects good physicochemical stability as significant repulsive forces tend to inhibit aggregation during occasional collisions with nearby nanoparticles (Zielińska et al. 2020). Keawchaoon and Yoksan, (2011), Hasani et al. (2015) and Hadidi et al. (2020) have recorded coordinating results.

Loading capacity (LC) and encapsulation efficiency (EE)

Loading capacity (LC) indicates the amount of oil loaded per unit weight of the nanocapsule. It is obvious from Table (3) that (LC) had increment rate with increasing the chitosan: oil ratio. The values started with 32.4% with the ratio (1: 0.08) to reach its maximum value at 72.3% with the ratio (1: 1.28). This result was in agreement with the findings regarding the loading of carvacrol into chitosan nanoparticles, Keawchaoon and Yoksan (2011).

Encapsulation efficiency (EE %) is the percentage of oil that is successfully entrapped into the nano capsule. Table (3) also illustrated that the decrease in encapsulation efficiency was a function of initial LEO concentration. EE decreased from 65.2% with the ratio (1: 0.08) to 25.6% with the ratio (1: 1.28) which mean that low mass ratios are more suitable for higher EE than higher mass ratios. This may be explained by the saturation of LEO loading into chitosan nanoparticles or the higher viscosity in capsule with increasing the oil concentration, or due to the encapsulation limitation (Nguyen and Le. 2021).

In vitro release of LEO from LEO/chitosan nanoparticles.

The in vitro release profile of the nano-encapsulated essential oil in different ratios of chitosan: oil is presented in Fig (7). The amount of oil released at different times was measured at 275 nm. As LEO concentration increased, the burst effect decrease and the accumulative release was reduced (Hosseini

etal., 2013). Results of the in vitro release profile of LEO from LEO/CN nanocapsule was calculated using the three ratios 1: 0.32, 1: 0.64 and 1: 1.28. Findings demonstrated an initial rapid burst release followed by successive slower release. This might be due to the diffusion of the LEO dispersed into the polymer.

The maximum amount (more than 40%) of the loaded LEO released within 25 h. in the first ratio LEO: CN (1: 0.32), 35% of the initial burst of encapsulated LEO was observed after the first 10 h., followed by slow release as only 7% released within the subsequent 15 h. The release amount within 25h is reduced in the second and third ratios (1: 0.64 and 1: 1.28) to reach 39% and 35% respectively as initial burst. Relatively faster oil release from the formulations at initial period might be due to the LEO molecules adsorbed on the surface of the capsule and oil captured near the surface or from the unencapsulated dissolved oil molecules in the aqueous phase of the nanoparticle dispersions. The second part of profile is related to slow release arises from the slow degradation of nanoparticles (Jayakumar et al., 2007, Anitha et al., 2011 and Das et al., 2012).

The release of LEO from capsules is affected by several factors including capsule composition (oil, polymer, and additives), physical and/or chemical interactions among components, their ratio and preparation methods (Lee and yeo, 2015). The amount of LEO released at intervals of time from nanoparticles occured by many mechanisms including surface erosion, disintegration, diffusion and desorption (Hariharan et al., 2006). Similar observations were reported by Dounighi et al. (2012) who concluded that the in vitro release of chitosan nanoparticles showed an initial burst release of approximately 60% in the first ten h, followed by a slow and reduced additional release for about 60 h. Likewise, Rwegasila et al. (2016) studied the release rate of panchovillin which indicated that the burst started at 42% in the first 6 h followed by a slow and sustained release up to 72 h.

CONCLUSION

Lemongrass essential oil (LEO) was extracted from air dried aerial parts of lemongrass plants by the hydro-distillation method. GC/MS analysis showed that Citral with its two components; α-Citral and β- Citral was the major compound representing 64.02% of total essential oil composition followed by Myrcene, Geranyl acetate and cis-Verbenol

LEO-loaded chitosan nanoparticles were prepared successfully by the ionic gelation method with sodium tripolyphosphate (TPP) as a cross-linking agent and were characterized with FTIR spectroscopy, SEM, TEM, DLS, and Zeta potential which confirmed the successful preparation of LEO/ chitosan nanocapsule formulation. Most of the LEO nanocapsules particles were nearly spherical in shape with smooth surface and particle size of 205 - 210 nm and 249 nm as observed by SEM and TEM micrographs, respectively. PDI values proved that these formulations were in the nano size with uniform particle distribution. Loading LEO on chitosan to produce LEO-chitosan nanoparticles increased particle size and reduced the surface positive charge. This reduction in zeta potential values was in positive correlation with the initial content of LEO. By increasing the weight ratio of LEO to chitosan, LC increased, while EE decreased. The in vitro release profile of LEO from LEO/chitosan nanoparticles showed an initial rapid release up to 35% after the first 10 h., followed by subsequent slower release as only 7% released within the subsequent 15 h. A study is now being prepared to prove the biological activity of this formulation as a natural and safe pesticide. The results of this study prove the ability of using chitosan-based nanoparticles technology as delivery systems.

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Table 1: Chemical composition (%) of the lemongrass, *C. citratus*, essential oil as determined by GC-MS.

Rt	Compound	%	Rt	Compound	%
7.52	Linalool	0.86	12.27	β-Citral	10.13
8.70	Citronellol	1.62	12.66	β-citral	17.57
9.22	Verbenol	1.97	12.92	Geranyl acetate	0.73
9.71	Cis-Verbenol	5.04	13.18	Geranyl acetate	9.26
11.27	α-Citral	9.93	13.74	Neryl acetal	4.06
11.40	α-Citral	4.38	14.89	Myrcene	11.61
11.65	α-Citral	13.11	15.45	Caryophyllene	0.31
12.16	β-Citral	8.90	15.88	α-Farnesene	0.52

Table 2: Particle size, Polydispersity Index, and Zeta potential of LEO-loaded CN

Chitosan: LEO Ratio (w/w)	Particle size (nm)	Polydispersity Index (PDI)	Zeta potential (mv)
1:0.08	206.9±1.15 ^{c}	0.459	+ 40.7
1:0.16	207.7±2.89 ^{c}	0.102	+39.6
1: 0.32	209.8±3.46 ^{c}	0.427	+33.4
1: 0.64	221.5±4.04 ^{b}	0.419	+ 31.2
1: 1.28	370.9±2.31 ^{a}	0.474	+ 33.6

Particle size was significantly affected by LEO concentration (F=590.526;P<0.05).

Table 3: Loading capacity (LC) and encapsulation efficiency (EE) of LEO in LEO-loaded chitosan nanocapsules.

Chitosan: LEO ratio (w/w)	UV–Vis spectrophotometry	
	EE%	LC%
1: 0.08	32.4±1.15 ^{d}	65.2±2.31 ^{a}
1:0.16	39.7±2.31 ^{d}	60.3±2.89 ^{a}
1:0.32	47.5±1.73 ^{c}	50.8±1.15 ^{b}
1:0.64	60.8±2.89 ^{b}	38.1±3.46 ^{c}
1:1.28	72.3±3.46 ^{a}	25.6±1.73 ^{d}
F	43.107	43.947

LC and EE were significantly affected by LEO concentration (P<0.05).

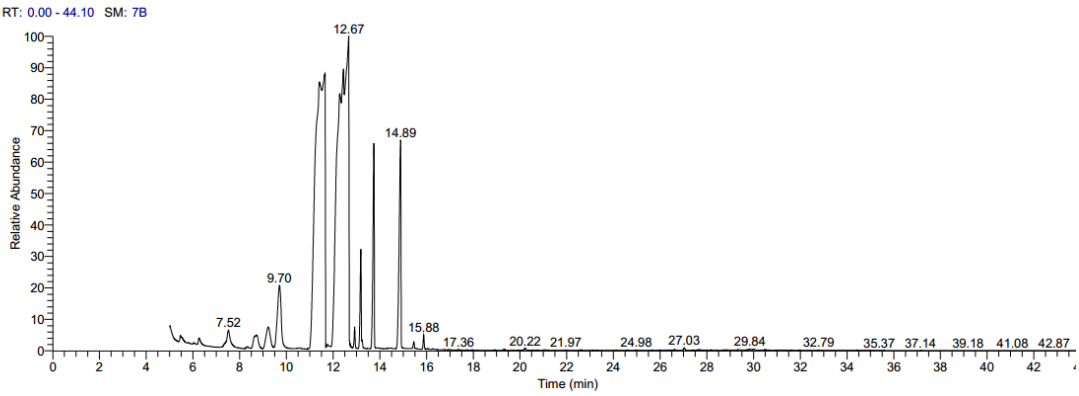

Figure 1: GC–MS profile of lemongrass oil (*C. citratus*).

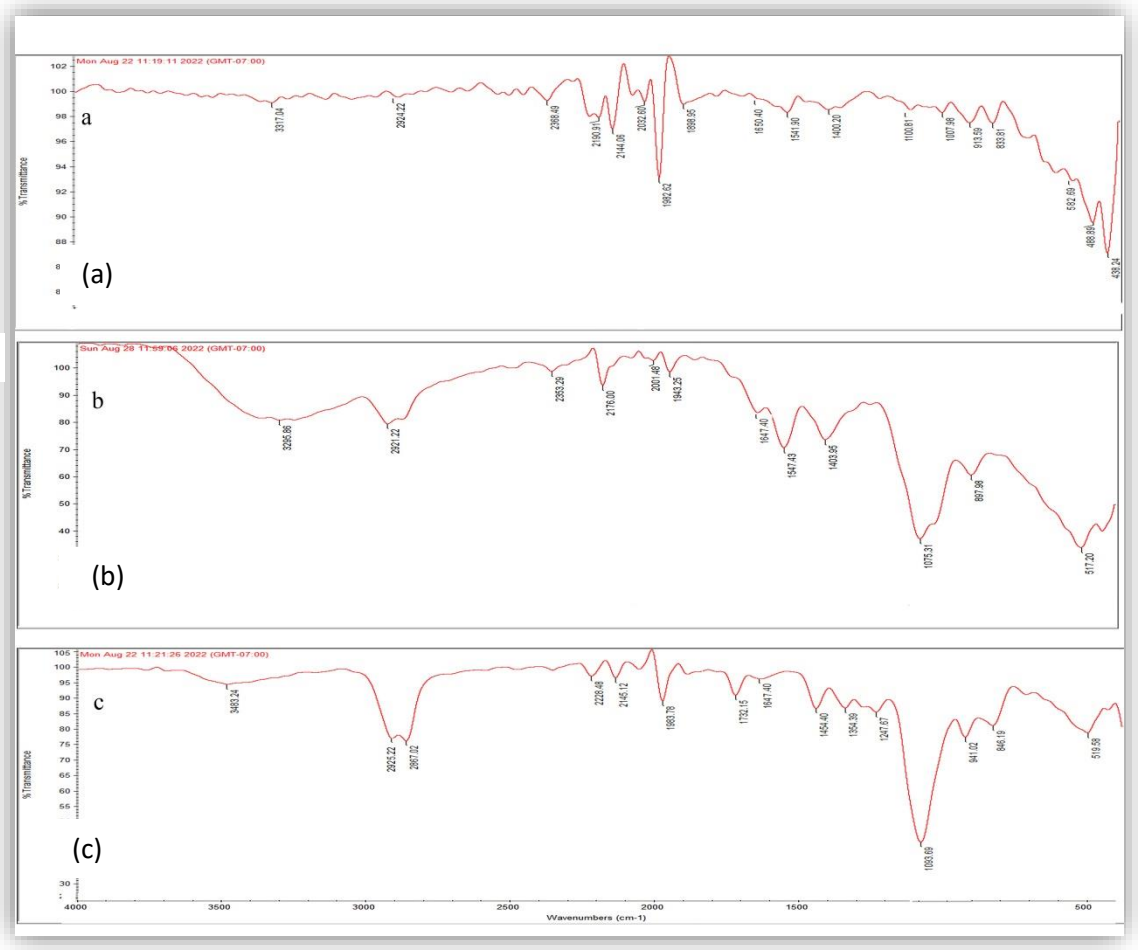

Figure 2: FTIR spectra of (a) chitosan powder, (b) chitosan nanoparticles (CN), (c) LEO-loaded CN with chitosan:LEO weight ratio of 1:0.32.

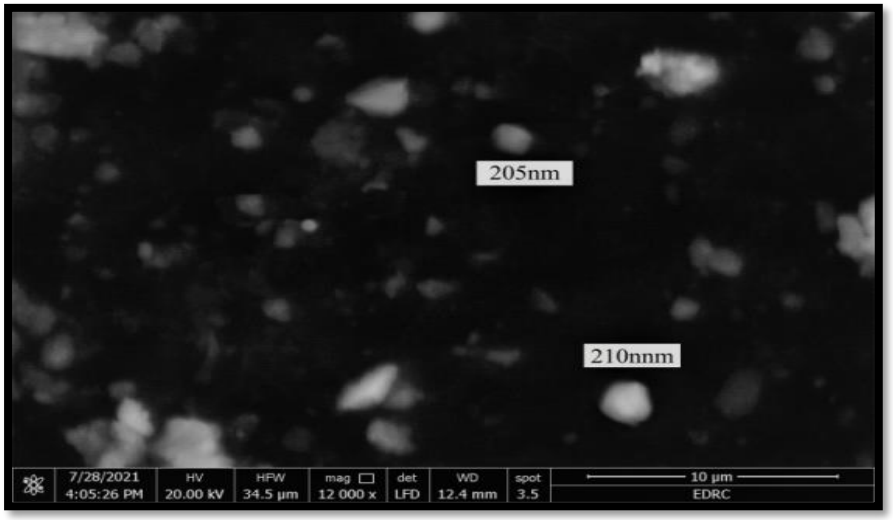

Figure 3: Scanning electron microscope (SEM) of LEO loaded CN with ratio chitosan:oil (1: 0.32).

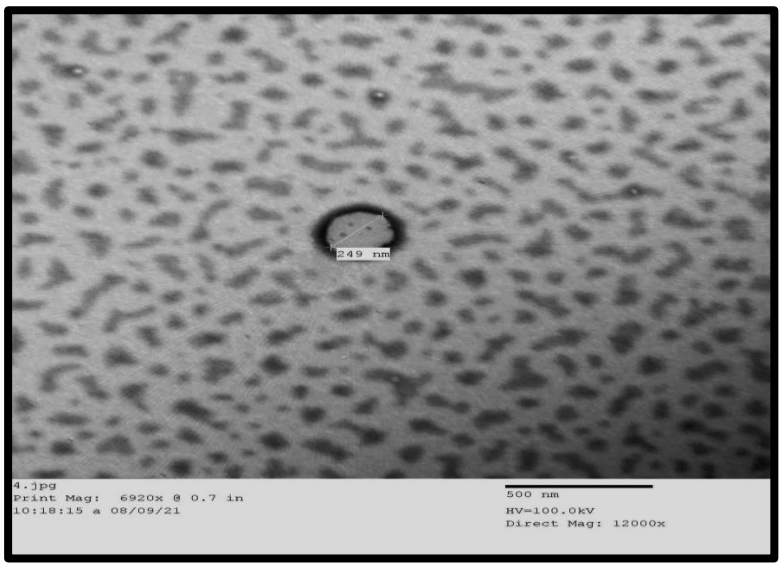

Figure 4: Transmission electron microscope images of the LEO/ CN

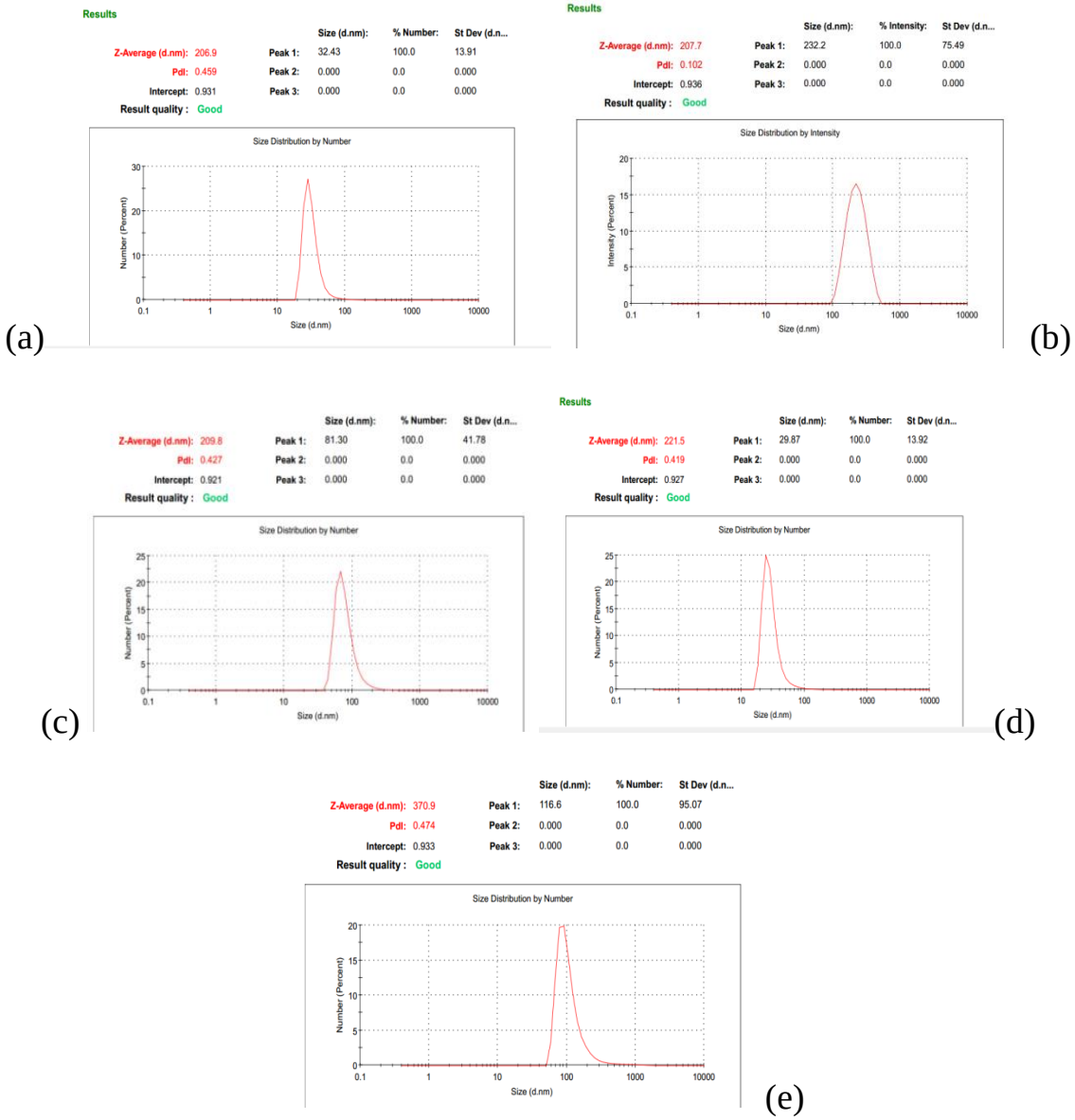

Figure 5: Particle size and PDI of LEO/ CN with different chitosan /LEO mass ratios (a) 1: 0.08, (b) 1:0.16, (c) 1:0.32, (d) 1:0.64 ,and (e) 1:1.28.

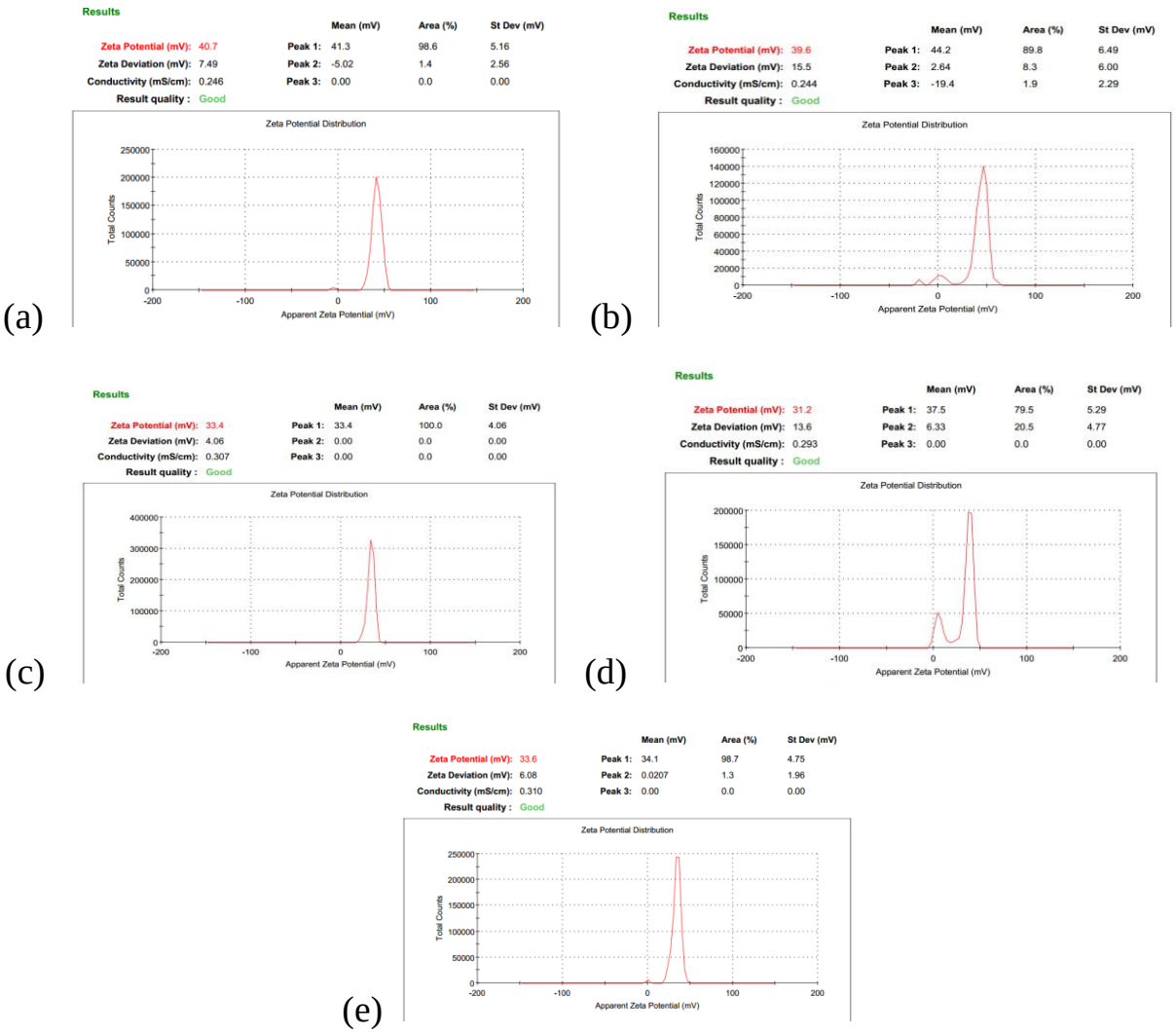

Figure 6: Zeta potential of LEO loaded in CN with different chitosan: LEO mass ratios.(A) 1:0.08, (B)1:0.16, (C) 1: 0.32, (D)1:0.64, and (E)1:1.28.

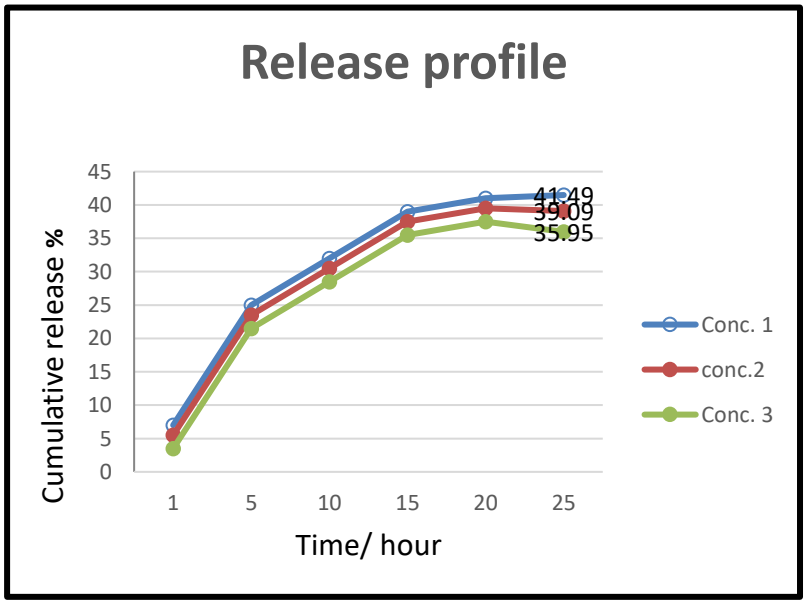

Figure 7: Release profile of LEO loaded in chitosan nanocapsule using three chitosan:LEO mass ratio.conc.1(1:032), conc 2 (1:0.64) and conc 3(1:1.28).

كبسلة زيت عشبة الليمون العطري في جزيئات الشيتوزان النانوية، التوصيف ودراسة الإطلاق في المختبر.

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الملخص العربى

زيت حشيشة الليمون العطري نشط بيولوجيا وله دور ملحوظ في المضافات الصيدلانية والزراعية والغذائية والعوامل الحافظة والأدوية والمكملات الغذائية. هدف الدراسة هو إنتاج تركيبة نانوية عالية الجودة وآمنة لتلبية الاحتياجات البشرية وكبسولة نانوية صديقة للبيئة للتطبيقات المحتملة على مصفوفات طبيعية مختلفة. كشفت دراسة GC-MS التحليلية لزيت عشبة الليمون العطري (LEO) أن Citral هو المركب الرئيسي الذي يمثل (64.02٪) من إجمالي تركيبة الزيت العطري يليه Myrcene، geranial acetate و cis-Verbenol. تم تغليف LEO في جسيمات الشيتوزان النانوية (CN) باستخدام الجيل الأيوني مع sodium tripolyphosphate(TPP) كعامل ربط ، كما تم التحقق من صحته بواسطة التحليل الطيفي ATR-FTIR. كانت معظم جزيئات الكبسولات النانوية LEO كروية الشكل تقريبًا مع سطح أملس وحجم جسيم يتراوح بين 205 - 210 نانومتر و249 نانومتر كما لوحظ بواسطة الصور المجهرية SEM وTEM، على التوالي، لكن دراسات تشتت الضوء الديناميكي (DLS) أظهرت أن متوسط حجم الجسيمات كان (206.9) - 370.9) نانومتر. أدى تحميل LEO على الشيتوزان إلى زيادة حجم الجسيمات وتقليل الشحنة الإيجابية للسطح. كان هذا الانخفاض في قيم Zetaالمحتملة له علاقة إيجابية مع المحتوى الأولي لـ LEO. من خلال زيادة كمية LEO المحملة في كبسولة الشيتوزان النانوية، زادت سعة التحميل (LC)، بينما انخفضت كفاءة التغليف (EE). أظهر الزيت المستخرج من الجسيمات النانوية LEO/الكيتوزان ملفًا أوليًا سريع الإطلاق يصل إلى 35% بعد أول 10 ساعات، يليه إطلاق أبطأ لاحقًا.

الكلمات الاسترشادية: