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Lemongrass Oil Containing Chitosan Microcapsules by Ionotropic Gelation

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Ionotropic gelation using sodium tripolyphosphate (STPP) as the crosslinker was successfully used to formulate lemongrass oil containing chitosan microcapsules. Formation of oil in aqueous chitosan polymer solution was followed by crosslinking with STPP. Optimum formulation for the synthesis of microcapsules was found varying the amounts of lemongrass oil (1-3 g), chitosan solution (1-2%) and STPP (0.5-1.0 g). Microcapsules were characterized by Fourier transform infrared spectroscopy, scanning electron microscopy and stage microscope. Encapsulation efficiency, oil content, oil load and release rates were determined by UV-visible spectroscopy. The SEM images indicated that the oil loaded capsules are spherical in shape and possess a smooth surface and their size varied between 100-1000 nm. FTIR spectra confirmed successful encapsulation of lemongrass oil within chitosan. At polymer: 1 g, oil: 3 g and crosslinker: 0.5 g gave microcapsules with higher stability and steady controlled release.

Keywords: Chitosan, Ionotropic gelation, Lemongrass oil, Microencapsulation, Sodium tripolyphosphate.

INTRODUCTION

Encapsulation is known as one of the natural techniques to protect biological structures. The encapsulation is used in formulations to introduce essential ingredients in a capsule or sphere, to protect them against oxidation, isomerization, degradation and to extend the bioavailability over a longer period [1,2]. Encapsulation can also be used for controlled/sustained delivery of functional substances when ingested in the body. This means that the unstable components must remain intact for a certain period in the digestive system and then, be released into the intestine in a range of physiological conditions. In general, nano/microencapsulation involves coating of emulsion droplets in fluidic dispersions in the nano and/or microsize regime [1].

Ionotropic gelation is one of the popular techniques of encapsulation [3]. It exploits the ability of polyelectrolytes to crosslink in presence of the polymers of opposite charge to form capsules [4]. The mechanism of capsule formation cannot simply be explained using electroneutrality as it depends on the three-dimensional structure and the other ionic groups present in the polymer. The factors affect the ability of the ions to conjugate with the ionic groups on polymers, which impart a selectivity to crosslink formation [3]. This technique has seized much attention as this is a simple and mild technique.

Essential oils are volatile, natural and contain complex compounds with a characteristic strong odour. These oils play important roles in plants as chemical defense, acting as insecticides and protection against pathogens [5]. However, their full potential cannot be harnessed due to their sensitivity to light, heat, moisture and oxygen. Encapsulation of oils help surmount these impediments [6].

Encapsulated essential oils have numerous applications in a variety of fields. Interest on natural pesticides have grown immensely during the recent years as alternatives to synthetic pesticides. Many essential oils like lemongrass, cinnamon, citronella oil, *etc*. possess properties necessitated for pesticides. Cinnamon oil encapsulated in polyvinyl alcohol has been used to produce insect-repellent food packaging film using low density polyethylene films [7]. Encapsulated fish oil in milk protein complexes has been used to fortify cheese with omega-3 fatty acids to improve the nutritional levels of cheese [8].

Lemongrass (*Cymbopogon citrates*) belongs to the Poaceae family and its oil is extracted from fresh or partially dried leaves using steam distillation. The characteristic smell of lemongrass oil (LGO) is due to the presence of citral. Other main compounds

are geranyl acetate, geraniol, myrcene, citronellal, nerol, neral, limonene and geranial. These are known for their antimicrobial, antiseptic, insecticidal and counterirritant properties [9]. Lemongrass oil (LGO) has shown antifungal properties against *A. flavus* isolated from stored rice and also reduced the aflatoxin production [9]. It has also shown potent *in-vitro* activity against *Candida* species [10].

Natural and synthetic polymers have been used in encapsulation. Natural polymers such as chitosan, starch, gelatin, and cellulose are biodegradable and environmentally friendly. Synthetic polymers such as melamine, phenols, urea and urethanes have also been used in encapsulation [2]. Chitosan is a linear polysaccharide obtained by deacetylation of chitin. Chitin is a naturally occurring polymer (mucopolysaccharide) and the second most abundant polymer in nature next to cellulose. It is found in shells of arthropods such as crabs, shrimps, lobsters and insects and also produced extracellularly by the cell walls of fungi and brown algae. *N*-Deacetylation of chitin yields chitosan and it is composed of linearly distributed β-(1→4)-linked D-glucosamine and *N*-acetyl-D-glucosamine. Chitosan is characterized by the degree of deacetylation. This polysaccharide has a strong positive electrical charge, which strongly attracts and bonds with negatively charged molecules. It is insoluble in water and organic solvents. It dissolves in acetic acid, nitric acid, hydrochloric acid, phosphoric acid and perchloric acid solutions [11]. Chitosan has been used to encapsulate cinnamon oil [12], cardamom oil [13], pepper seed oil [14], neem oil [15], *etc*. Objective of this study is to formulate lemongrass oil containing chitosan microcapsules using ionotropic gelation method.

EXPERIMENTAL

Chitosan (CS), medium molecular weight with viscosity of 36.5 cps was purchased from Biotech Surinodo, Indonesia. Acetic acid was purchased from Sigma-Aldrich (*m.w.* 60.05 g/mol with 99.5% assay). Tween 80 (*m.w.* 604.822 g/mol, density of 1.06-1.10 g/mL) was used without further purification. Lemongrass oil (LGO) was purchased from Wrook Infinite (Pvt.) Ltd., Sri Lanka. Industrial grade sodium tripolyphosphate (STPP) was used without further purification.

Gas chromatographic (GC) analysis of lemongrass oil (LGO): The GC analysis of LGO was performed using GC-2025 Gas chromatograph Shimadzu GC system. Nitrogen was used as the carrier gas at a constant flow rate of 30 mL/min. The inlet temperature was maintained at 250 ºC. The oven temperature was programmed from 50 ºC to 240 ºC as at 50 ºC, 2 min, from 50 ºC to 240 ºC temperature ramp at the rate of 8 ºC/min. The detector (FID) temperature was maintained at 250 ºC. The diluted samples (1/100 v/v, in hexane) of 2 µL were injected. The identification of the separated compounds was conducted using the library search facility.

Formulation of oil encapsulated chitosan microcapsules (LGO-CS MCs) using ionotropic gelation method: Chitosan (1%) solution in 1% acetic acid (w/v) (100 mL) was taken into a beaker. To this, oil was added dropwise with slow agitation (200 rpm) using an overhead stirrer (IKA RW 20 digital) at room temperature. The mixture was agitated using high speed (1000 rpm) for 10 min to obtain a good emulsion. Then the speed was decreased to 200 rpm and STPP was added dropwise. The solution was stirred at the same speed for further 30 minutes. The microcapsules were centrifuged at high speed (6000 rpm) for 15 min. The supernatant was discarded and the resulting pellet was washed with ethanol. Microcapsules were freezed dried using the freeze dryer (LABONCO) and stored in the refrigerator until further use.

Optimization of process parameters: Process parameters were altered to find the optimum formulation for the microcapsules. The optimum formulation for LGO containing microcapsules were achieved by changing the amount of polymer (1-2%), LGO (1-3 g) and STPP (1-2%).

Measurement of particle size of microcapsules: The particle sizes of the prepared microcapsules were measured using the particle size analyzer (CILA-Nano DS). The size was determined using water as the dispersing medium.

SEM analysis: The microcapsules were sputtered with gold and surface characteristics of microcapsules were studied using scanning electron microscope (ZEISS EVO LS15).

FTIR analysis: The FTIR measurements of chitosan (CS) film, lemongrass oil (LGO), chitosan microcapsule (CS-MC) and LGO-CS microcapsule were performed on FTIR spectrometer (Perkin-Elmer, Spectrum Two). The spectra were obtained in the range of 4000-750 cm^{-1}. Lemongrass oil loaded and empty capsules were made into KBr pellets before analyzing in the FTIR instrument. IR spectrum of LGO was obtained using the ATR accessory of the instrument.

Lemongrass oil release studies: The study was conducted according to the method described by Devi *et al*. [15]. Briefly, a series of LGO solutions (1-10 mg/L) in Tween 80 (0.1%, w/v) were made. The solution with the highest concentration of oil was scanned in the range 200-400 nm (UV-visible spectrophotometer-Agilent Technologies, Cary 60) to obtain the wavelength with maximum absorption. A prominent peak was observed at 225 nm for LGO. A calibration curve for the oil was made using the above concentration series by plotting the absorbance value at λ_{max} against the concentration. A known weight of microcapsule (1 g) were placed in Tween 80 solution (0.1%, w/v) (100 mL). The solution was shaken from time to time. The solution was maintained at room temperature during the whole experiment. An aliquot (5 mL) was taken from the solution at 0.5 h intervals for 8 h and assayed spectrophotometrically at λ_{max} of the oil. In order to maintain a constant volume, fresh Tween 80 (5 mL) was added to the solution after every measurement. Each determination was conducted in triplicate.

Determination of encapsulation efficiency, oil content and oil load: Encapsulation efficiency (EE), oil load and oil content were determined using the method described by Devi & Maji [15]. A known weight of wet microcapsule (1 g) was ground in a mortar. It was transferred into a volumetric flask containing Tween 80 solution (0.1%, w/v) (100 mL) and kept overnight. An aliquot of known volume (5 mL) was taken from the solution and assayed spectrophotometrically at 225 nm. The encapsulation efficiency, oil content and oil load were calculated using the calibration curve and the equations given below:

$$\text{Encapsulation efficiency (\%)} = \frac{w_2}{w_3} \times 100$$

$$\text{Lemongrass oil content (\%)} = \frac{w_2}{w_1} \times 100$$

$$\text{Lemongrass oil load (\%)} = \frac{w_3}{w_4} \times 100$$

where w_1 = weight of microcapsules; w_2 = actual amount of oil in a known amount of microcapsules; and w_3 = amount of oil introduced in the same amount of microcapsules.

Statistical analysis: Data are presented as mean ± standard deviation. The statistical analysis (analysis of variance, ANOVA) was conducted using the PSPP statistical software for windows.

RESULTS AND DISCUSSION

GC analysis of lemongrass oil (LGO): The purchased LGO was pale yellow in colour. According to the GC analysis (Table-1), the major constituents of LGO are citral-a (44.31%), citral-b (34.12%), geranyl acetate (3.53%) and geraniol (6.93%). Similar compositions have been reported in previous studies [16-18].

TABLE-1
MAJOR CONSTITUENTS OF LEMONGRASS OIL

Compound	Relative abundance (%)
Myrcene and limonene	1.08
cis-Omicene	0.29
γ-Terpinene	0.13
Methyl heptanone	0.74
Citronellal	0.38
Camphor	0.34
Linalool	0.32
Linalyl acetate	1.10
Isobornyl actetate	0.64
β-Cargophyllene	2.23
α-Farnesene	0.24
α-Humulene	0.35
Citral-b	34.12
Citral-a	44.31
Geranyl acetate	3.53
Geraniol	6.93
Methyl eugenol	0.34
Cedrol	0.47
Eugenol	0.14
α-Farnesol	0.67

Morphology of LGO-CS-MCs: Fig. 1 gives a microscopic image of the LGO-CS-MCs. Encapsulation of LGO occurs due to chemical or physical interactions formed between LGO and the chitosan (CS) polymer strands. It has been reported that aldehyde (citral), which is the major constituent of LGO can form reversible imine bonds with the amino group of the CS polymer strands [19]. The polymer strands arrange around the oil vesicles formed in the medium during the emulsion formation step. When STPP is added to the medium, it forms crosslink between free ammonium groups of chitosan and phosphate group of STPP. This crosslink formation encapsulates the oil vesicles within a framework of chitosan with pores of various sizes [12].

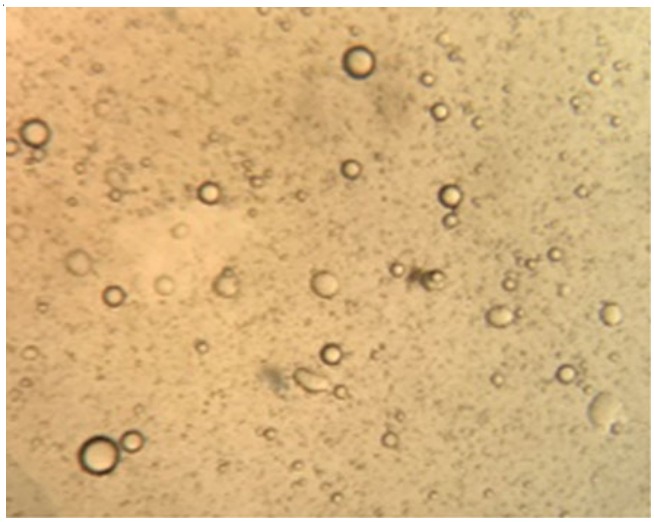

Fig. 1. Microscopic image of the LGO-CS-MCs formed at 3 g of LGO, 1% of CS and 0.5 g of STPP

Optimization of process parameters: The optimum conditions for the formation of microcapsules were determined by varying concentrations of chitosan (1, 1.5 and 2%), LGO (1, 2 and 3 g) and STPP (0.5, 0.75 and 1 g). At 3 g of oil and 2% of chitosan a lot of oil were floating on the top of the emulsion. When the oil loading increased to 3 g, there is not enough polymer in the medium to cover all the oil vesicles. At 2% chitosan concentration, the viscosity of solution increases reducing the efficiency of the dispersion force thus resulting in more free oil on the top of the emulsion. At low oil load, microcapsules had a thicker wall while the wall got thinner with increasing oil load.

Fig. 2 shows the change in wall thickness with variation of oil loading and polymer concentration. At higher oil load, the polymer tries to coat all the oil vesicles at the expense of the wall thickness. At 2% polymer concentration a thicker wall was observed as there is excess polymer to cover the oil vesicles thus causing multiple layers of polymer around the oil vesicles. It was observed at lower oil load and polymer concentration, microcapsules were smaller whereas at higher oil load and polymer concentration, the particle size increased. High amount of oil and polymer decrease the efficiency of dispersion of the oil which results in bigger particles. Similar observations have been made in previous studies [15].

The role of STPP is to make crosslink between ammonium groups of chitosan and phosphate group of STPP [12]. At low concentration of STPP, the wall thickness was low and the thickness increased with increasing concentration of STPP. When a high amount of crosslinker is present in the medium, it tends to crosslink more polymer thus making thicker walls. Fig. 2 shows the variation of wall thickness with the change of STPP concentration. Applying excess STPP, causes aggregation of microcapsules and separate them out from the emulsion as a precipitate. The visible thickness of the wall increased with increasing STPP concentration. This is due to the presence of more STPP to crosslink with protonated amine groups thus

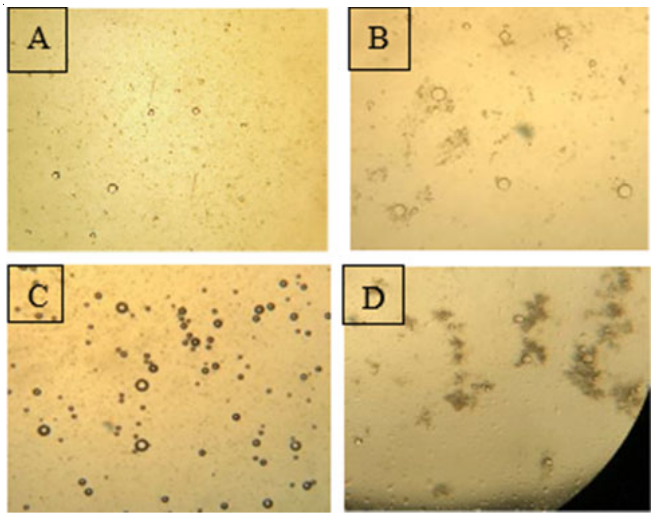

Fig. 2. Wall thickness of MCs with variation of CS:LGO:STPP (g); (A) 1:1:0.5, (B) 1:2:0.5, (C) 2:1:0.5, (D)1:1:1

making thicker walls [20]. Good formation and a good yield of microcapsules were obtained at 3 g of LGO, 1% of chitosan and 0.5 g of STPP (Fig. 2c).

Particle size and SEM studies: According to the DLS measurements (Fig. 3) LGO-CS-microcapsules are polydisperse (100-1000 nm). Fig. 4 shows the SEM images of CS-MC and LGO-CS-MC. Oil loaded microcapsules are spherical in shape and have a smooth surface, whereas the CS-MC appear in a layered structure.

FT-IR studies: The IR spectrum of chitosan film shows major peaks for N-H and O-H *str.* (3400 cm^{-1}), C-H *str.* (2919 cm^{-1}), amide-II carbonyl *str.* (1649 cm^{-1}) and N-H bending vibration of amine-I (1582 cm^{-1}) (Fig. 5a) [20]. The new peak at 1150 cm^{-1} in CS-MC (Fig. 5b) evidences the crosslinking of chitosan with phosphate group of STPP [12]. Furthermore, N-H *str.* (3400 cm^{-1}) and N-H bending vibration of amine-I (1582 cm^{-1}) of chitosan film have shifted to 3412 and 1530 cm^{-1}, respectively indicating the formation of linkage between the phosphoric group of STPP and ammonium groups of chitosan in CS-MC [12].

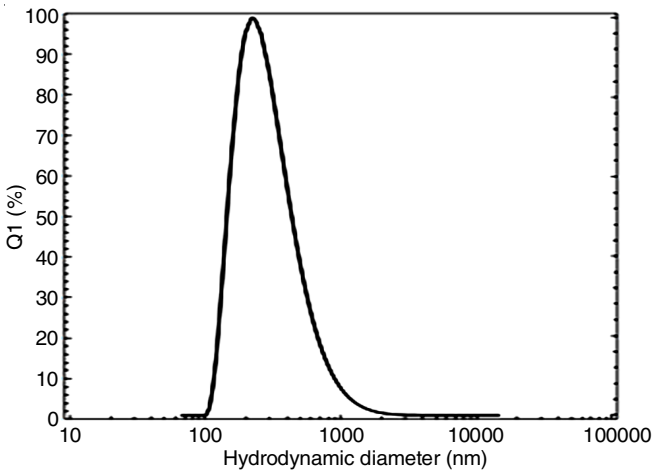

Fig. 3. Particle size distribution of dried LGO-CS-MC

In the IR spectra of LGO (Fig. 5c), aldehyde C-H *str.* were observed at 2850 and 2750 cm^{-1}, C-H *str.* peaks at 2924 cm^{-1}, C=O *str.* peak at 1719 cm^{-1} and C-O peak at 1120 cm^{-1}. No significant difference was observed in the position of the major bands of the oil, in the oil loaded microcapsules. The carbonyl peak at 1750 cm^{-1} of LGO was observed at 1745 cm^{-1} in LGO-CS-MC spectrum (Fig. 5d) . Thus, it is concluded the successful encapsulation of oil in chitosan. Similar peaks for microcapsules, LGO and chitosan have been observed in previous studies [21-23].

Effect of variation of process parameters on encapsulation efficiency (EE) and oil content: Table-2 shows the effect of variation of process parameters on the behaviour of microcapsules. Oil content and EE increases with increasing oil load. As oil loading increases, higher quantity of microcapsules form with larger sizes. This causes an increase in the oil content and the EE of the microcapsules.

When the polymer concentration increases, oil content and EE increase up to an optimum value and then decreases. The increase of oil content and EE is caused by the abundance of polymer in the medium to encapsulate more oil vesicles.

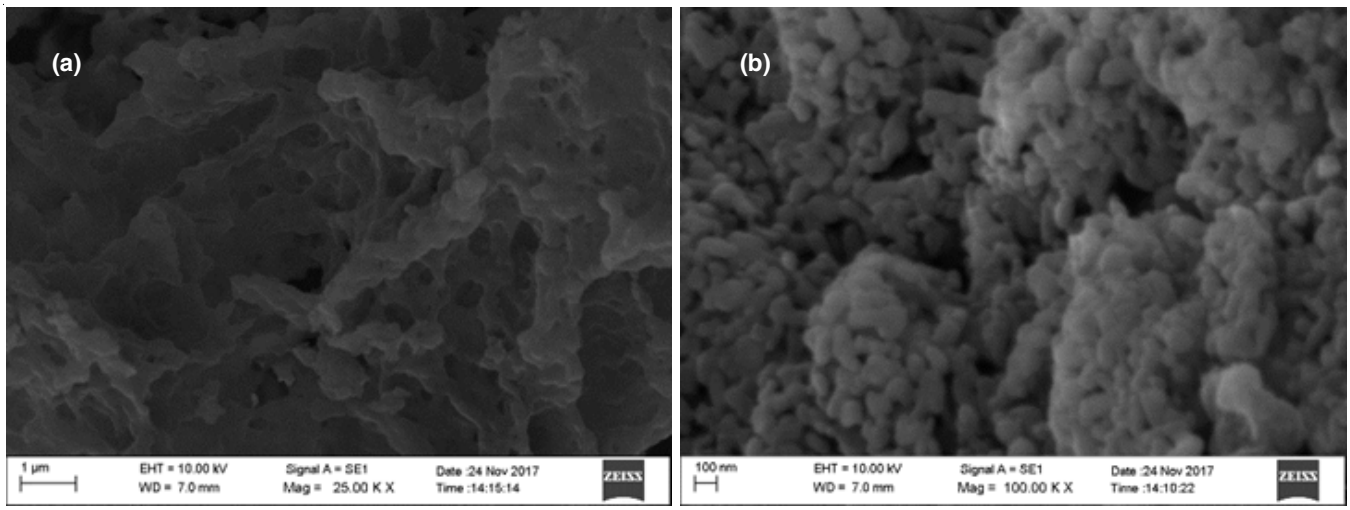

Fig. 4. Scanning electron microscopic images of (a) CS-MC (1.0 g of CS, 0.5 g of STPP) (b) LGO-CS-MC (3.0 g of LGO, 1.0 g of CS, 0.5 g of STPP)

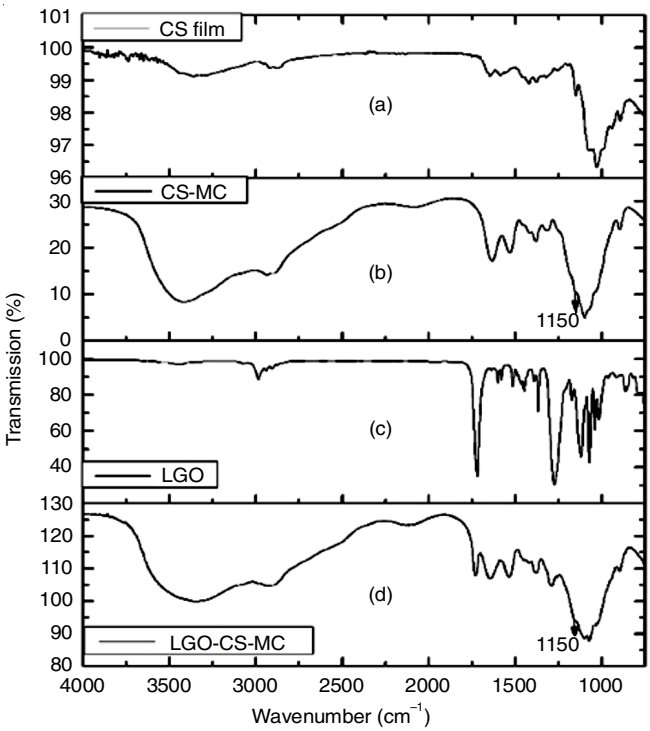

Fig. 5. FTIR spectra of LGO, CS film, CS-MC and LGO-CS-MC

TABLE-2
EFFECTS OF VARIATION OF OIL LOADING, CS CONCENTRATION AND STPP CONCENTRATION ON THE BEHAVIOR OF LGO-CS-MCs

Amount (g)			Oil content (%)	EE (%)
CS	LGO	STPP		
1.0	1.0	0.50	38.66 ± 0.46^{a}	71.88 ± 2.23^{a}
1.0	2.0	0.50	52.16 ± 0.29^{b}	88.60 ± 1.34^{b}
1.0	3.0	0.50	91.83 ± 0.11^{c}	93.85 ± 3.18^{c}
1.0	1.0	0.75	83.43 ± 0.16^{b}	74.56 ± 3.34^{b}
1.0	1.0	1.00	95.94 ± 0.26^{c}	56.67 ± 5.23^{c}
1.5	1.0	0.50	85.66 ± 0.08^{b}	75.95 ± 2.42^{b}
2.0	1.0	0.50	96.33 ± 0.05^{c}	54.51 ± 4.56^{c}

^{a,b,c}Indicate significant difference among the given values (ANOVA, Tukey's test, $p < 0.05$). Each value represents the mean ± standard variation (n = 3).

When the polymer concentration is too high, the viscosity of the medium increases. This decreases the efficiency of the dispersion force on emulsion formation. This lowers the oil content and the EE.

The oil content and EE increase with the increasing STPP concentration then both parameters decrease. The increase may be due to the formation of a more compact solid-matrix structure due to increased STPP concentration, which leads to the increased number of microcapsules formed. However, when the STPP concentration exceeds a critical value, aggregation of microcapsules occurs. This decreases the oil content and EE [20].

Effect of variation of process parameters on release profile: Fig. 6 shows the release profile of microcapsules with increasing oil loading. Increasing oil loading increases the release rate of microcapsules. As the oil loading increases amount of oil vesicles in the medium increases. Due to the scarcity of the polymer compared to the previous conditions, chitosan

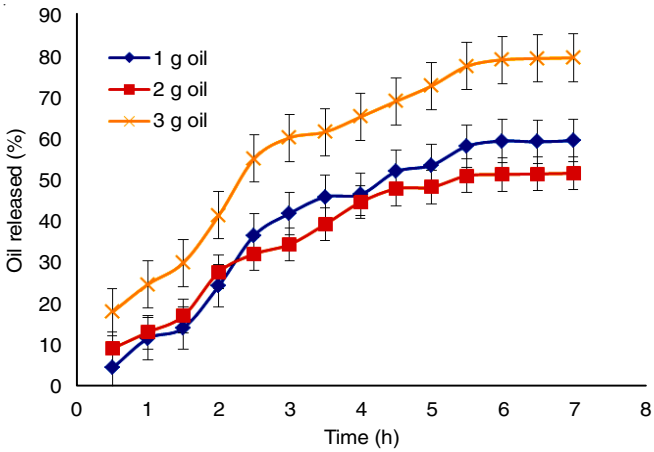

Fig. 6. Effect of variation of oil loading on release profile

polymer cover all the oil vesicles at the expense of wall thickness. This increases the release rate [15].

With the increase of polymer concentration, the release rate of microcapsules decreases. Fig. 7 displays the effect of polymer concentration on release profiles of microcapsules. Due to the abundance of chitosan in the medium with increasing polymer concentration, excess polymer remains in the medium after encapsulation of the oil vesicles. With further addition of STPP from this point onward causes the excess polymer to crosslink around the covered oil vesicles causing the thickness of the wall to increase. This causes the release rate to decrease in microcapsules [15].

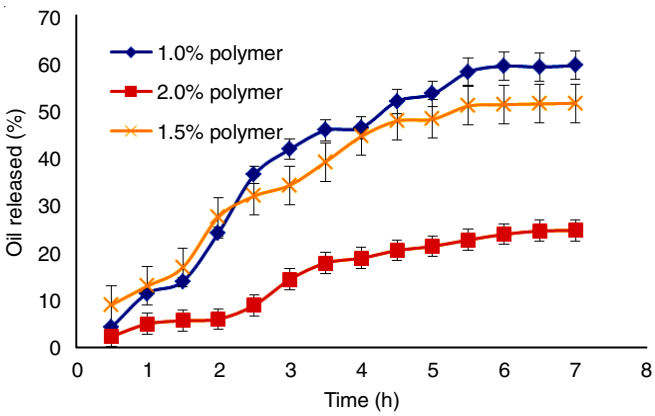

Fig. 7. Effect of variation of CS concentration on release profile

Fig. 8 shows the effects of variation of STPP concentration on release profile. With higher concentration of STPP the amount of crosslinking in the polymer matrix is high thus the wall of MC becomes compact. This lowers the diffusion rate of the oil through the microcapsule wall which in turn decreases the release rate.

Release kinetic study of LGO-CS microcapsules: The release study data of microcapsules synthesized at the optimum conditions were plotted using different kinetic models. Zero order kinetics, first order kinetics, Higuchi's model, Korsmeyer - Peppas model were used to assess the release mechanism of LGO-CS-MCs. Table-3 represents the r^2 values obtained with each model. The kinetic model with the highest correlation

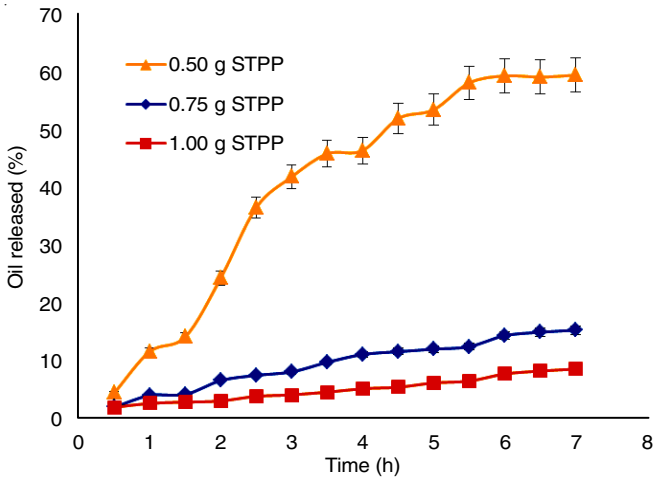

Fig. 8. Effect of variation of STPP concentration on release rate

coefficient (r^2) value is the most appropriate model, which can be used to explain the release process. The best fit with highest correlation value is the Korsmeyer-Peppas model followed by Higuchi's model, zero order kinetics and first order kinetics. A value of 0.67 was obtained for 'n' value for the microcapsules which indicate the oil release follow non-fickian or anomalous diffusion.

TABLE-3
r^2 VALUES OF VARIOUS KINETIC MODELS

Model	Zero order	First order	Higuchi's model	Korsmeyer-Peppas model
r^2 value	0.9457	0.896	0.965	0.9695

In the release study, a burst release of the oil from the microcapsules can be seen. The higher 'n' value also confirms this release to be an anomalous release. This burst release could be caused due to the geometry of the microcapsules, surface characteristics of the coating material, heterogenous distribution of the oil within the polymer matrix and properties like pore density, *etc*. [24].

Conclusion

The aim of this study was to develop LGO-CS-MCs using the ionotropic gelation method with chitosan (CS) as the coating material and STPP as the crosslinking agent. Lemongrass oil (LGO) contains citral-a (44.31%) and citral-b (34.12%) as major components in the oil. The size of LGO-CS-MCS were 100-1000 nm. Varying the process parameters had a clear impact on the size of microcapsule, encapsulation efficiency (EE) and release rate of microcapsules. The optimum formulation for the synthesis of LGO-CS-MCs is chitosan (1 g): LGO (3 g):STPP (0.5 g). Release profile follows the Krosmeyer-Peppas model with a 'n' value of 0.67. This confirms the burst release of oil from the polymer coating following a non-fickian or anomalous diffusion process.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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