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Preparation, Characterization of Eugenol loaded nanoemulsions and their Rheological characteristics

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Abstract:

Food grade nanoemulsions encapsulating lipophilic components such as essential oils have potential for utilization in beverage and food industries to improve the health benefits of food products. Eugenol (EU) nanoemulsions were prepared with variable proportion of EU to canola oil (CA), emulsified and stabilized with purity gum ultra (PGU), a newly developed succinylated waxy starch. The nanoemulsion showed decrease in droplet size with increasing EU concentration in the EU:CA mixture. However, prepared nanoemulsions (1:9 –5:5 % EU-CA) showed negligible change after one month storage. The nanoemulsion droplets showed round shape as visualized by using Scanning electron microscope (SEM). On the other hand, all prepared nanoemulsion showed shear thinning behaviour after one month storage. These results suggest the formation of stable and small droplet size EU loaded nanoemulsions using modified starch as emulsifier. The current study favours the small sized food grade nanoemulsion in different food systems because of their shear thinning behaviour. This research article provides basis of essential oil

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like Eugenol loaded nanoemulsion formulation, characterization and flow behaviour with emphasis on systems suitable for utilization within the food and pharmaceutical industry.

Key words: Nanoemulsion, Canola oil, Eugenol, Purity Gum Ultra, Scanning electron microscope.

1- Introduction:

The efficacy of phytochemical and its bioavailability can be limited by poor aqueous solubility and sometimes it causes side effects due to limited solubility[1]. Therefore, the improvement of lipophilic compounds solubility and their bioavailability remains one of the most challenging aspects [1,2,3]. In the past attempts has been done to develop novel drug delivery systems for herbal drugs, because synthetic drug based strategies are expensive, may have side effects and decreased pathogen sensitivity due to multiple uses To overcome such barriers encapsulation of plant bioactive considered being a better strategy that could minimize their presystemic metabolism and serious side effects by accumulation to non-targeted areas[4,5,6]

Nanoemulsions are commercially valuable delivery systems because they have unique characteristics of small size and high surface area, optical clarity, and reduced rate of gravitational separation and flocculation. However, droplet size of nanoemulsions may change by Ostwald ripening[7] (growth of larger droplets at the expense of small droplets). Donsi, Annunziata, Vincensi, & Ferrari, (2012)[8] overcome Ostwald ripening of carvacrol, D-Limonene and trans-cinnamaldehyde nanoemulsions by the addition of sunflower oil in lipid phase that resulted in stable nanoemulsions. Surfactants are usually small amphiphilic molecules that lower surface tension between the oil and water phase. Food grade casein and starches have also shown encapsulation properties. However, starches are neglected due to their bigger size but, recently scientists have

developed modified starches that have low molecular and greater surface activity. For example, Purity Gum Ultra (PGU) produced stable, small droplets (254 nm) of orange oil in water emulsions at low surfactant ratio i.e., 1% wt/wt while with gum Arabic the droplet diameter was (497 nm) at high surfactant ratio 5% wt/wt.[9] The objective of our study was to use PGU for the preparation of eugenol loaded food grade nanoemulsion, their characterization and rheological characteristics.

2- Materials and Methods:

2.1- Materials:

Eugenol (EU) was purchased from Jishui County Man Herbal Medicinal Oil Refinery Co., Ltd. (Jiangxi, China). Canola oil (CA) purchased from a local market and used without further purification. Purity Gum Ultra (PGU), a succinylated waxy maize starch, was purchased from National Starch (Bridgewater, NJ, USA). All other chemical were of analytical grade and purchased from sigma (St. Louis, MO, USA).

2.2- Preparation of Emulsions

An aqueous solution of PGU 2% (w/w) was prepared by dispersing the dried powder in deionized water at room temperature and stirred overnight to enhance hydration of the starch prior to homogenization. The mixture of EU:CA at ratios of 1:9, 3:7 and 5:5 (10% v/v) were used as core materials. Oil mixture and aqueous starch phases were premixed with a high-speed homogenizer (Ultra-Turrax T25 IKA Janke and Kunkle, GmbH and CO KG, Germany) at 13,500 rpm for 2 min at room temperature. These coarse emulsions were finely dispersed with a high pressure homogenizer (IKA-Labor Pilot 2000/4, IKA-Werke GmbH and Co. Staufen, Germany.) at pressures 150 MPa for 5 processing cycles. During the process a heating exchanger was used to control the inlet, operational, and outlet temperatures at 15 °C.

2.3- Particle Size Measurements

Emulsion particle size were measured by dynamic light scattering and phase analysis light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, U.K.), respectively, using 1 mL emulsion samples diluted 100x with deionized water to avoid multiple light scattering effects. Measurements were performed after 1, 15, and 30 days of storage. For dynamic light scattering, the particle size data are reported as Z-average mean diameter and polydispersity index (PDI).

2.4- Scanning electron microscopy of nanoemulsion (SEM)

Scanning electron microscopy (SEM) Morphology of films was analyzed by a scanning electron microscope (SEM, S-4800, Hitachi, Japan). Samples were attached to double-sided adhesive tape and then mounted on the specimen holder. The samples were sputter coated with 10 μm thickness of gold under vacuum and scanned with an accelerating beam voltage of 1 kV.

2.5- Rheological Measurements.

Rheological measurements of CO nanoemulsions were performed at 25 °C using Physica modular compact rheometer (MCR 301, Ashland, USA) with a cone and plate geometry (cone diameter = 50 mm, angle = 1°, gap = 0.1 mm). For each measurement, 1 mL of the emulsion sample was loaded on the rheometer. The viscosity of nanoemulsions was measured by a steady state flow program with the shear rate ranging from 100 to 1000 s−1 during 5 min. Experimental flow curves were fitted to a power law model

$$\eta = \gamma - K\, n\ 1 \quad (1)$$

where η was the viscosity (Pa·s), γ was the shear rate (s−1), K was the consistency index (Pa·sn), and n was the index that

provided information about the flow behavior related to the effect of shear rate. There exist three value ranges for n: $n < 1$ for a shear-thinning fluid, $n = 1$ for a Newtonian fluid, and $n > 1$ for a shear-thickening fluid.

3- Results and Discussions:

In order to prepare eugenol loaded nanoemulsions it was necessary to blend it with oil of high density like LCT (Long chain triglyceride) to prevent the phenomenon of Ostwald ripening that cause's emulsion instability. Therefore, in current study we mixed EU with CA (LCT) as Ostwald ripening inhibitor for long term stable nanoemulsion as shown in Table: 1. (Terjung et al., 2012)[10] also prepared eugenol loaded nanoemulsion by adding medium chain glyceride as ripening inhibitor to attain emulsions which were stable for one month. It was observed that droplet size decreased with increasing EU concentration in the emulsion mixture as shown in Table: 1. These results suggest that EU acted as co-surfactant and lowered droplet size significantly. The droplet size reduced from 205 – 155 nm and polydispersity index (PDI) changed from 0.001 – 0.15. On the other hand, addition of EU was limited and upto 5:5 % mixture of EU-CA was possible to make nanoemulsions (Figure 1). After that the nanoemulsion formed showed separation of layers and particle size grew larger as shown in Table: 1. Therefore, the loading of emulsion with EU is limited by the amount and solubility properties of the other lipid phase.

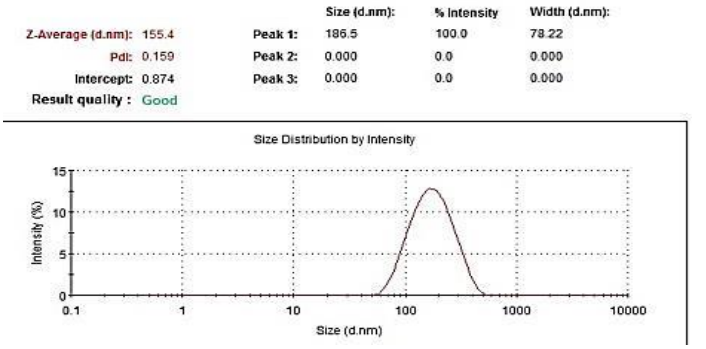
Table 1: Emulsion formulations, corresponding particle sizes and polydispersity index (PDI) of nanoemulsions prepared using 2% wt/wt PGU, 150 MPa pressure and 5 processing cycles.

Emulsion Formulation (V/V %)	**EU: CA**	
	Particle Size(nm)	**PDI**
1:9 %	205.27 ±0.188	0.21±0.001

3:7 %	196.43±1.887	0.20±0.006
5:5 %	155.40±1.766	0.15±0.15
7:3 %	248.53±0.377	0.32±0.001

CA: canola oil, EU: Eugenol

Figure 1: showing particle size distribution of 5:5 % EU-CA nanoemulsion



Storage stability of these 1:9 to 5:5 % EU-CA nanoemulsions showed quite consistent droplet size for one month. After 30 days of storage the nanoemulsions showed only 20 – 30 nm rises in particle diameter (Data not shown). These results were in accordance to the findings of (Liang et al., 2012)[11] that prepared peppermint oil loaded nanoemulsion using modified starches and were stable for a period of one month. After storage stability test we investigate the morphology of small sized EU-CA nanoemulsion (5:5 % EU-CA) using scanning electron microscope.

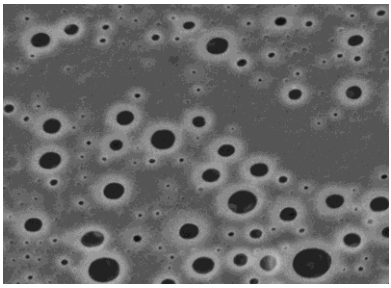


Figure 2: Scanning electron microscope image of 5:5 % EU-CA nanoemulsions.

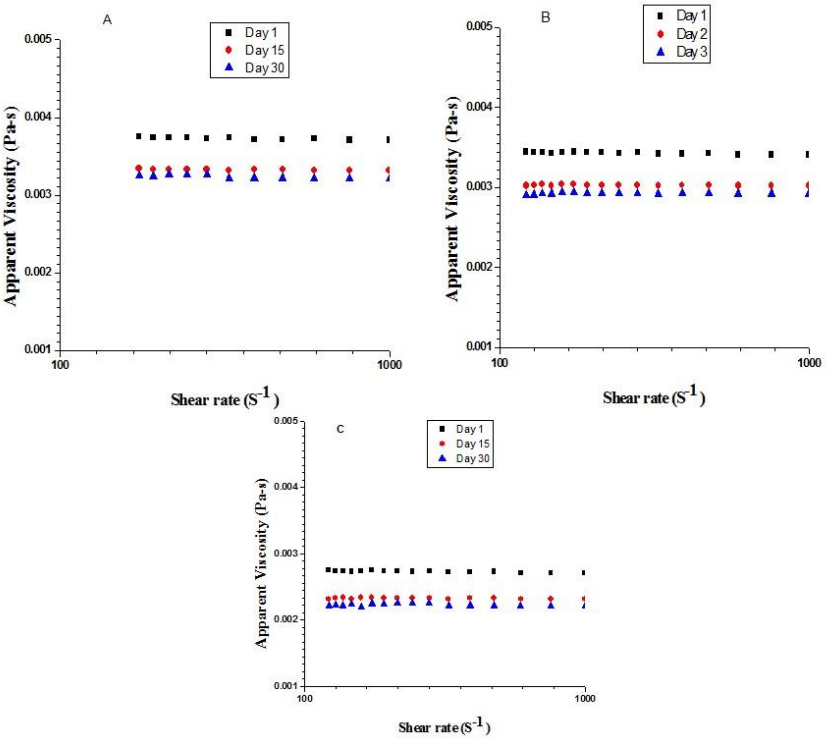


Figure: 3 Effects of storage time on the rheological properties of nanoemulsions containing variable ratios (A) 1:9, (B) 3:7 & (C) 5:5 % v/v EU:CA at room temperature.

To evaluate the stability of EU nanoemulsions rheological properties were also investigated. The viscosities of EU nanoemulsions based on shear rates have been shown in Fig: 3. Shear-thinning property is a frequent phenomenon that could be observed, when emulsion based delivery systems used in foods and beverages. It was fairly excellent for lowering the viscosity under flow during consumption (Liang et al)[11]. The nanoemulsions showed different degrees of shear-thinning behaviour. The square of the correlation index (R^2) was > 0.98, recommended the suitability of power law used to interpret the rheological properties of nanoemulsions. In all emulsion formulations K value decreased as observed in viscosity curves

in Figure 3. However, flow behaviours (n) increased for all nanoemulsion formulations, suggesting the shear-thinning nature of nanoemulsions (Table 3). In case of 5:5 EU:CA nanoemulsion, n value changed from 0.69 to 0.785 after one month storage. This trend of viscosity change favoured the Newtonian nature of nanoemulsions during storage and these findings are in accordance with other studies[27]. The emulsion formulations showed loss of shear-thinning and viscosities during storage and were in agreement to the findings of Liang et al.,[11] who observed similar phenomenon due to increase in particle size of nanoemulsions. The current emulsion formulations showed extended stability (30 days) with no visual coalescence or creaming phenomenon.

Table: 3 Rheological parameters using power law model for CO nanoemulsions prepared with different CO:CA concentrations.

EU:CA (v/v %)	Storage time (Days)	K (Pa-s)	N	R^2
1:9	1	0.003751	0.629	0.9973
	15	0.003322	0.779	0.9924
	30	0.003221	0.781	0.9827
3:7	1	0.003452	0.649	0.9982
	15	0.003021	0.770	0.9962
	30	0.002901	0.776	0.9934
5:5	1	0.002759	0.690	0.9855
	15	0.002321	0.783	0.9803
	30	0.002222	0.785	0.9999

4- Conclusions:

In conclusion, stable Eugenol loaded nanoemulsions were prepared using EU and CA oil mixture. These nanoemulsions

remained stable against coalescence and phase separation for an extended storage period (one month). The morphology characterization of these small sized nanoemulsions using scanning electron microscope suggests the round shape of particles. However, rheological data suggest smaller the droplet size greater will be the shear thinning. These findings can lead to more rational design of nanoemulsion based delivery systems for essential oils and the desired function of their constituents in food and pharmaceutical products.

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