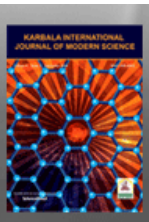

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In-vitro evaluation of high dosage of curcumin encapsulation in palm-oil-in-water, nanoemulsion stabilized with a sonochemical approach

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Keywords

Curcumin Nanoemulsion, Ultrasound, Particle size, Encapsulation efficiency, In-vitro

RESEARCH PAPER

In-vitro Evaluation of High Dosage of Curcumin Encapsulation in Palm-oil-in-water, Nanoemulsion Stabilized with a Sonochemical Approach

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Abstract

Curcumin is unstable under different environmental conditions. To increase the bioavailability and stability of curcumin, it is proposed in the present study to encapsulate it in palm oil-in-water nanoemulsions using ultrasound. The present work deals with maximum curcumin encapsulation into oil forms stable nanoemulsion using a food-grade surfactant and examines its antioxidant assay and in-vitro study. The synthesized curcumin nanoemulsion (CuNE) shows particle size in the range of 14.7 ± 3 nm, 95 ± 0.5% encapsulation efficiency, and provides stability against different environmental parameters. Furthermore, in-vitro analysis of high dosage CuNE shows more than 80−90% retention during simulated gastric and intestinal conditions.

Keywords: Curcumin nanoemulsion, Ultrasound, Particle size, Encapsulation efficiency, Antioxidant activity

1. Introduction

Curcumin is a low molecular weight polyphenol bioactive compound extracted from the curcumin longa tree, primarily found in South and East Asian countries. Curcumin shows good inflammatory, anticancer, antioxidant, antimicrobial, wound healing, and many other unrevealed drug properties, useful in the pharmaceutical and food industry [1−8]. In modern days, mainly curcumin is used in the preparations of drugs. Some researchers mention that the curcumin has a potential application in functional food and chemoprevention treatment [4−7]. However, due to curcumin's low bioavailability and solubility in water, it has limited use in the pharmaceutical and food industry. Also, curcumin was found to be unstable due to various processing parameters such as heat, pH, and ionic strength [9]. The curcumin compound's self-oxidation process occurs due to various forms of by-products, such as acids and pentadione at physiological pH [10]. Furthermore, the direct addition of curcumin into functional foods may hamper its flavor, color, and texture compared to encapsulation form [11].

Several attempts have been made in the past to improve the bioavailability and biosolubility of curcumin in the form of a metal complex [12], liposomal curcumin [13], phospholipid complex [14], and nanoparticles [7,8]. However, under varying processing conditions, these curcumin delivery forms are unstable emulsions with lower encapsulation effectiveness and antioxidant activity. The encapsulation strategy can be used to get around these limitations. An emulsion is a great approach for encapsulation technology in the food industry [9,12,13]. In today's world, nanosized emulsions have a wide range of applications, including food preservatives, sports drinks, cosmetics, and pharmaceutical formulations [14−16]. Nanoemulsion-based encapsulation is highly resistant to aggregation and separation, and allows for controlled delivery of bioactive compounds [2,9,12,17−19]. Among all

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approaches, the synthesis of nanoemulsions with the aid of sonoprocess is becoming popular in last few decades. Ultrasound is one of sonoprocess technique that has gained attention due to its low operation cost, low energy requirement, ease of operation, and improved control over its formulation variables [20,21]. The phenomenon of ultrasound consists of formation and breakdown of vapor cavities in the aqueous media. The acoustic field is created by ultrasonic waves passing through the medium, resulting in a fine dispersion of the two immiscible liquids. Localized turbulence, which breaks up primary droplets into nanoscale size, can be attributed to the fine dispersion of two phases [1,20,22,23]. Many researchers have recently focused on bioactive compound-based nanoemulsions such as resveratrol, limonene, and tocopherols [24], β-carotene [25], phytosterols and lycopene [20,21], itraconazole [22], thalidomide [23]. Also, researchers using high-energy technologies have reported curcumin encapsulation in the form of nanoemulsion (concentration 10–80 mg/mL of oil used) [4,6,7,24–28].

In the present study, we have investigated the higher loading of curcumin (40–150 mg/mL of oil) into O/W nanoemulsion and their stability in terms of particle size and zeta potential values as a function of processing parameters such as temperature, pH, and salt concentration. The production of the curcumin nanoemulsion (CuNE) was carried out using ultrasound approach in the presence of stabilized materials such soy lecithin or lecithin and Tween 80. For the synthesis of nanoemulsion, the ultrasound processing parameters were also optimized. Particle size analysis and optical microscopic image analysis were used to characterize the nanoemulsions. The effect of pH, ionic strength, and heat treatment on particle size and stability of nanoemulsion demonstrates improved stability, as well as high encapsulation efficiency and anti-oxidant properties of the nanoemulsion. An in-vitro nanoemulsion analysis was also performed.

2. Material and methods

2.1. Materials

Curcumin, palm oil, soy lecithin, and Tween 80 were procured from the local market of Chelyabinsk city, Russian federation. Distilled water was used throughout the experiments.

2.2. Preparation methods of curcumin

Initially, as reported by Ahmad et al. [2], To prepare a lipid phase of an emulsion, 40–150 mg of curcumin powder were dissolved in palm oil at 50 °C with continuous mechanical agitation at 400 rpm for 5 min. Stirring was continued until the majority of the curcumin gets dissolved in the oil. Later, the aqueous phase was created by dissolving two different emulsifiers in distilled water, namely soy lecithin or Tween 80. An ultrasound (Volna UZTA 063/22 OM, Biysk) probe reactor with a power of 400 W of frequency at 22 ± 1.65 kHz was used in all the experiments. High intensity (80% amplitude of total power) sonication was used to mix and emulsify both the aqueous and oil phases. The temperature was monitored with a digital thermometer during the emulsification process and kept below 50 °C. To reduce the risk of overheating during sonication, the total sonication time was divided into four cycles of three minutes each, with jacketed cool water surrounding the reactor.

2.3. Characterization of CuNE samples

Nanoemulsion stability was measured according to the method described by Sari et al., [24]. The emulsions were placed in a hot water bath at 80 °C for 30 min before being transferred to an ice bath for 15 min before centrifugation (Hettich Zentrifugen, Mikro 22R) at 5000 rpm for 30 min.

2.3.1. Determination of pH, particle size distribution, PDI and zeta potential of nanoemulsion samples

An electrochemically assembled pH meter was used to measure the pH of the prepared emulsions. Nanotrac FLEX (St. Petersburg, Russia) particle size analyzer was used to evaluate the particle size distribution through the DLS method. In the particle size analysis, the emulsions were diluted with distilled water (1:100) for homogenous particle suspension. Particle size distribution was obtained by mounting the suspension in Nanotrac external probe where, light passing from the sample is used to measure the particle size distribution. All measurements were repeated three times.

2.3.2. Encapsulation efficiency of CuNE samples

Encapsulation efficiency is frequently used to describe the encapsulation of bioactive compounds. Encapsulation efficiency was calculated using the method reported by Surassmo et al. [29], with some modification. 15 mL of prepared nanoemulsion was passed through filter membrane cap and centrifuged at 5000 rpm and 5 °C for 30 min. After centrifuge permeate was collected and measured the UV (Shimadzu UV-2700, Japan) absorbance at 520 nm wavelength. All measurements were done in triplets.

$$Encapsulation\ Efficiency\ (\%) = \frac{Total\ phenolic\ content - phenolic\ content\ after\ certrifuge}{Total\ phenolic\ content} \times 100$$

2.3.3. Effect of ultrasound parameter on CuNE samples

For the ultrasound process parameter optimization, the samples were prepared at an applied power of 80% (400 W), under different sonication times ranging from 3 to 12 min. To understand encapsulation of curcumin in the emulsion was measured as a function of sonication time in particle size and optical microscope (All measurements done in triplets). Optical microscope images at 70× magnification were taken at different time intervals.

2.3.4. Antioxidant activity in terms of DPPH assay

In order to investigate the oxidation inhibition capacity of an emulsion, a 0.1 mM solution of 2,2, diphenyl -picryhydrazul (DPPH) was prepared [30]. To prepare the samples for the antioxidant UV absorbance study, 20 μL of nanoemulsion sample was added to 280 μL of DPPH radical solution. These samples were incubated in the dark for 30 min before being measured for UV absorbance at 520 nm. The controls were prepared with the same procedure but without nanoemulsion. Using a UV-spectrophotometer (Shimadzu UV-2700, Japan), the sample at 517 nm absorbance was measured against the blank sample.

$$\text{DPPH scavenging activity } (\%) = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

2.3.5. Effect of processing parameter on CuNE stability

The influence of pH, ionic strength, and temperature on chemical and physical stability of curcumin nanoemulsion was investigated. To examine the effect of temperature on nanoemulsion stability; 10 mL of nanoemulsion was transferred into glass test tubes and subjected to different temperatures,

Table 1. Formulation for nanoemulsion preparation.

Experiment No	Lecithin (gm)	Tween80 (gm)	Curcumin concentration (gm)	Stability
CuNE 1	2	–	0.04	Unstable
CuNE 2	2	–	0.04	Unstable
CuNE 3	4	–	0.15	Stable
CuNE 4	2	–	0.15	Unstable
CuNE 5	4	–	0.04	Stable
CuNE 6	–	5	0.04	Stable
CuNE 7	–	2	0.15	Unstable
CuNE 8	–	5	0.15	Stable
CuNE 9	4	–	0.15	Unstable

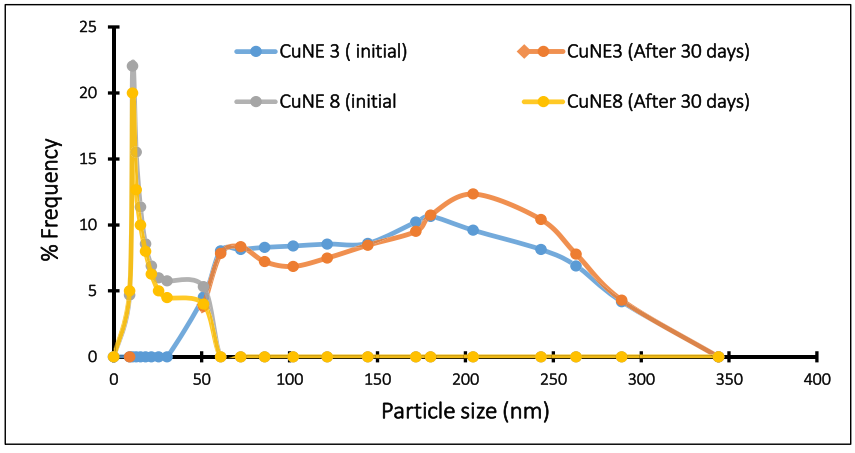

Fig. 1. Particle size distribution of stable nanoemulsions.

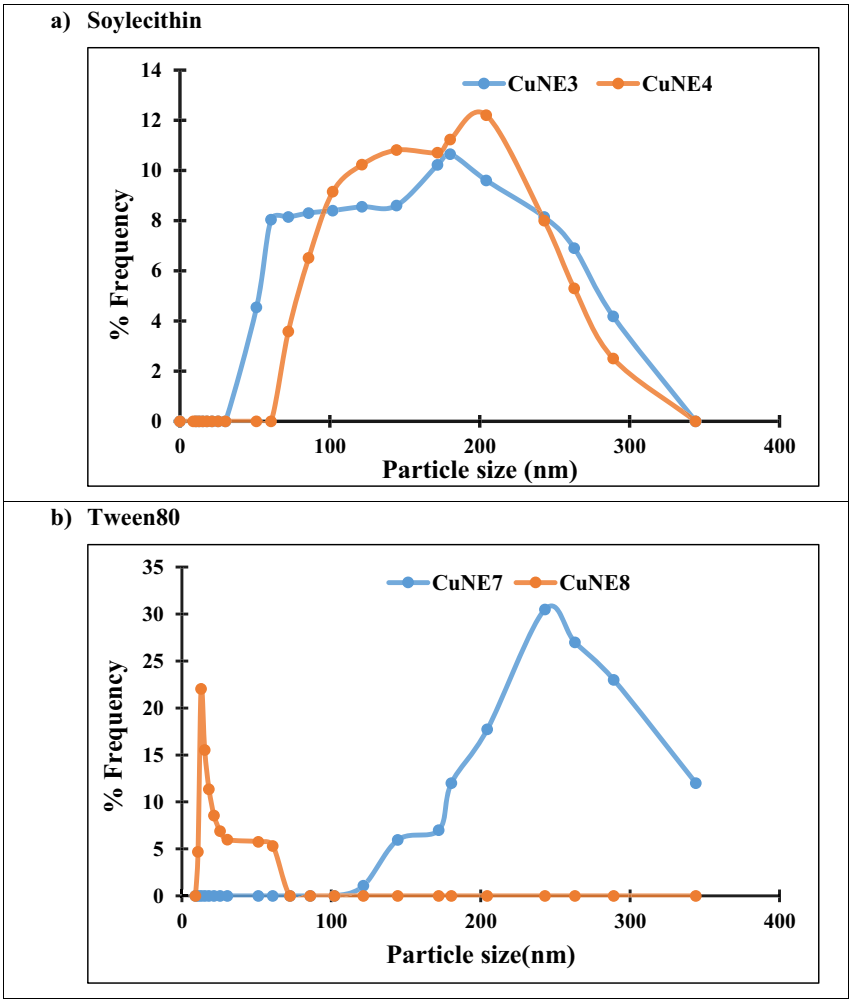

Fig. 2. Effect of surfactant and their concentration on particle size distribution of curcumin nanoemulsion.

including pasteurization (63 °C for 30 min), boiling (85 °C for 10 min). For ionic strength, nanoemulsions with varying ionic strengths were created by adding salt concentrations ranging from 0.1 to 1.0 M at pH 7.0. Dissolved the salt nanoemulsions were stirred for 30 min. To observe the effect of pH (3, 5 and 7), sample were added to different pH. All treated samples were subjected to stability, particle size, and zeta potential analysis.

2.3.6. In-vitro analysis of curcumin nanoemulsions

Potential bioavailability and bioactivity were evaluated according to the method described by Kumar et al., [31]. The following two phases were formed in order to model the in-vitro digestion process. In the first phase, i.e., the stomach phase, a 3:1 ratio (CuNE: simulated gastric fluid (SGF)) was used. SGF with 125 nM NaCl, 7 mM KCl, 45 mM NaHCO, and 0.32% pepsin, pH 1.5. The following mixture was shaken in a water bath for 2 h at 37 °C. To disable the enzyme, samples were removed every 30 min. Further, the second phase, the small intestine phase, i.e., simulated intestinal fluid (SIF) containing 4 mg/mL pancreatin and 25 mg/mL bile salts at pH 6.0, was then mixed to SGF incubated at 37 °C for 3 h for intestinal digestion. Every 30 min sample is taken to measure the absorbance for release of curcumin using the calibration curve obtained from section 2.3.2.

Table 2. Effect of surfactant concentration on nanoemulsion in terms of particle size distribution and PDI, Zeta potential.

Experiment number	Particle size (nm)	PDI	Zeta potential (mV)
CuNE 3	180 ± 05	0.387	−70
CuNE 4	204.4	0.56	−69.5
CuNE 5	171.5 ± 0	0.309	−70.5
CuNE 6	144.4 ± 05	0.329	−70.2
CuNE 7	243.3	0.43	−15
CuNE 8	12.77 ± 0.3	0.15	−25

Table 3. Encapsulation efficiency and Antioxidant activity of nanoemulsion samples.

Sample	Total phenolic content (μg GAE/mL)	Total phenolic content after centrifuge	Encapsulation efficiency (%)	DPPH assay (%)
CuNE 3	2390 ± 0.3	501.9 ± 0.2	79	108
CuNE 8	2392 ± 0.3	117.3	95	125

2.4. Statistical analysis of CuNE

The data is expressed as mean ± standard error of the mean (SEM) for each group. Statistical analysis was done using GraphPad Prism version 8.0 software (GraphPad software, 2019). Significance of difference $p \leq 0.05$.

3. Results

Increased consumer awareness of the nutritional and healthy food intake has resulted in most dietary and food industries developing novel products. The study mainly focuses on enhancing the solubility of products and protecting curcumin from environmental degradation. The inner oil phase of the current o/w nanoemulsion system was prepared by incorporating different concentrations of curcumin ranging from 0.04 to 0.15 g in edible palm oil, as shown in Table 1. The sample was dissolved in ethanol and centrifuged for 15 min at 3000 rpm to determine the curcumin loading %. It is collecting the sample for UV absorbance to determine the curcumin loading %. 0.03 to 0.011 g of curcumin is found to be solubilized in oil and thus available for encapsulation.

3.1. Physical characterization of curcumin nanoemulsion

We formulate nine different CuNE and their stability results in the data shown in Table 1. Palm oil is a medium chain triglyceride (MCT) compound with lower water solubility than short chain triglycerides (SCT). It has a polydispersity index (PDI) of 0.3–0.357. During a 72-h storage period, nanoemulsions prepared with modified starch and low surfactant concentration form a trans-parent creamy layer on the upper portion of an emulsion. Fig. 1 shows that particle size remains constant from the first day to the last 30 days of storage for CuNE3 and CuNE8 sample, indicating no aggregation of emulsion particles; thus, emulsions can be considered stable (No significant of $p < 0.05$). As shown in Table 1, nanoemulsions 3, 5, 6, and 8 were more stable without aggregation and settling than the other nanoemulsion samples. Table 1 shows the value of stable nanoemulsions with stability and pH. At room temperature, the gravitational stability of nanoemulsions against droplet aggregation was observed. In practice, a stable emulsion is formed when the particle size is less than 200 nm and the polydispersity is less than 0.4. Furthermore, lower oil concentration and high water solubility provide resistance to Ostwald ripening. CuNE3 particle size ranges from 180 to 204 nm (within a 12% variation) and CuNE8 particle size ranges from 12.77 to 13 nm (within a 2% variation) as shown in Fig. 1 and so consider this sample for further analysis.

3.2. Effect of surfactants and their concentrations on nanoemulsion stability

A surfactant's ability to form and stabilize oil droplets in a nanoemulsion is determined by its

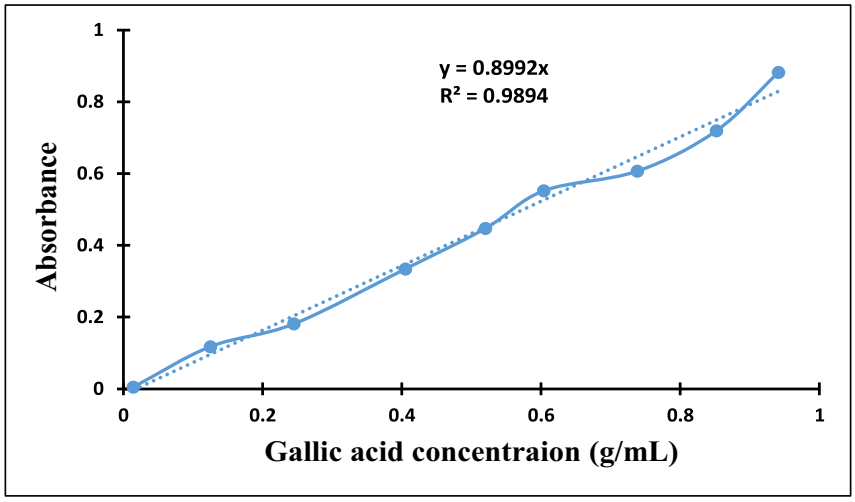

Fig. 3. Standard calibration curve for calculation phenolic content.

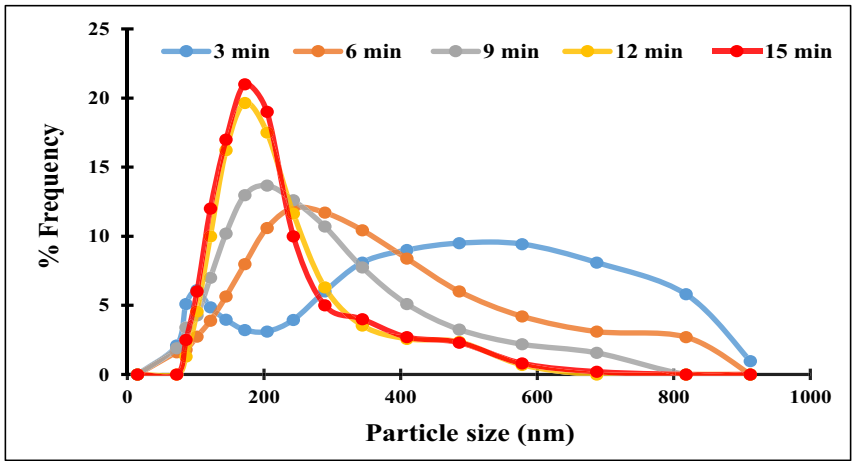

Fig. 4. Effect of ultrasound sonication time on curcumin nanoemulsion with respect to particle size distribution.

molecular structure and physicochemical properties. A graph of surfactant concentration versus particle size distribution was used to determine the effect of surfactant concentration on emulsion particle size (Fig. 2). It was discovered that increasing the surfactant concentration aided in particle diameter reduction by repelling the forces that prevent particle aggregation and mixing up to a certain threshold value, after which the decrease in particle size was limited due to low dilution effect and formation pressure during mixing. Droplet size reduction raise the interfacial tension between oil and water molecules, making further oil droplet breakup difficult. The particle size ranged from 180 to 204 nm for CuNE3 samples, while the particle size ranged from 12.77 nm to 243.4 nm CuNE8 samples, indicating an undesirable particle size distribution. The observation in Fig. 2 revealed that the type of surfactant and its concentration determine the particle size of a nanoemulsion. A low mass emulsifier lecithin surfactant that forms a fast coating on the surface of an oil droplet is effective during the emulsification process.

An oil droplet surfactant is adsorbed during the high and low-pressure cycles of ultrasonic mixing.

Furthermore, the structure of lecithin contains opposing phosphate groups, which creates a repulsive electrostatic force between the emulsion molecules and promotes stability against coalescence and effects on PDI, as shown in Table 2. Tween 80 is a nonionic surfactant with a low molecular weight that is made up of polyethoxylated sorbitan and oleic acid. It has a hydrophilic group of polyoxyethylene and a lipophilic group of oleic acid, which

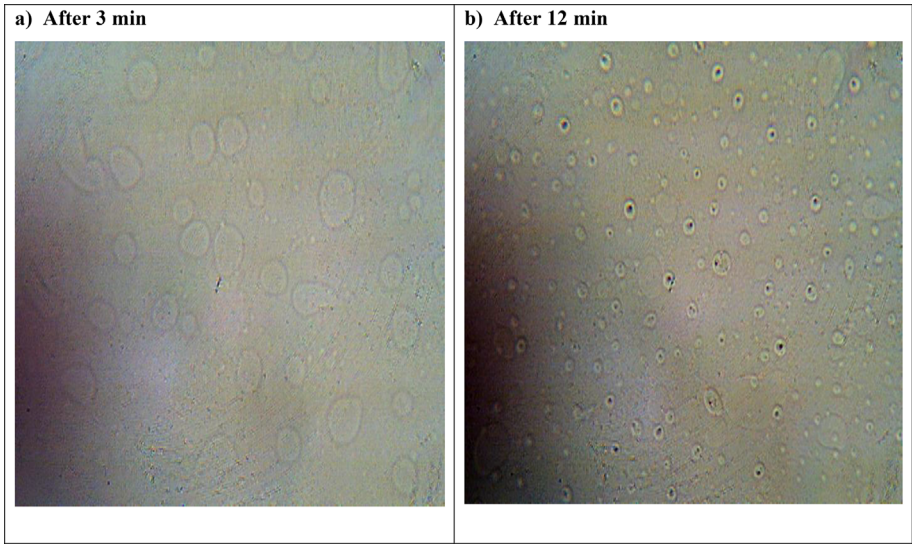

Fig. 5. Oil droplet growth of curcumin nanoemulsion samples as a function of sonication time after a) 3 b) 12 min.

Table 4. Effect of processing condition on nanoemulsion samples.

Processing conditions	Sample details	Particle size (nm)		Zeta potential (mV)	
		CuNE 3	CuNE 8	CuNE 3	CuNE 8
	Control CuNE	180.4	13.5	−70	−25
Effect of heat treatment	63 °C for 30 min	204.4	18	−63.7	−22
	85 °C for 10 min	24.3	25.5	−59	−20
Effect of pH	pH 3	244	15.19	−15.1	0.85
	pH 5	171.9	12.77	−70	−25
	pH 7	204	16	−62.6	−23
Effect of ionic strength	M 0.1	173	13	−56 to −65	12 to −23
	M 5	185	15		
	M 1	185	15.77		

gives it the remarkable ability to form a stable nanoemulsion even at low concentrations. Table 2 shows that curcumin nanoemulsion containing Tween80 has a PDI in the range of 0.15–0.1 and single narrow distributions regardless of concentration [30].

The zeta potential of nanoemulsion particles is the potential surface charge that provides stability against multiphase reactions under repulsive force between particles. Table 2 shows that all nanoemulsion samples have zeta potentials less than −30 mV. Regardless of concentration, the lecithin nanoemulsion sample has the lowest zeta potential value. The main reason for this is that lecithin, which has a pH of around 6.5, contains various types of negative surface charge phospholipids. These negative phosphate groups subsidize the droplet's surface charge, causing the zeta potential to become more negative. Tween80, on the other hand, is made up of non-ionic surfactants with free fatty acid and oil groups, which causes a decrease in the zeta potential value of samples. In addition, Tween 80 free

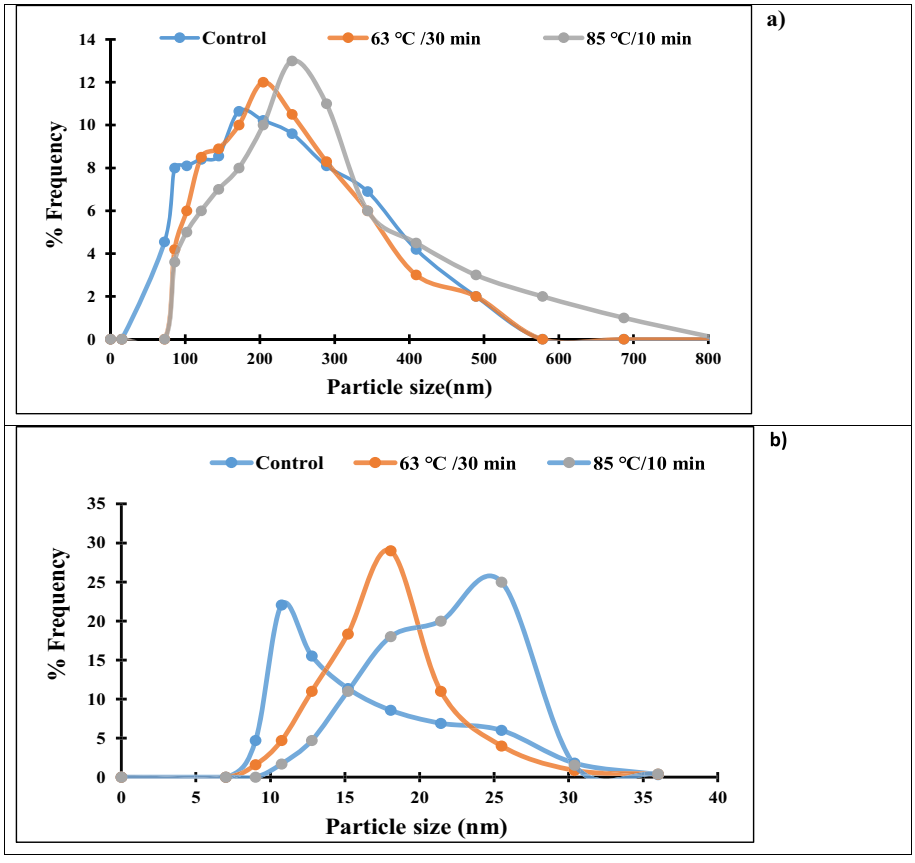

Fig. 6. Effect of heat treatment on nanoemulsion stability with respect to particle size for a) CuNE 3 and b) CuNE 8.

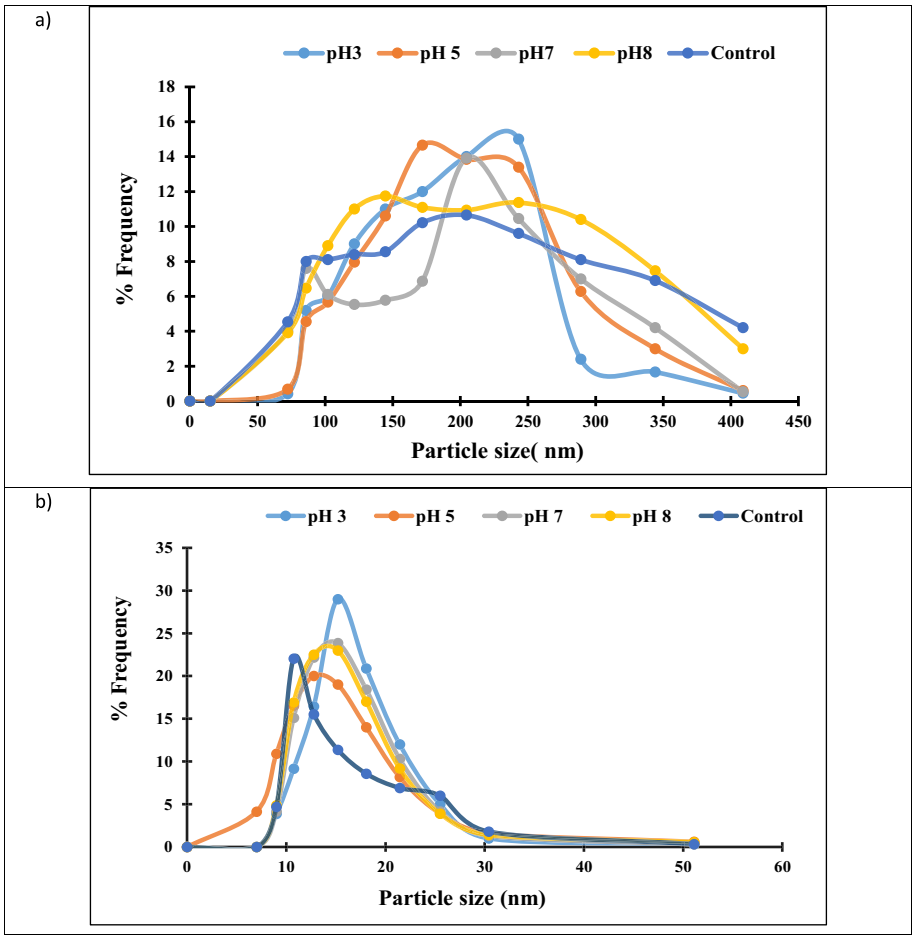

Fig. 7. Effect of pH on nanoemulsion stability with respect to particle size a) CuNE3 and b) CuNE8.

hydroxyl groups adsorb and orient on the surface of oil droplets to decrease their charge. Tween 80 also contains a lot of hydrophilic polyoxyethylenes, which gives it a repulsive force action on droplets. As shown in Table 2, there is a significant difference in zeta potential value between the two surfactant based nanoemulsions [27].

Only CuNE3 and CuNE8, which are based on lecithin and Tween 80, exhibit stable nanoemulsions against gravitational force (Maximum loading curcumin 0.15 g). As a result, these CuNE were chosen for further investigation into encapsulation efficiency, sonication parameters, processing parameters, and antioxidant activity on nanoemulsion.

3.3. Encapsulation efficiency CuNE samples

The total phenolic content of the nanoemulsion was used to calculate encapsulation efficiency. The Folin-Ciocalteus reagent was used to determine the total phenolic content of a stable nanoemulsion. The total phenolic content was determined before and after 30 min of centrifugation at 5000 rpm (Table 3). The percentage oxidation inhibition and encapsulation efficiency of the stable emulsion CuNE3, and CuNE8 were determined.

In this method, by standard calibration curve of gallic acid at different concentrations with R^2 value 0.9894 as shown in Fig. 3.

CuNE 8 has a higher encapsulation efficiency than lecithin, as shown in Table 3. Because of the low surface tension between oil droplets, flocculation and reaggregation are avoided, improving curcumin solubility. As seen in the optimization of sonication time, polydispersity provides information about the homogeneity of the distribution of sizes was low (0.3) for all samples, indicating the formation of monodisperse systems. According to this system, sonication would improve encapsulation efficiency. If the concentration of Tween 80 increases curcumin stability, then it also increases the reflects in encapsulation efficiency [31].

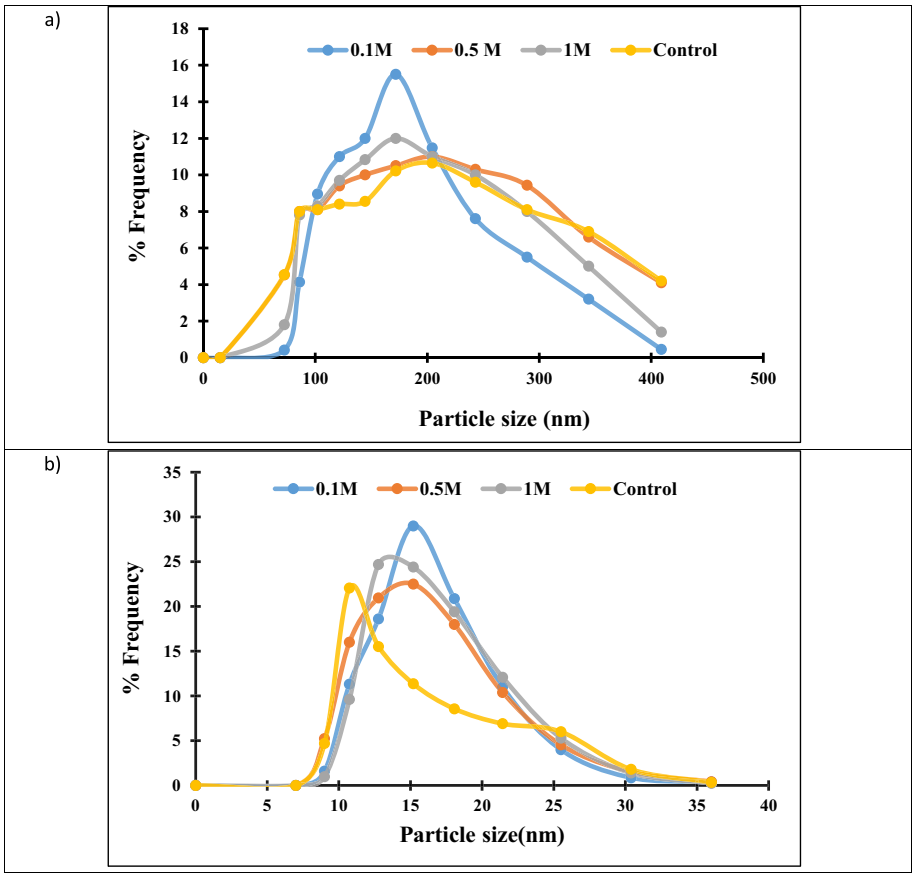

Fig. 8. Effect of ionic strength on nanoemulsion stability with respect to particle size a) CuNE 3 and b) CuNE 8.

3.4. Kinetics of nanoemulsion concerning sonication time and their effect on particle size and optical image

Because of the shearing effect of ultrasound, sonication aids in the appropriate mixing of all emulsion components by decreasing large oil droplets into smaller droplets. Ultrasound also contributes to the formed emulsion controlled stability. Furthermore, as previously discussed, different types and concentrations of emulsifiers govern the optimization of a stable oil droplet. Optimizing the power of ultrasound probes is also important for reducing energy loss and production costs. Taking this into account, in the current study, 80% of the applied power (400 W) was used for nanoemulsion preparation. To investigate the effect of sonication time on particle size distribution, particle size analysis of a nanoemulsion was performed at various processing times ranging from 3 to 15 min with a step size of 3 min (Fig. 4). The analysis was precise in determining that as sonication time increases, the narrow particle size distribution in the range. Sonication time has helped to ensure that the oil phase is dispersed uniformly in an aqueous phase. Furthermore, the particle size distribution curve for samples treated for 12 and 15 min overlaps, indicating that 12 min is the optimal sonication time. The optical microscopic images were used to examine the effect of sonication time on the morphological structure of an emulsion. According to Fig. 5, sonication aids in the disruption of droplet diameter and is associated with an increase in sonication time encapsulation of curcumin in nanoemulsion [24].

3.5. Effect of processing conditions on nanoemulsion stability

3.5.1. Effect of heating or temperature on nanoemulsion stability

In general, it has been observed that most food ingredients deteriorate at higher temperatures. CuNE3 and CuNE8 stable nanoemulsions were considered for testing different process conditions such as temperature, ionic strength, and pH. Table 4 provides a summary of the effects of all processing conditions for easier comparison.

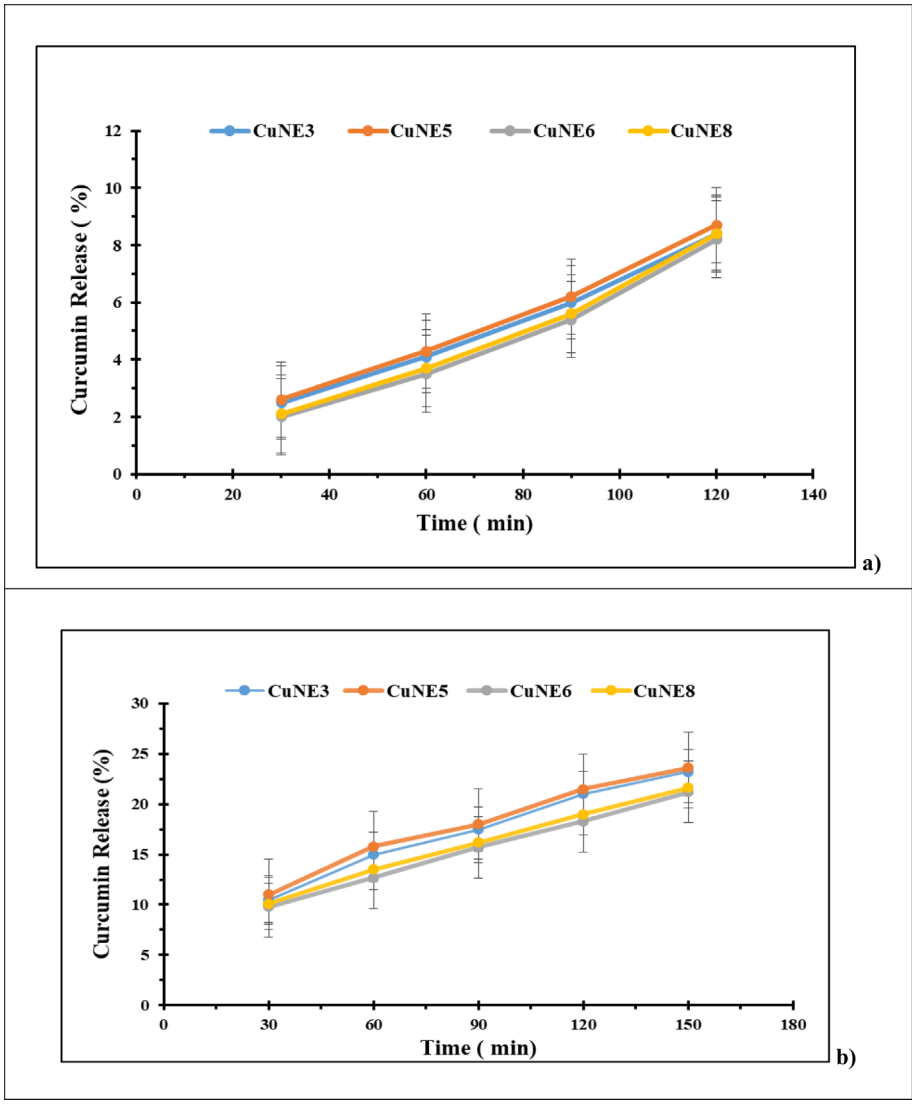

Fig. 9. In-vitro release of curcumin from nanoemulsion sample under a) SGD and b) SIF.

Fig. 6 shows that an CuNE8 has particle sizes ranging from 10 nm to 35 nm, whereas CuNE3 has particle sizes ranging from 0 nm to 850 nm. The larger particle size of CuNE3 can be attributed to a structural change in the lecithin molecule above 70 °C, which causes a decrease in emulsion viscosity and slight aggregation in an emulsion. The effect of temperature change, on the chemical and physical stability of nanoemulsions against aggregation was investigated. They discovered that temperature has no visible effect on the particle size of an emulsion. The unchanging particle size with temperature change can be attributed to the decrease in Tween 80 hydrophilicity caused by the formation of the hydrogen bond. Emulsion stability was also confirmed for two different pasteurization temperature conditions using zeta potential analysis. Denaturation of lecithin and Tween 80 occurred at these temperatures, resulting in a change in the particle surface charge [24] (see Table 4). The surface charge control values were −70 and −25 CuNE 3 and 8, respectively. For CuNE 3 and 8, the surface charge decreased to −63.7 mV, −59 mV, and −22 mV, −20 mV after heat treatments of (63 °C 30 min) and (85 °C 10 min), respectively.

3.5.2. *Effect of pH on nanoemulsion stability*

Two acidic conditions (pH 3, pH 5) and one neutral condition (pH 7) were found to have an effect on the particle size and zeta potential of nanoemulsions. To satisfy the gastrointestinal juice pH condition, acidic pH conditions were chosen (Fig. 7). The aggregation of emulsion particles near the isoelectric point of the surfactant is responsible for the

increase in particle size of nanoemulsion at lower pH. Table 4 shows, the change in particle size and zeta potential at different pH levels.

pH on nanoemulsion stability as measured by zeta potential; the study reported emulsion stability at pH 6 and 7. However, at higher and lower pH, particle growth or accumulation contributes to hydrolysis or charges repulsion effects [24,27].

3.5.3. Effect of ionic strength on nanoemulsion stability

The role of ionic strength for food-grade nanoemulsion depends on the nature of the food products. Salts are common food additives. As a result, understanding the behavior of prepared nanoemulsions under different ionic strengths is critical.

The zeta potential value for both CuNE3 and CuNE8 approaches zero as electrostatic repulsion between the molecules decreases, resulting in a charge reduction of the molecule in it (Table 4). As a result, at high salt concentrations, salt causes destabilization of the nanoemulsions. As seen in Fig. 8, the particle size of the nanoemulsion was changing. This instabilization can be attributed electrostatic repulsion between protein droplets and salt, which causes particle aggregation. For low salt concentrations, nanoemulsion demonstrated better resistance towards Van Der Waals bonds; this could attributed to strong electronic repulsion between molecules [24].

3.6. Antioxidant activity of curcumin nanoemulsion

Table 3 shows the antioxidant ability of samples with and without CuNE as g of Trolox Equivalents (TE) per g of nanoemulsion. In general, regardless of surfactant concentration, the antioxidant capacity of nanoemulsions measured by the Ferric Reducing Antioxidant Power (FRAP) assay did not show significant changes. However, the antioxidant capacity values obtained from the DPPH assay are greater than those obtained from the FRAP assay. In this regard, reducing some species necessitates oxidizing others. Curcumin oxidation can occur at three active sites via electron transfer and hydrogen abstraction.

During free radical reactions, phenoxy radical form due to the easily abstractable hydroxyl phenolic group from curcumin that create the resonance for stabilization. Curcumin may be subjected to various types of a derivative that cause autoxidative degradation, which may affect the encapsulation efficiency of NE. According to Table 3, CuNE3 and CuNE8 samples have antioxidant capacities in DPPH of 108 and 125%, respectively [32]. Table 3 show that a link between nanoemulsion encapsulation efficiency and antioxidant activity. The greater the encapsulation efficiency, the greater the antioxidant activity. CuNE3 and CuNE8 samples exhibit the same trend for encapsulation efficiency and antioxidant activity due to their surfactant nature. Because lecithin contains phosphate ions that can interact with the hydroxyl phenolic of curcumin, it completes the formation of H-bonds, allowing for the effective capture of bioactive molecules within a chain of lipophilic lipids. Lecithin has been shown to reduce the rate of peroxyl radical permeation through the nanoemulsion interface. The results indicate that lecithin CuNE has superior antioxidant activity. Similarly, the antioxidant activity of Tween80 containing CuNE varies according to concentration in terms of scavenging assay. Again, we can see from Table 3 that Tween80 containing CuNE has a higher antioxidant and encapsulation efficiency than lecithin. Because of Tween80, slow release of curcumin at high loading. It has been reported that a high loading or concentration of surfactant, particularly Tween 80, can form strong micelles capable of capturing the bioactive complex inside, enhancing the protection of encapsulated curcumin and thus increasing the systems antioxidant capacity. One of the primary reasons for more antioxidants is the sonication method, which embedded curcumin in a very compact nanoemulsion and thus controlled its solubility within it.

3.7. In vitro analysis of nanoemulsion samples

Polyphenols are known to need to pass through the entire digestive process before they can have any physiological effect. To better understand the potential positive effects of biologically active substances on human health, it is critical to reveal how the digestion process influences their stability and subsequent absorption. An active encapsulation scheme requires the stability of nanoemulsion particles in the gastric location. It is necessary to protect the bioactive from the harsh gastric intestinal environment. Lipids and surfactants are digested during digestion, and the molecules of curcumin released are analyzed. After 2 h of gastric digestion, the results showed that 8.4, 8.7, 8.2, and 8.7% of curcumin was released from the nanoemulsions samples CuNE3, CuNE5, CuNE6, and CuNE8, respectively, demonstrating more than 90% retention of curcumin (see Fig. 9a). This high retention could be attributed to less significant adsorption of gastric enzyme onto absorb surfactant particles, which form a layer on oil droplets. Tween 80 and lecithin have higher steric hindrances and surface activity, which

could have resulted in a less favorable situation for enzymes if the enzyme fragments had not been allowed to adsorb on the oil droplets [31].

Curcumin is found to be released at a higher rate in simulated intestinal environmental conditions (SIF) than in gastric conditions. The percentage of curcumin released after 2.5 h of intestinal digestion was 21.6 and 23.6% for Tween 80 and soy lecithin nanoemulsion samples, respectively (see Fig. 9b). The increased rate of curcumin release could be attributed to the action of bile salts, which are anionic biosurfactants found in the SIF. This salt has a high affinity for surfactant molecule displacement at the interface of oil droplets. At the same time, they aid in the removal of digestion products from the interface by solubilizing them in the bulk aqueous phase. It observed that the dependent on emulsifier type and concentration affects the increased bioavailability of curcumin [31].

4. Conclusion

The current study describes the formation of a stable curcumin nanoemulsion with a high curcumin loading using ultrasound (0.15 g). The effect of different concentrations and sonication times of lecithin and Tween 80 surfactants on nanoemulsion stability was observed. Curcumin nanoemulsions were found to have a 125% increase in antioxidant inhibition. In conclusion, CuNE8 outperforms CuNE3 in terms of encapsulation efficiency and antioxidant capacity, as well as stability. Furthermore, CuNE8 had a narrow particle size distribution, with an average particle size in the range of 10–30 nm for all processing conditions, such as heat, pH, and ionic conditions, compared to 100–400 nm for CuNE3. In-vitro studies show that CuNE3 and CuNE8 improve bioavailability and long-term curcumin release.

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