Preparation of eugenol nanoemulsion by the spontaneous method

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Abstract

Background*: The present study aims to enhance the water solubility of eugenol through nanoemulsion formulation with spontaneous method and evaluate the particle size, surface load, thermodynamic stability, and drug release of the nanoemulsion.*

Materials and Methods: Eugenol, oleic acid, and surfactant tween 80 were mixed using a vortex for 1 minute. Isopropyl alcohol was added as the aqueous phase and the mixture was polymerized using a magnet on a stirrer for 3 minutes. Thermodynamic stability studies were conducted on selected nanoemulsions.

Results*: from the selected nanoemulsions, those with the smallest particle sizes, measuring (95,101,81,77) nm were identified. Additionally, all formulations exhibited a surface charge of 25 mv. eugenol nanoemulsion formulation demonstrated a reduced release rate compared to the solution state, indicating the potential for sustained drug release.*

Conclusion*: The spontaneous formation of nanoemulsion is regarded as a highly cost-effective approach that offers time and material savings, along with the production of stable nanoemulsions. this characteristic renders it particularly suitable for industrial applications necessitating high-volume production.*

Keywords: **nanoemulsion, eugenol, essential oil, surfactant, particle size, spontaneous method, stability**

1-Introduction

Eugenol is a major component of clove oil, obtained from dried flower buds of Eugenia caryophyllata Thunb. It has various applications in medicine, food and cosmetic industries, and agriculture. In dentistry, eugenol is commonly used as a cavity-filling cement with local antiseptic and analgesic effects. (10) However, it has also been studied for its antimicrobial, antiviral, antifungal, antiparasitic, and insecticidal properties. Additionally, eugenol has anti-inflammatory, antioxidant, and potential anticarcinogenic effects. It can be used alone or in combination with conventional therapies. Therefore, eugenol has a wide range of pharmacological activities and potential therapeutic uses. (2) However, due to limited aqueous solubility, it has poor bioavailability. Its therapeutic potential can be enhanced by developing eugenol nano-formulations like liposomes, nanoparticles, microemulsions, and micelles. (3) nanoemulsions are the easiest to formulate and handle and they can be

obtained at low cost. They are suitable in the presence of lipophilic or low water-soluble compounds, such as EOs, that require dispersion in water media, i.e., pesticides or foodstuff ingredients. Such delivery systems could enhance the bioavailability, and thus the effectiveness, of active compounds through their solubilization into small oily droplets. (4)

Nanoemulsions are stable dispersions of immiscible liquids with droplet sizes around 500 nm. They offer advantages such as enhanced stability, large surface area per unit volume, improved interfacial and wetting behavior, and adjustable rheology. two main methods of preparing nanoemulsions: high-energy methods involving ultrasonication and high-pressure homogenization, and low-energy methods involving phase inversion temperature and phase inversion composition. (5)

The spontaneous method for preparing nanoemulsions involves mixing two immiscible liquids using surfactants and co-surfactants to reduce interfacial tension. High shear conditions, such as homogenization or ultrasonication, are applied to induce turbulence and create small droplets. The selection of appropriate surfactants and co-surfactants is critical for stability. Controlling formulation parameters, such as surfactant concentration and homogenization speed, allows for control over droplet size. This method is scalable and finds applications in drug delivery, food technology, and cosmetics.

Thus, this study aimed to formulate and produce stable eugenol nanoemulsions via the spontaneous emulsification method. The spontaneous emulsification method was selected because it is an effective low-energy method to encapsulate the sensitive compounds in food and pharmaceutical products. (1)

2. Materials and methods

2.1. Preparation method of nanoemulsion

To prepare the nanoemulsion Eugenol (MERK, Germany), Tween 80, and Tween 20 (both from MERK, Gernsheim, Germany) were used as non-ionic surfactants. Nine series of formulas were created with different percentages of surfactant Tween 20 and 80 (6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%, 22%), and different oils, including 2% each of oleic acid(MERK, Germany), flax(IRAN, Barij), and sesame(IRAN, Barij). Water, isopropyl alcohol (MERK, Germany), and pleoxamer (2%) were also included in the formulations. Among the formulations made with different percentages of surfactant and different oils, 6 formulations were selected as nanoemulsions based on their size and stability. The prepared nanoemulsions included 20-22% w/w surfactant, 50-56 w/w distilled water, 2% w/w Eugenol, 2%w/w oleic acid, 20-22%v/v isopropyl alcohol, and poloxamer. Table 2-1 shows the composition of 6 selected formulas. Briefly, To prepare the nanoemulsion, eugenol, oleic acid, and surfactant (Tween 20 and 80) were first mixed with a vortex(Iran, HastaranTeb) for 1 minute, and then cosurfactant (isopropyl alcohol), was added with water and placed on a stirrer (Germany, Schott for 3 minutes.

Table 2-1. The ingredients of the selected eugenol nanoemulsion formulation

Formula	water	Isopropyl (v /v)	oleic w/v	Tween 80(w/v)	eugenol (v/v)	Tween 20(w/v)	Poloxamer2 % (W/V)
F 1	56%	20%	2%	20%	2%	-	-
F2	54%	20%	4%	-	2%	20%	-
F 3	50%	22%	4%	-	2%	22%	-
F 4	56%	20%	2%	20%	2%	-	1.21
F5	54%	20%	4%	-	2%	20%	1.08
F 6	50%	22%	4%	-	2%	22%	1

2.2. Characterization of Nanoemulsion

Thermodynamic stability studies

The thermodynamic stability studies for the nanoemulsions include the following tests:

1.*Centrifuge test:* The nanoemulsions are subjected to centrifugation(Germany, Eppendorf) at 3500 rpm for 10 minutes.

2. *Heating-cooling cycle test:* Formulations that pass the centrifuge test are then subjected to 6 cycles between refrigeration temperature (4°C) and elevated temperature (45°C).

3. *Freeze-thaw cycle test:* The nanoemulsions that pass the heating-cooling cycle test are further tested for stability by subjecting them to 3 cycles between freezing temperature (-21°C) and room temperature (25°C). Stability is determined by observing whether phase separation, turbidity, or color change occurs during these freeze and thaw cycles [14].

Long-term stability studies

Formulations that are stable in thermodynamic tests were chosen for long-term stability studies. These formulations were stored for more than one month at room temperature (25°C) and refrigerator temperature (4°C).

Investigating particle size and dispersion

A Malvern Nanosizer (Malvern Instruments, Worcestershire, UK) was used to measure droplet size and the size distribution of nanoemulsion. [15].

Release of Essential Oil

The release of Eugenol was determined by using the dialysis method. One ml of the Eugenol-loaded nanoemulsions was placed in the bags and immersed in Ethanol 40% v/v. All sets were incubated at 37 ºC. Sampling has been done at the times 2, 4, 6, 12, and 24 h after immersion. the absorbance was measured at 284 nm using a UV-VIS spectrophotometer (Jenway, Barcelona, Spain). The same procedure was also performed on pure Eugenol dissolved in ethanol.

The amount of eugenol in the selected formulation was examined for two months

The eugenol content in the selected formulations was quantified over two months by combining 100 µl of nanoemulsion in a volume of 100 cc with 40% ethanol (V/V) and measuring the absorbance of the diluted formulations at a wavelength of 284 nm. Subsequently, utilizing the formula derived from the calibration curve, the drug concentration at the initial time, after one month, and after two months was determined. This meticulous comparison of the eugenol concentrations at different time points provides valuable insights into the stability and longevity of the formulations over the specified duration.

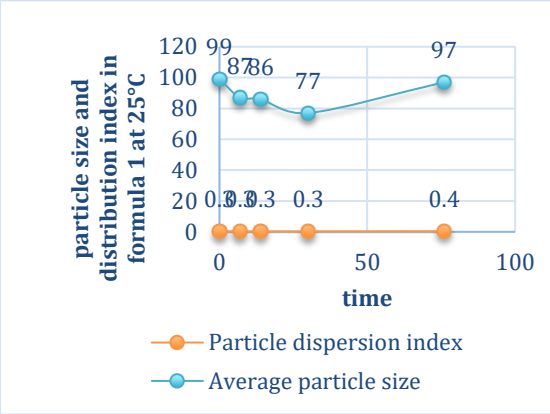
3-Result

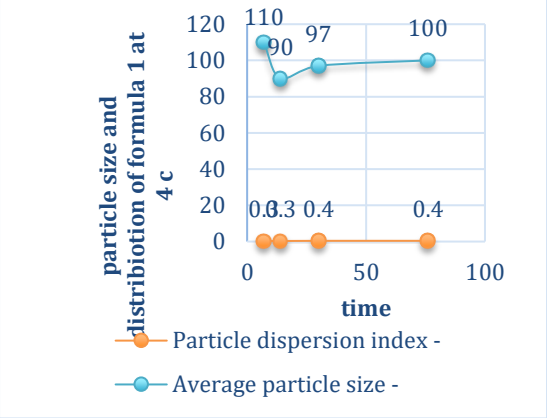
Results of long-term stability studies

The stability of formulations was assessed over one month, with storage conducted under varying conditions including room temperature (25°C) and refrigeration (4°C). The duration of storage encompassed intervals of 0, 7, 14, 30, and 76 days. The primary focus of this inquiry was the particle size and particle size distribution. The relevant findings from this investigation are presented in Figure 3-(1-6).

Upon analysis of the obtained data, it was determined that there was no significant increase observed in the particle size or particle size distribution of the formulations, consistently observed at all designated time points. This consistent observation over various time intervals strongly indicates that the formulations retained stability over a prolonged period under the tested storage conditions.

In summary, the meticulous evaluation of particle size parameters unequivocally demonstrates the robust stability of the formulations, rendering them suitable for extended storage without noteworthy alterations in their essential characteristics.


particle size and distribution index in formula 1 at 25°C
time
Particle dispersion index
Average particle size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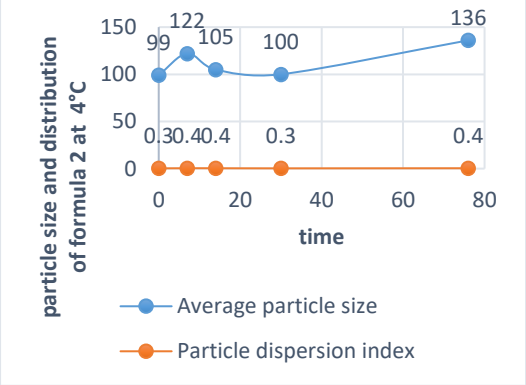
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time
Average particle size
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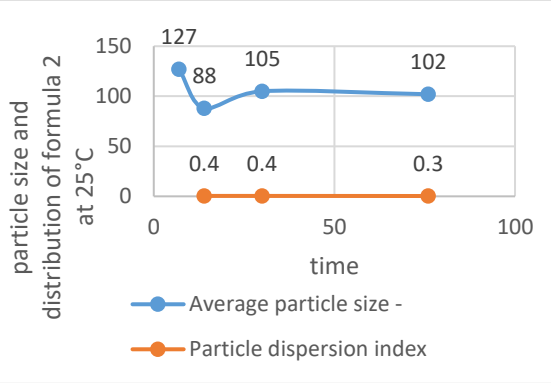
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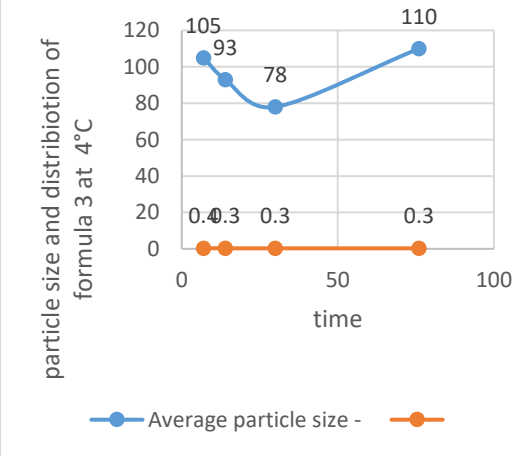
particle size and distribiotion of formula 3 at 4°C
time
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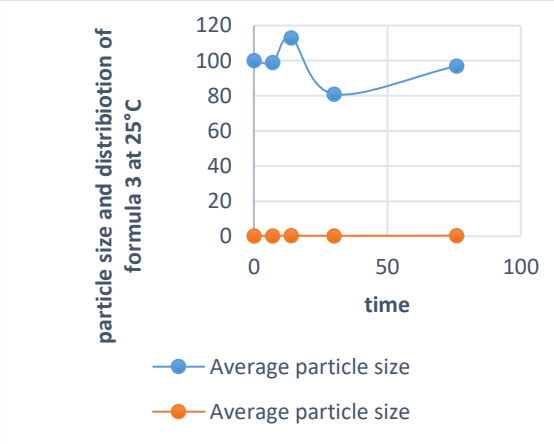
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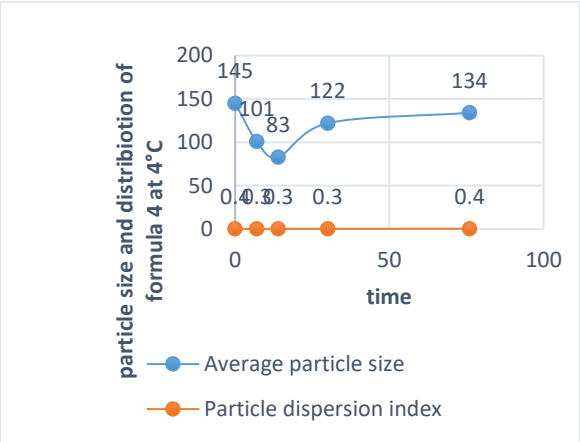
time
Average particle size
Average particle size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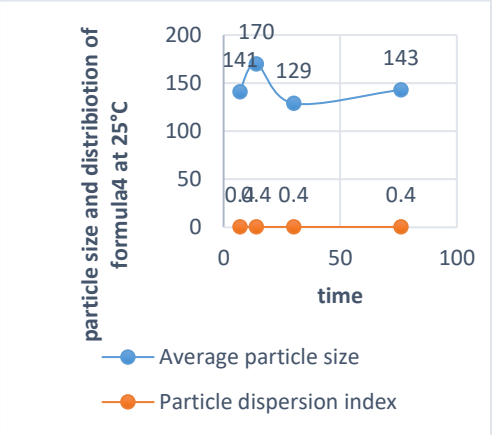
time
Average particle size
Particle dispersion index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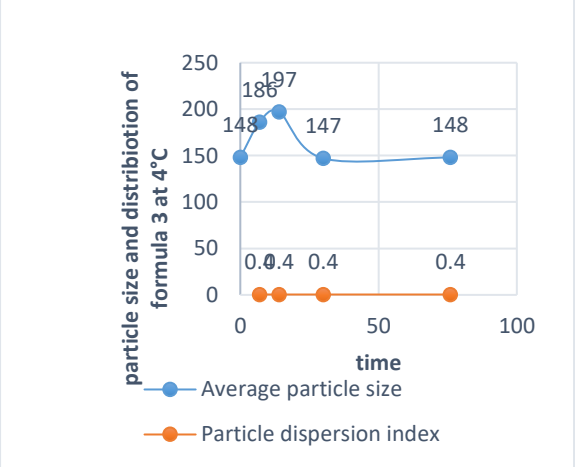
time
Average particle size
Particle dispersion index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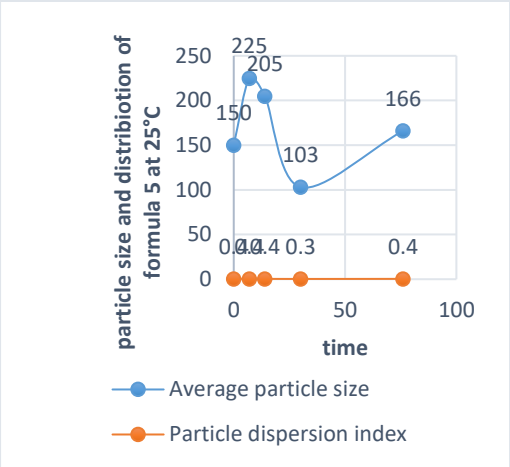
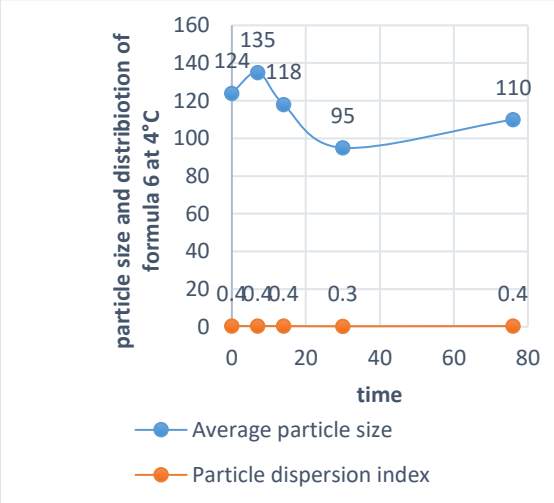
time
Average particle size
Particle dispersion index


particle size and distribiotion of formula 5 at 25°C
time
Average particle size
Particle dispersion index


particle size and distribiotion of formula 6 at 4°C
time
Average particle size
Particle dispersion index

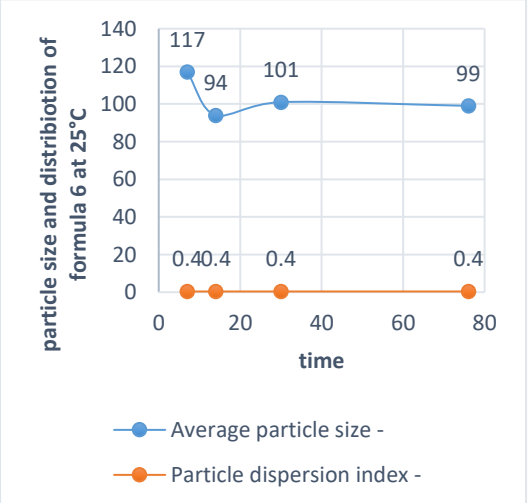
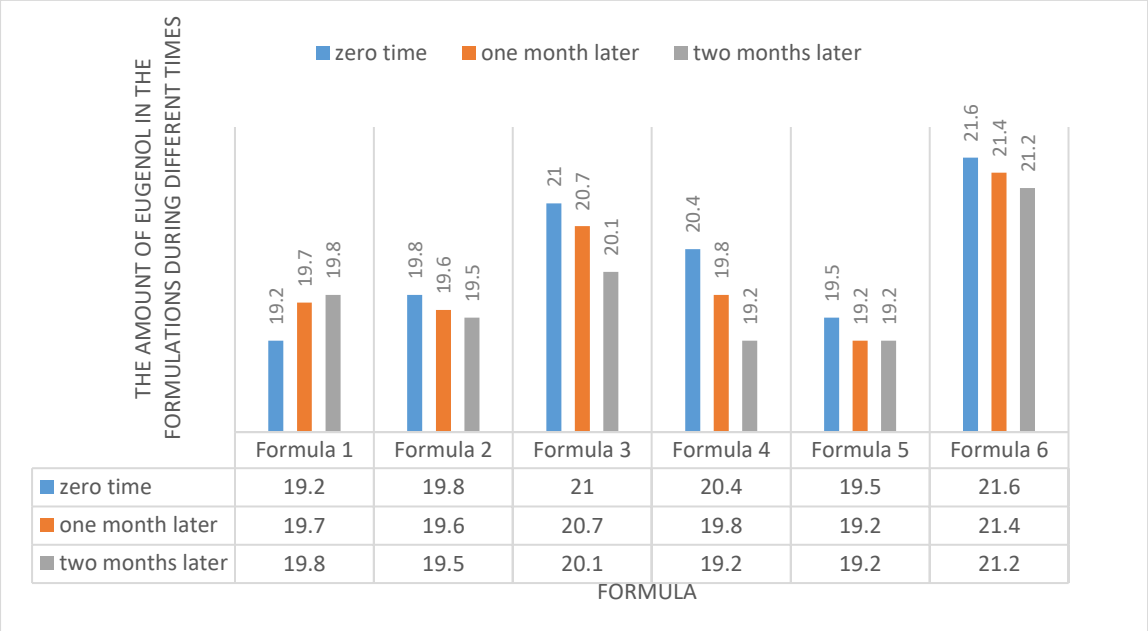

particle size and distribiotion of formula 6 at 25°C
time
Average particle size -
Particle dispersion index -

Figure 3-(1-6). particle size and distribution index of formula (1,2,3,4,5,6) at 4°C and 25 °C

The amount of eugenol in the formulations during different times

The absorption of eugenol at a concentration of 20 µg/ml was measured at a wavelength of 284 nm at specific time intervals (0, 30, 60 days). These absorbance values were then utilized in the calibration curve formula to determine the eugenol concentration in the formulation over the two months. The obtained results are summarized in Figure (3-7).



	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
zero time	19.2	19.8	21	20.4	19.5	21.6
one month later	19.7	19.6	20.7	19.8	19.2	21.4
two months later	19.8	19.5	20.1	19.2	19.2	21.2

Figure3-7. The amount of eugenol in the formulations during different times

Investigation of drug release from eugenol nanoemulsion

Release tests were conducted, and the graph illustrating the cumulative drug release percentage from nanoemulsions over 24 hours was depicted (refer to Figures 3-8). Simultaneously, the release of the ethanol-soluble drug was investigated for comparative analysis.

Upon scrutinizing the obtained curve, it was discerned that the preparation of the drug in nanoemulsion form led to a decreased release rate compared to its solution state. Specifically, after 6 hours, only 57% of the drug

was released from the nanoemulsion, whereas in the solution state, nearly 80% of the drug was released within the same timeframe.

This observation signifies that the nanoemulsion form of the drug exerts a retarding effect on the release rate, resulting in a lower percentage of drug release during the initial 6 hours. This aligns with existing literature [5, 7, 8, 9] and underscores the influence of nanoemulsion formulation on modulating the kinetics of drug release.

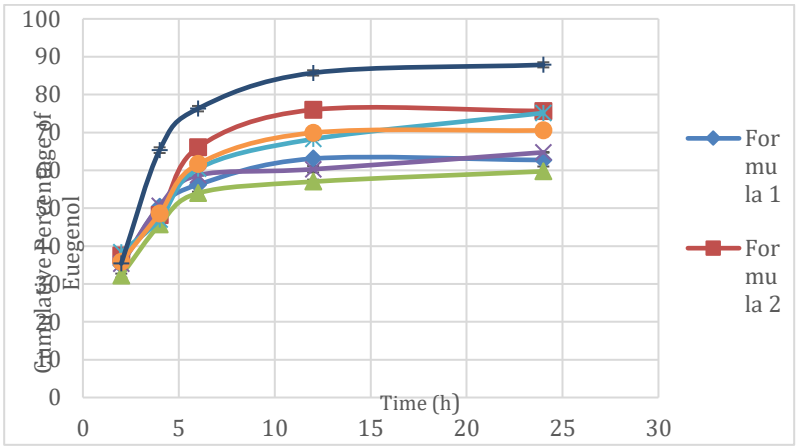


Figure 3-8 Cumulative drug release diagram from nanoemulsion and ethanol solution

Validation of UV spectrophotometry method

Utilizing the absorbance values of standard samples, a calibration curve was constructed by plotting the sample absorbance at the wavelength of 284 nm against the concentration of eugenol. The resulting equation of the line was determined as $\gamma + 0.0334\chi = 0.0226$ (refer to Figure 3-9). This equation encapsulates the quantitative relationship between the absorbance and the concentration of eugenol, providing a reliable basis for subsequent concentration determinations in the experimental samples. The construction of the calibration curve follows standard scientific procedures, ensuring accurate and reproducible results in the analysis of eugenol concentrations.

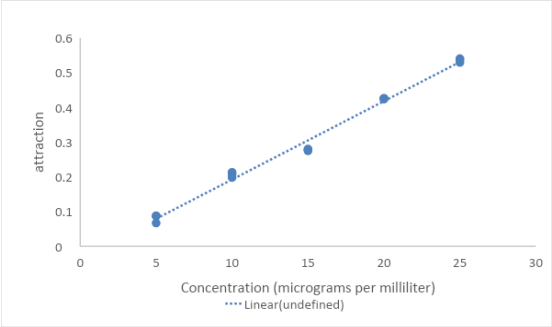


Figure 3-9 Calibration curve of eugenol absorption against concentration

The accuracy and precision of the UV method for the eugenol solution are reported in (Table 3.1)

Table 3.1- accuracy and accuracy of UV spectrophotometry method for eugenol solution

Standard concentration µg/ml	Accuracy (RSD%)	Validity
5	11	100.9 ± 2
10	3	106 ± 0.9
15	0.3	91.7 ± 0.5
20	0.1	101.4 ± 0.2
25	0.8	100.6 ± 0.9

3. Conclusion

In this study, we conducted research on the development of a drug delivery system that is both highly efficient and cost-effective, with a particular focus on its potential for future industrialization. Our first step was to create an oil-in-water (O/W) nanoemulsion that had the desired particle size and used minimal raw materials. We utilized a spontaneous method for nanoemulsion production, which is known for its efficiency in terms of time and cost compared to high-energy methods. This method resulted in formulations with improved thermodynamic stability. The concentration of surfactants, specifically Tween 80 and Tween 20, played a crucial role in the formation of transparent and stable nanoemulsions. We found that as the concentration of surfactants decreased, the nanoemulsion became cloudier and had larger particle sizes. Importantly, we observed that the percentages of Tween 20 and Tween 80 in the formulations did not have any antimicrobial effects. Overall, our research provides valuable insights into the development of a drug delivery system that is highly efficient and cost-effective. These findings have significant implications for the future industrialization of drug delivery systems.

According to the study by Gosh et al, they designed a eugenol nanoemulsion to have an anti-Staphylococcus aureus effect in fruit juice. They used various concentrations of eugenol (6%, 3%, 2%, 1% v/v), different percentages of surfactants (18%, 12%, 6% w/w) Tween 20 and 80, and 6% sesame oil. They employed an ultrasonic method to create the nanoemulsion. The optimal formulation for particle size reduction was identified to have a higher surfactant concentration (18%) and eugenol concentration (3%) [12].

The researchers utilized gum arabic and lecithin as emulsifiers and found that lecithin and ethanol reduced particle size in eugenol nanoemulsions. Arabic pitch had no significant effect on particle size. Poloxamer, added to increase stability, unexpectedly resulted in larger particle sizes. The introduction of oleic acid enhanced eugenol solubility and reduced particle size but did not exhibit antimicrobial effects. Flaxseed and sesame oil were discarded due to small size and micelle formation, and isopropyl alcohol was used as a cosurfactant to achieve clear and stable nanoemulsions. All formulations showed thermodynamic stability without particle deposition, phase separation, or turbidity. Shahid Jameel developed a nanoemulsion formula with clove essential oil, achieving a particle size of 29.1 nm and zeta potential of 31.4 for thermodynamic stability [16].

Long-term studies were conducted to measure the absorption levels of different formulations for two months. These studies consistently showed that all formulations had similar concentrations of eugenol.

In addition, release studies were conducted to determine how quickly the formulations released eugenol. It was found that pure eugenol was completely released within 6 hours, while the eugenol nanoemulsion released only 57% of eugenol within the same timeframe [13].

Due to the incorporation of the drug into oily nanoparticles, the release from the nanoemulsion base demonstrated a slower release profile.

As the corresponding author, I confirm on behalf of all authors that there is no conflict of interest.

Declarations

Competing interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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