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Black Seed Oil-Alginate Nanoemulsion Characteristics Utilising an Ultrasonicator at Pilot Plant Scale

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Abstract | Black seed oil (BSO) contains thymoquinone, an active ingredient that is well-known for its antioxidant property and used in food and traditional medicine. BSO is encapsulated in micrometre-sized alginate beads (AB), to increase its palatability. This encapsulation is established and produced on a small scale in the lab. To achieve the ideal BSO alginate nanoemulsion during large-scale manufacturing, it is vital to use suitable parameters where two parameters were manipulated: the flow rate of the pump and the percentage of the power (amplitude) of the ultrasonicator. The droplet size, PdI, and zeta potential of the nanoemulsion were investigated. The zeta potential values for BSO nanoemulsions ranging from -53.83 ± 1.50 to −63.50 ± 0.66 mV. All zeta values were below -30 mV, demonstrating that the nanoemulsions are stable emulsions. Each amplitude and flow rate produced BSO alginate nanoemulsion within the targeted droplet size, which is below 500 nm of the sonication process, except at flow rates 144, 216 and 288 mL min^{-1} at 30 % power of amplitude. The droplet size was found to be smaller at a lower flow rate. The smallest droplet size was achieved at 72 mL min^{-1} i.e., 346.57 nm to the power of 90% of ultrasonicator amplitude. For every flow rate of 70% and 90% power of sonication, the PdI of BSO alginate nanoemulsion was less than 0.700, The PdI ranges for these parameters are from 0.262 ± 0.005 to 0.627 ± 0.045. The higher the flow rate and the low percentage of ultrasonicator power, the larger the particle size of the BSO alginate nanoemulsion obtained. As the applied power of the amplitude increases from the optimal value (70%), the size of the emulsion particle decreases. It is discovered that the BSO alginate nanoemulsion particle size is influenced by the pump flow rate and ultrasonicator power.

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Introduction

Thymoquinone, a vital component of black seed oil (BSO), is well-known for its antioxidant and medicinal properties. BSO has a bitter taste, making it difficult for consumers mostly youngsters and the elderly to eat it daily. To improve its palatability, BSO is therefore enclosed in micrometre-sized alginate beads. This encapsulation was previously established on a lab scale. The conventional mixing method to create a stable BSO-alginate emulsion was utilising a magnetic stirrer paired with a sonicator. When making BSO beads on a big scale, this sonication method using small amount is unsuitable. Pharmaceutical development must provide a high-quality product and manufacturing method, in order for the product to continuously deliver on its intended performance according to ICH guidelines Q8 (R2) International Council for Harmonisation (ICH, 2009). Adherence to these guidelines is crucial for meeting regulatory requirements set forth by the National Pharmacy Regulatory Agency (2015). It is the responsibility of the manufacturer to ensure that the sampling strategy and acceptance criteria are sufficient to establish that the manufacturing process is well-controlled and robust to consistently produce medicinal products that fulfil requirements (National Pharmacy Regulatory Agency, 2015).

Ultrasonic waves are known to mechanically reduce particle size through the cavitation process, which is characterised by repeated stresses generated by the production and dissolution of bubbles in the fluid (Kashaninejad *et al*., 2020). Due to its limited processing capacity and high energy consumption, ultrasonication has not been widely used on an industrial scale (Carpenter *et al*., 2018). BSO in alginate emulsion needs to be emulsified using the jacketed flow cell ultrasonicator for large volume emulsion. This equipment was utilised to create a nanoemulsion, which is necessary to create the beads with the right attributes, by emulsifying the oil with an alginate solution. The machinery must also be able to withstand the rigours of the production process.

In terms of the functional and operational parameters, the employment of a flow cell ultrasonicator irradiating downward in a 1 L vessel was qualified for the enhancement of the sonochemical activity. These variables included input power of the ultrasonicator and the flow rate of the BSO emulsion into and out of the flow cell ultrasonicator. The ultrasonicator's capacity to emulsify BSO and alginate solution to create nanoemulsion was tested in this study. Thus, this study aims to achieve an ideal BSO alginate nanoemulsion characteristics through large-scale manufacturing which adheres to guidelines set by the regulatory body. These BSO alginate nanoemulsion ideal characteristics were achieved by manipulating the flow rate of the pump and the percentage of the power (amplitude) of the ultrasonicator.

Materials and Methods

Materials

BSO (Blessed Seed Sdn. Bhd., Kuantan, Malaysia), gluronic acid-rich (high-G) sodium alginate (SA) (KIMICA Corporation, Chuoku-Tokyo, Japan), T80 and calcium chloride (Merck Schuchardt OHG (Hohenbrunn, Germany). The sample solutions were prepared using distilled water.

Emulsion formulation

Coarse emulsion (CE) formation: The study was carried out by mixing 2% (w/v) polymer with distilled water at room temperature to create sodium alginate solution (SAS). Conditions for the emulsification process and oil concentration used to create O/W emulsions were determined after early trials (Mohd Rus *et al*., 2021). At pilot scale, BSO alginate CE was prepared using 100L mixing tank (Gardner Global Ent., Malaysia). The machine consists of a 100L stainless steel mixer tank agitator with a solid-shaft SS 316 and motorised top entrance. In a single batch of 45 kg BSO alginate emulsion, purified water, SA and T80 were dissolved in the mixing tank and then emulsified with BSO with a stirrer and a H spinning at 500 rpm and 1500 rpm, respectively.

Stable emulsion formation: The CE of BSO alginate was sonicated with an ultrasonic processor to produce a stable emulsion and these parameters were optimised in the preliminary assay (Mohd Rus *et al*., 2021). The equipment consists of stainless steel sonicator flow reactor with a chiller (cooler), pump to circulate cooling water, probe, controller for sonicator amplitude, time controller, cycle controller, circuit breaker, temperature monitoring and amplitude changing rode (Gardner Global Ent., Malaysia). To create a nanoemulsion, which is essential for producing BSO alginate beads with suitable characteristics, the flow cell ultrasonicator equipment was used to emulsify the oil with an alginate solution.

The probe has a power of 2000 watts and a working frequency of 220V/50Hz, a chiller (cooler) with a capacity of more than 10 L and a temperature of > 5°C for water usage. All interior contact materials are made of stainless steel 316L. The temperature of BSO in an alginate emulsion were not rise above 40 °C due to the water jacket on the jacketed flow cell sonicator. Using a peristaltic pump, the CE of BSO alginate was transferred from the 100 L mixing tank to the flow cell ultrasonicator, where approximately 250 mL of sample was taken and examined every 5 minutes at the outlet of flow cell ultrasonicator. In a controlled environment, the BSO in an alginate emulsion were sampled and evaluated for droplet size, droplet size distribution, and zeta potential.

The experiment was done at various flow rates with various amplitude powers to create stable emulsions, determining the ultrasonicator's capacity to emulsify BSO and alginate solution to get nanoemulsion. For a processing volume of 1000 mL, the ultrasonication cell was run in circulating mode at varied flow rates of 72, 144, 216, and 288 mL min^{-1} with an amplitude power of either 30%, 50%, 70%, or 90%.

Characterisation of emulsion

Droplet size measurement: The Metasizer 2000 (Malvern Instrument Ltd., UK) was used to measure droplet size using the laser light diffraction method. Because the Z-average computation is mathematically stable and the Z-average result is noise-insensitive, the emulsion droplet size was expressed as the Z-average (Zuki *et al*., 2019). The polydispersity index (PdI) determines the uniformity of the nanoemulsion droplet size (Branco *et al*., 2020). Prior to measurement, the emulsions were diluted with distilled water at a ratio of 1:200.

Zeta potential

Dynamic light scattering was used to determine the emulsions' zeta potential (Malvern Zetasizer, Malvern Instruments Ltd., Worcestershire, UK). Prior to measurement, the emulsions were diluted with distilled water at a ratio of 1:200. By identifying the electrical charge on the oil droplets in the nanoemulsion, the physicochemical characterisation of nanoemulsion is essential for evaluating the colloidal dispersion stability of nanoparticle (Sharma *et al*., 2019).

Statistical analysis

The software Graph Pad Prism version 8 (California, USA) was used for statistical analysis. The data were analysed using two-way ANOVA to compare multiple pairs of the group at once and multiple comparisons with Dunnett analysis were performed at a significant level of $p \leq 0.05$. The results were expressed as means ± standard deviations (error bar) (n=3).

Results and Discussion

Effect of flow rate and different amplitude power for BSO alginate nanomulsion in sonication process

Cavitation and gas bubble collapse are brought on by hydrodynamic shear forces, which are produced by the ultrasonic process (Silva *et al*., 2018). A highly concentrated cavitational activity zone is created directly under the tip of the probe type sonicator by applying ultrasound to a small area at the end of the tip (Son *et al*., 2020). About 250 mL of BSO alginate nanoemulsion sample was collected during the sonication process for each variable flow rate and power of the amplitude as stated in the section above to examine the droplet size, PdI, and zeta potential of the BSO alginate nanoemulsion. Data were analysed statistically by two-way ANOVA and the results were reported as mean ± standard deviation (n=3), as shown in Tables 1, 2, 3.

Table 1: *Z-average of the BSO alginate nanoemulsion at various flow rates and sonicator power (amplitude). Data are shown as mean ± standard deviation (n=3).*

Amplitude (Power)	Z-average (nm)			
	Flow rate (mL min^{-1})			
	72	144	216	288
30%	455.03 ± 33.51	586.53 ± 4.08	690.27 ± 64.42	782.33 ± 32.42
50%	395.30 ± 27.46	485.17 ± 62.11	490.07 ± 12.31	494.23 ± 37.17
70%	385.10 ± 3.05	450.83 ± 69.03	465.73 ± 31.27	470.97 ± 24.59
90%	346.57 ± 9.65	379.50 ± 8.25	410.43 ± 27.33	422.63 ± 19.03

BSO alginate nanoemulsion's particle size

The goal of the study was to identify the key factors and their interrelationships. The flow rate of the pump and the percentage of the power (amplitude) of the sonicator were the two variables in this investigation. The Design Expert® version 11 program (Stat-Ease Inc., Minneapolis, USA) was used to create the four-level factorial. The Zetasizer Nano S (Malvern, UK) and Zetasizer Nano software v3.30 (Malvern, UK) were used to measure the particle size of each component of the BSO alginate nanoemulsion. According to Table 1, all amplitudes and flow rates except for those at flow rates of 144, 216, and 288 mL min^{-1} at 30 % power of amplitude generated BSO alginate nanoemulsion within the required droplet size, which is below 500 nm of the sonication process.

Table 2: *PdI of the BSO alginate emulsion at various sonicator flow rates and power (amplitude). (n=3) Data are shown as mean ± standard deviation (n=3).*

Amplitude (Power)	PdI			
	Flow rate (mL min^{-1})			
	72	144	216	288
30%	0.615 ± 0.024	0.685 ± 0.028	0.785 ± 0.074	0.424 ± 0.009
50%	0.574 ± 0.048	0.743 ± 0.055	0.714 ± 0.071	0.793 ± 0.063
70%	0.262 ± 0.005	0.428 ± 0.038	0.569 ± 0.016	0.627 ± 0.045
90%	0.557 ± 0.019	0.543 ± 0.108	0.518 ± 0.047	0.615 ± 0.024

Table 3: *Zeta potential of the BSO alginate nanoemulsion with varying sonicator flow rate and power (amplitude). Data provided as mean ± standard deviation (n=3).*

Amplitude (Power)	Zeta potential (mV)			
	Flow rate (mL min^{-1})			
	72	144	216	288
30%	-54.97 ± 1.17	-54.33 ± 1.40	-56.00 ± 0.35	-60.13 ± 0.85
50%	-53.83 ± 1.50	-63.50 ± 0.66	-58.37 ± 1.14	-58.50 ± 0.78
70%	-55.17 ± 0.96	-57.77 ± 0.67	-57.67 ± 1.62	-60.03 ± 1.50
90%	-55.50 ± 1.30	-56.20 ± 2.04	-57.43 ± 0.86	-57.67 ± 1.85

Emulsions with droplet sizes between 20 and 500 nm are referred to as nanoemulsions (Goindi *et al*., 2016; Gupta *et al*., 2016). Droplet aggregation and gravity separation are prevented by the thermodynamic stability of nanoemulsions (Montes de Oca-Valos *et al*., 2017) at a decreased flow rate, it was discovered that the droplet size was smaller. At a flow rate of 72 mL min^{-1}, or 346.57 nm to the power of 90% of sonicator amplitude, the lowest droplet size was attained. Additionally, ultrasonication produces highly reactive free radicals during the intense cavity collapse in the liquid medium, which might travel to the surface or inside the oil droplets, oxidizing the oil molecules (Carpenter *et al*., 2018).

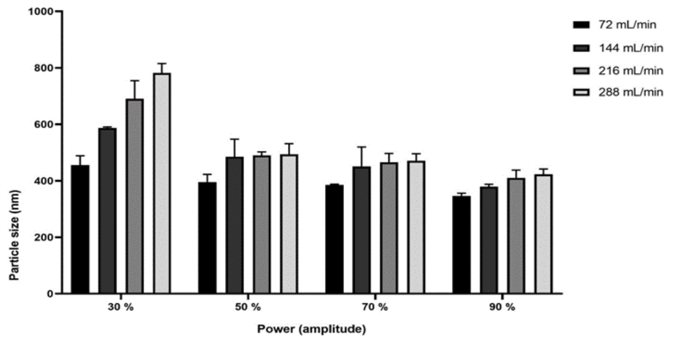

Figure 1: *The BSO alginate nanoemulsion particle size of a flow cell sonicator at various flow rates and power (amplitude). Data are shown as mean ± SD (error bar) (n=3).*

In order to maximize emulsion physical stability while maintaining the chemical characteristics of oil molecules or emulsions, the sonication time must be optimized (Carpenter *et al*., 2018). This result demonstrates that if the sonication procedure is not optimized, 1 L of sonicator vessel might have an uneven impact on the BSO alginate emulsion. As the sonochemical activity grew, the cavitational activity zone eventually encircled the probe body and became a circular shape (Son *et al*., 2020). The same researchers stated that strong ultrasonic reflections that began at the vessel bottom and eventually reached the liquid surface were found to be the cause of the large, brilliant activity zone's circular form. In addition, the horizontal boundaries, however, had a negative effect on the cavitational activity zone and sonochemical activity when the probe was placed close to the vessel's walls (Son *et al*., 2020). No appreciable decrease in sonochemical activity was seen when the probe was positioned very close to the liquid surface (Son *et al*., 2020).

BSO alginate nanoemulsion's polydispersity index

The particle size trend for the BSO alginate nanoemulsion was depicted in Figure 2. In comparison to 30%, 50%, and 70%, the amplitude of 90% demonstrated the best trend since a large amplitude imparts greater disruptive force. The ultrasonic process, which uses a lot of energy to produce hydrodynamic shear forces, is what causes cavitation and gas bubble collapse (Bing *et al*., 2018). As the experimental settings were changed, such as adjusting the pulse, the cavitational activity zone gradually surrounded the probe body and took on a circular form, indicating that the sonochemical activity increased (Son *et al*., 2020). The PdI of the BSO alginate nanoemulsion was less than 0.700 for every flow rate of 70% and 90% power of sonication, as shown in Table 2 and Figure 2. These parameters' PdI ranges are 0.262 ± 0.005 to 0.627 ± 0.045. This is due to the discovery that a huge, sparkling activity zone developed at the vessel bottom before moving to the liquid surface because of powerful ultrasonic reflections (Son *et al*., 2020). With a large particle size distribution (such as polydisperse), PdI values greater than 0.7 are acceptable (Mudalige *et al*., 2019).

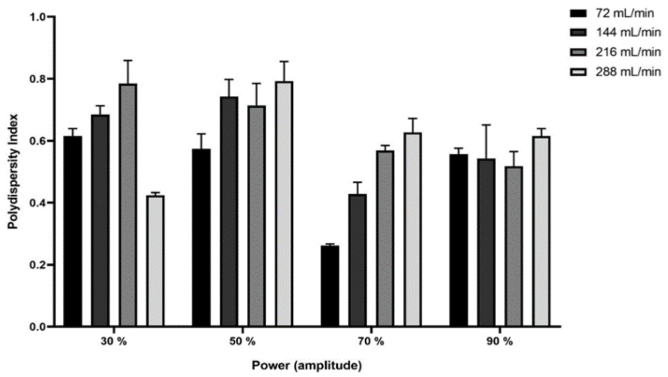

Figure 2: *The flow cell sonicator's PdI of BSO alginate nanoemulsion at various flow rates and power (amplitude). Data are shown as mean ± SD (error bar) (n=3).*

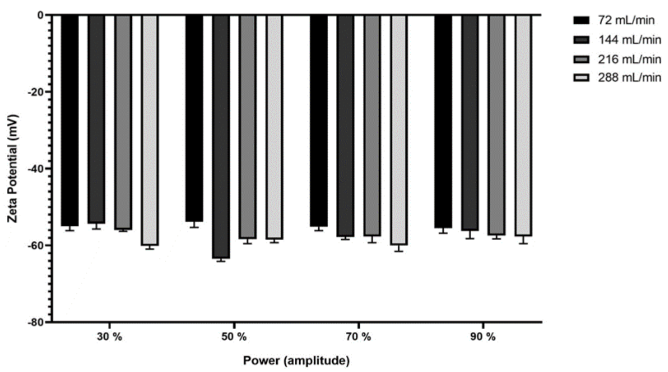

Figure 3: *The zeta potential of a flow cell sonicator's BSO alginate nanoemulsion at various flow rates and power (amplitude). Data are shown as mean ± SD (error bar) (n=3).*

BSO alginate nanoemulsion's zeta potential

Zeta potential measurement is an essential step in determining an emulsion's stability. With high electronegative values ranging from -53.83 ± 1.50 to −63.50 ± 0.66 mV, the information concerning zeta potential values for BSO nanoemulsions reported in Table 3 demonstrated the negative potential. The outcomes of the statistical analysis shown in Figure 3 show that the nanoemulsions are stable emulsions since all zeta values were below -30 mV. When the zeta potential is above +30 mV and below -30 mV, the repulsive interactions between particles are stronger than the attraction forces (Bhattacharjee, 2016). According to Joseph and Singhvi (2019), these nanoemulsions display a potent enough repulsive force in the direction of increased physical colloidal stability. As a result, the negative potential values obtained through this sonication procedure ought to be sufficient to provide the nanoemulsions a high level of stability. The zeta potential and interfacial tension of the biopolymer solutions were often decreased throughout the sonication process, resulting in stable emulsions with smaller droplet sizes.

Conclusions and Recommendations

According to the findings, the BSO nanoemulsion's particle size would increase with higher BSO alginate nanoemulsion flow rates and lower sonicator power percentages where the targeted size of the nanoemulsion were below 500 nm and PdI value below 0.700. The results of this investigation indicate that the flow rate and applied sonicator power have an impact on the BSO alginate nanoemulsion's particle size. Additionally, this research verified the earlier hypothesis that the particle size of a BSO alginate nanoemulsion will be affected by the interaction of several process variables.

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Novelty Statement

The novelty of this study lies in the optimization of parameters, specifically the flow rate of the pump and the percentage of ultrasonicator power, to achieve stable and sub-500 nm droplet-sized black seed oil (BSO) alginate nanoemulsions, which is crucial for large-scale manufacturing and enhancing the palatability of BSO, a traditional medicine and antioxidant-rich ingredient. This research reveals the precise control required for producing stable BSO alginate nanoemulsions with desirable properties, offering valuable insights into their potential applications in the food and pharmaceutical industries.

Author's Contribution

Shaiqah Mohd Rus: Writing review and editing, writing original draft, visualization, validation, methodology, investigation, formal analysis, data curation.
Anika Z.M.R.: Writing review and editing, visualization, funding acquisition.
Awis Sukarni Mohmad Sabere: Writing review and editing, writing original draft, visualization, validation, supervision, resources, project administration, methodology, investigation, funding acquisition, formal analysis, data curation, conceptualization.
Mohd. Rushdi Abu Bakar: Conceptualization, visualization, supervision.
Farahidah Mohamed: Conceptualization, visualization.
Abd Almonem Doolaaneaa: Visualization, validation, supervision, resources, project administration, methodology, investigation, funding acquisition, formal analysis, data curation, conceptualization.

Conflict of interest
The authors have declared no conflict of interest.

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