Hydrodynamic cavitation: A feasible approach to intensify the emulsion cross-linking process for chitosan nanoparticle synthesis

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ABSTRACT

Chitosan nanoparticles (NPs) exhibit great potential in drug-controlled release systems. A controlled hydrodynamic cavitation (HC) technique was developed to intensify the emulsion crosslinking process for the synthesis of chitosan NPs. Experiments were performed using a circular venturi and under varying operating conditions, i.e., types of oil, addition mode of glutaraldehyde (Glu) solution, inlet pressure ($P_i$), and rheological properties of chitosan solution. Palm oil was more appropriate for use as the oil phase for the HC-intensified process than the other oil types. The addition mode of water-in-oil (W/O) emulsion containing Glu (with Span 80) was more favorable than the other modes for obtaining a narrow distribution of chitosan NPs. The minimum size of NPs with polydispersity index of 0.342 was 286.5 nm, and the maximum production yield ($P_y$) could reach 47.26%. A positive correlation was found between the size of NPs and the droplet size of W/O emulsion containing chitosan at increasing $P_i$. Particle size, size distribution, and the formation of NPs were greatly dependent on the rheological properties of the chitosan solution. Fourier transform infrared spectroscopy (FTIR) analysis indicated that the molecular structure of palm oil was unaffected by HC-induced effects. Compared with ultrasonic horn, stirring-based, and conventional drop-by-drop processes, the application of HC to intensify the emulsion cross-linking process allowed the preparation of a finer and a narrower distribution of chitosan NPs in a more energy-efficient manner. The novel route developed in this work is a viable option for chitosan NP synthesis.

1. Introduction

Chitosan (CS) is a linear amino polysaccharide typically used in the form of microcapsules, microspheres, or nanoparticles (NPs) for drug delivery systems due to its excellent biocompatibility, biodegradability, and nontoxicity \cite{1,2}. The emulsion cross-linking (ECLK) approach is one of the most popular ways to synthesize chitosan microspheres; ECLK typically includes a water-in-oil (W/O) emulsification process and a cross-linking reaction process \cite{3,4}. In this conventional approach, emulsification and cross-linking processes are completed by mechanical stirring, and the particle size of CS spheres prepared is typically at micrometer level \cite{5,6}. Moreover, the size distribution is very broad \cite{7,8}. These characteristics lead to poor targeting and low bioavailability \cite{8,9}. Furthermore, NPs with diameters of less than 1.0 μm have many advantages compared with microparticles, such as sustained and controlled drug release, site-specific targeting, and high surface to volume ratio \cite{2,8,9}. Thus, several technologies have been developed to intensify or improve this conventional process to produce uniform or monodisperse chitosan NPs.

For instance, Lv \textit{et al.} \cite{9} reported that the uniform W/O emulsion containing CS was obtained using membrane emulsification, and then, glutaraldehyde (Glu) was added by stirring for 10 h. Uniformly sized nanospheres with polydispersity index (PDI) of 0.027 were formed. Akamatsu \textit{et al.} \cite{10} used the uniform W/O emulsion containing CS prepared by membrane emulsification to cross-link Glu with stirring for 2 h. Monodisperse particles with diameters of 1.1–12.0 μm were obtained. Takahashi \textit{et al.} \cite{11} used electrostatic emulsification to produce uniform emulsion droplets with diameters of less than 300 nm; these

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were used to cross-link Glu for 24 h. The size of the prepared particles was equal to the diameter of the emulsion droplets. Riegger et al. [12] utilized an ultrasonic processor to assist in the formation of nano-sized W/O emulsions containing CS, which were used to cross-link Glu with stirring for 18 h. The obtained particle size was in the range 109.9–200.3 nm. However, these developed processes are time-consuming and difficult to perform in a continuous operation. Moreover, preparing uniformly sized NPs in a highly efficient manner or scaling up these techniques is still difficult [9,11,13]. The enhancement of the cross-linking reaction process has been neglected in the three abovementioned intensified/improved processes. Thus, a method to prepare uniformly sized NPs needs to be developed.

Hydrodynamic cavitation (HC) is an emerging technique used to intensify physical and chemical processes [14], e.g., heat and mass transfer [15], emulsification [16,17], esterification [18], and trans-esterification [19,20]. HC has been used to produce uniform and stable nano-size emulsions [16,17,21–25]. Tang and Sivakumar [16] used a liquid whistle hydrodynamic cavitation reactor to generate stable water-in-oil-in-water emulsions and observed that the droplet size of such emulsions was obtained at ca. 600 nm with a narrow droplet-size distribution PDI of 0.35–0.40. Ramisetty et al. [17] found that uniform nanoemulsions with a droplet size of 170 nm can be produced using HC, and the PDI decreased with increasing operating inlet pressure. Parthasarathy et al. [21] obtained the palm oil-based emulsions with the minimum droplet size of 476 nm and PDI of 0.5 under HC conditions. Detailed and extensive studies on emulsification using cavitation have been conducted by Patil and Gogate [22] and Sivakumar et al. [23–25]. Thus, HC has a great potential to intensify the emulsification process and the cross-linking reaction process for the synthesis of uniformly sized NPs.

However, no published report exists on the use of HC-intensified emulsion cross-linking process for chitosan NP synthesis. HC has shown its potential application in the synthesis of nanomaterials [26–28]. HC was used to intensify the ionic cross-linking process for the preparation of chitosan NPs by Zhang et al. [27], who found that the size of NPs prepared by HC was lower by 20.5% compared with that obtained by stirring. Also, Sonawane et al. [28] reported that orifice-based HC reduced particle size to 37 nm; calcite synthesized without HC was 101 nm in size. HC has the advantages of being able to process high volumes in a continuous operation, producing NPs in an energy-efficient manner, and being comparatively easy to scale up [17,29]. Therefore, studying the HC-intensified synthesis of chitosan NPs based on the conventional emulsion cross-linking process is of great significance.

The current study is a continuation of our previous research [30], in which a uniform W/O nanoemulsion containing CS was prepared using an ultrasonic reactor. The main objective was to evaluate the feasibility of introducing controlled HC to the emulsion cross-linking process to produce uniformly sized chitosan NPs. Another objective was to study the effect of various factors on NP properties, e.g., mean particle size, size distribution, surface morphology, or production yield. The influence of HC-induced effects on the molecular structure of oil was studied. The HC, acoustic, and conventional processes were compared in terms of NP properties and energy efficiency.

2. Materials and methods

2.1. Chemical reagents

CS with a molecular weight ($M_w$) of 400 kDa and degree of deacetylation (DD) of 90.3% was purchased from Zhongfayuan Biological Technology Co., Ltd. (Guangdong, China). Glutaraldehyde (Glu) solution (25% in H$_2$O, $\rho = 1.06$ g/mL), paraffin oil (PO), and kerosene (KS) were purchased from Xilong Scientific Technology Co., Ltd. (Guangdong, China). Crude palm oil (CPO), refined palm oil (RPO), soybean oil (SO), and corn oil (CO) were purchased from Julong Grain and Oil Co., Ltd. (Tianjin, China). *Jatropha curcas* seed oil (JCSO) was purchased from Shenyu New Energy Co., Ltd. (Yunnan, China). Span 80 (HLB = 4.3, C.P.) was purchased from Damao Chemical Reagent Factory (Tianjin, China). All other chemicals were of analytical grade. Deionized water was prepared by a Millipore apparatus (Millipore, Billerica, USA) and used in all experiment.

2.2. Synthesis of chitosan NPs using HC-intensified process

2.2.1. Formulation of W/O emulsions entrapping CS based on HC process

The HC-induced formation of W/O nanoemulsion containing CS was
performed with reference to our previous work [30]. First, CS was dissolved in an aqueous acidic media at a given pH value to prepare the solutions, and Span 80 was added to the oil phase with stirring at 55 ± 5 °C for 5 min. Then, CS solution was added dropwise into the oil phase and stirred at 55 ± 5 °C for 10 min to form a coarse W/O emulsion containing CS (emulsion A). The stirring rate was controlled at 600 rpm using a mechanical stirrer (Power is 130 W) with a digital display (S312, Yuxiang Instrument Ltd., China). The same experimental conditions in the preparation of emulsion A were used to prepare emulsion B, which was formed as follows: Glu solution (25% in H2O) was added dropwise into the same oil to form a coarse W/O emulsion containing Glu.

Then, the coarse emulsion A was further subjected to the HC process. The experimental set-up of HC with a circular venturi was schematically depicted in Fig. 1. The HC-produced system consisted of a holding tank, a centrifugal pump (operated at 1300 W), control valves (V1, V2, V3, V4, and V5), pressure gauges (P1 and P2), a circular venturi tube, and a capacitive target flowmeter. The inner diameter and the inner height of the holding tank were ca. 14 and 31 cm, respectively. The throat diameter and its length in circular venturi were 3.2 and 11.0 mm, respectively. As the centrifugal pump stated to run, the operating inlet pressure of venturi was adjusted by controlling V2 and was measured by P1. The HC-induced emulsification process was maintained for 20 min, during which a thermostat was applied to keep the operating temperature at 55 ± 5 °C by circulating cooling water (7 °C) through the jacket. Then, the uniform submicron or nano-size W/O emulsion A containing CS was formed.

Fig. 2. Variations of HC-prepared chitosan nanoparticles in MD (a), PDI (b), Py (c), and visual observation of the sediments before washing (d) and after washing (e), when the type of oil phase was changed.
2.2.2. Preparation of Glu cross-linked chitosan NPs through the HC process

The emulsion B containing Glu was continually added at 26 mL/min to the abovementioned nano-size emulsion A in the holding tank. Then, the droplets of CS-loaded emulsion A and the droplets of Glu-loaded emulsion B were further mixed and collided during the HC process. Subsequently, cross-linking reaction occurred, and CS was solidified as NPs suspended in the oil phase. Such HC-induced cross-linking reaction process was maintained at 55 ± 5 °C for 60 min. The suspensions were collected in glass beakers by opening the V5 and the water-draining nut of the pump. After cooling quickly with an ice water bath, the suspensions were centrifugated at 8000 rpm for 10 min. Finally, the sediment (i.e., chitosan NPs) collected in the centrifuge tubes was successively washed with petroleum ether and ethanol thrice and dried by vacuum drying at 50 °C for 12 h.

Fig. 3. SEM images of chitosan NPs produced at using different type of oil phase during HC-induced emulsion cross-linking process. PO and KS represent the paraffin oil and kerosene, and JCSO, CPO, RPO, SO and CO represent the *Jatropha curcas* seed oil, crude palm oil, refined palm oil, soybean oil and corn oil, respectively.
Unless otherwise specified, the standard formulation conditions were as follows. The operating inlet pressure \( P_{in} \) was kept at 3.0 bar. The volumes of the oil phase used for emulsions A and B were fixed at 2800 and 560 mL, respectively. The phase volume ratios (water to oil, \( \rho_{w} \)) for emulsions A and B were fixed at 12.5%, i.e., the volume of CS solution for emulsion A was 350 mL, and the volume of Glu solution for emulsion B was 70 mL. The Span 80 vol fractions (\( \rho_s \)) for emulsion A and B was fixed at 3.2%, i.e., 90 mL for emulsion A, and 18 mL for emulsion B. The initial CS concentration (\( c_s \)) was 10 g/L, and the acidic solvent for CS was sodium acetate–acetic acid buffer with an initial pH level of 3.6.

2.3. Characterization of the chitosan NPs and W/O emulsions

2.3.1. Analysis of particle size and PDI for chitosan NPs

The powder of chitosan NPs was dispersed into the deionized water following sonication for 10 min. Subsequently, a 1.0 mL water suspension was taken out and added into the sample cell. Then, the particle size and PDI of chitosan NPs were analyzed at a scattering angle of 90° with a refractive index of 1.33 at 25°C by using a dynamic light scattering (DLS) on the Zetasizer nano S90 (Malvern Instruments, UK). The size distribution of chitosan NPs was represented by the PDI value.

2.3.2. Droplet size and size distribution for W/O emulsions

The droplet size and size distribution of HD-produced W/O emulsions containing CS were measured using DLS on the Zetasizer nano S90 (Malvern Instruments, UK), as described previously [30]. The Z-average diameter represents the mean droplet size. PDI represents the accumulated analysis of width measurements for droplet size distribution.

2.3.3. Production yield of the chitosan NPs

After washing, the HD-produced chitosan NPs were dried at 50°C for 12 h in a vacuum drying oven (DZ-2AII, Taiste Instrument Ltd., China), and the total amount of dried chitosan NPs was weighed. Then, the production yield (\( P_y \)) of chitosan NPs was calculated as follows [5]:

\[
P_y(\%) = \frac{W_s}{W_1 + W_2} \times 100
\]

where \( W_s \) is the weight of the dried NPs, \( W_1 \) and \( W_2 \) are dry weights of the initially added Glu and CS, respectively.

2.3.4. Scanning electron microscopy (SEM) observation

The surface morphology of chitosan NPs was observed by using a scanning electron microscope (Phenom ProX, Eindhoven, Netherlands). Small amounts of chitosan NPs were fixed with double-sided adhesive on holders and were coated with a thin layer of gold in SBC-12 Mini Sputter Coater (KYKY Technology Ltd., China). The SEM test was performed at 10 kV acceleration voltage.

2.3.5. Physico-chemical characteristics

The change in chemical bonds of oil molecules was tested using Fourier transform infrared spectroscopy (FTIR, Perkin Elmer, USA) [30,31]. The FTIR spectra of pure palm oil and palm oil after the formation of HC-produced chitosan NPs were studied, and FTIR spectrophotometry was carried out with a scan range of 4000–400 cm\(^{-1}\). The aim of this test was to investigate the structural and bonding changes in palm oil when it was used as the oil phase and with HC-induced effects during chitosan NP synthesis.

3. Results and discussions

3.1. Selection of the oil phase

From a practical application perspective, the particle size and the PDI representing the size distribution of chitosan NPs have an important relationship with their function and purpose, e.g., target-oriented release for the drug [9,32]. The oil phases used as a suspension media in the emulsion cross-linking process can greatly affect the droplet size, uniformity, and stability of W/O emulsions due to their different physical properties (e.g., viscosity) [7,9,30], which can indirectly affect the particle size, the size distribution, and the surface morphology of chitosan NPs during the subsequent cross-linking reaction process.

Fig. 2a and b illustrate the variations of mean diameter (MD) and PDI of chitosan NPs with the type of oil phase used in the HC-intensified process. Obvious differences were found in the MD and PDI values of the HC-prepared NPs when paraffin oil (PO), kerosene (KS), and other vegetable oils (e.g., crude palm oil, CPO) were used as oil phases. MD and PDI values after using PO and KS were greater than those obtained after using vegetable oils except Jatropha curcas seed oil (JCSO). This result can be ascribed to the fact that the measured viscosity (\( \mu \)) of vegetable oils in this study in the range 44.8–61.2 mPa-s at 25°C was typically larger than that of KS (2.2 mPa-s) and PO (30.1 mPa-s). A higher shear stress can be generated in the water–vegetable oil–Span 80 emulsion system at the same emulsification strength during the HC process [30,33]. Such shear stress was more favorable for the dispersal of the water phase in the vegetable oil phase, thereby resulting in the formation of a finer and a more uniform W/O emulsion containing CS for Glu, i.e., emulsion A or B. Moreover, the higher viscosity for vegetable oils can lead to greater resistance when the dispersed water droplets approach one another [34]. These abovementioned conditions were conducive for obtaining a finer and a more narrowed size distribution of chitosan NPs during the subsequent HC-intensified reaction process. A similar result that the MD of CS microparticles using SO was smaller than that using PO was reported by Akbuğa and Durmaz [35], where CS microparticles were prepared by conventional ECLK. Nevertheless, the MD of chitosan NPs at 835.8 nm with PDI of 0.956 for JCSO was the highest compared with other vegetable oils, which was possibly due to the formation of unstable W/O emulsion A and B under identical experimental conditions that led to aggregation or coalescence among the internal phase water droplets, thus easily producing some undesired agglomerates during the following HC-intensified reaction (Fig. 3c). Herein, the PDI value was greater than 0.7, indicating a very broad size distribution for chitosan NPs [36].

Fig. 2c shows the variations of the production yield (\( P_y \)) of chitosan NPs with type of oil phase using the HC-intensified process. Unexpectedly, the \( P_y \) of PO (I.e., the most commonly used oil in the conventional approach) at 9.89% and KS at 2.36% was much less than that of any other vegetable oil, which was confirmed by the variations of sediments before and after washing with petroleum ether and ethanol for three times in the centrifuge tubes (Fig. 2d and e). For this result, the nano size W/O emulsion containing CS formed with PO or KS was typically equipped with high-viscosity, non-Newtonian pseudoplastic property [24,30], which could substantially increase the mass transfer resistance to the dispersed water droplets containing CS or Glu approaching each other, thus greatly reducing the opportunities of collision among the dispersed water droplets containing CS or Glu in the oil phase. Hence, the crosslinking reaction probabilities between the aldehyde groups of Glu and the –NH\(_2\) groups of CS was greatly reduced. Indeed, the measured \( \mu \) of water-in-PO emulsion (75.6 mPa-s) was considerably greater than that of water-in-CPO (37.5 mPa-s) at 55°C in this study. Nonetheless, the \( P_y \) of chitosan NPs at 61.62% for JCSO was the highest compared with the other vegetable oils. This result can be due to the formation of some undesired heavy agglomerates (Fig. 3c); the sediments became more difficult to be dried fully at the same drying time, which was found in the experiment, and residual moisture left in the particles might have contributed to the increase in weight.

Fig. 3 presents the surface morphological features of chitosan NPs with type of oil phase using the HC-intensified process. All the oil types except JCSO could be used to produce chitosan NPs during HC-intensified ECLK, among which the particles obtained were discrete with an ultrafine (e.g., submicron or nanoscale) size, a narrow distribution, a regular shape, and a smooth surface. However, as stated above, the \( P_y \) of chitosan NPs using PO or KS was less than 10% (Fig. 2c), and...
Effects of the addition mode of Glu solution on MD, PDI, $P_r$, and surface morphology of HC-prepared chitosan NPs.

| Addition mode of Glu solution | Addition rate (mL/min) | Addition time (min) | MD (nm) | PDI | $P_r$ (%) | General aspects observed |
|-------------------------------|------------------------|---------------------|---------|-----|----------|-------------------------|
| Glu solution added dropwise   | 2.8                    | 25.0                | 1489.7 $\pm$ 32.6 | 0.968 $\pm$ 0.032 | 35.57 $\pm$ 2.82 | Particles with non-uniform size, and with heavy agglomerates |
| Glu solution added dropwise   | 5.6                    | 12.5                | 1012.6 $\pm$ 35.4 | 0.973 $\pm$ 0.041 | 39.92 $\pm$ 2.17 | Particles with non-uniform size, and with a matte surface |
| Glu solution added dropwise   | 11.2                   | 6.3                 | 712.9 $\pm$ 33.5 | 0.852 $\pm$ 0.033 | 45.28 $\pm$ 4.15 | Particles with non-uniform size, and with a matte surface |
| W/O emulsion B (without Span80) | 26                     | 24.3                | 768.5 $\pm$ 0.712 | 0.075 $\pm$ 0.082 | 38.21 $\pm$ 3.22 | Agglomerate behavior among particles occurred |
| W/O emulsion B (without Span80) | 52                     | 12.2                | 452.3 $\pm$ 0.675 | 0.082 $\pm$ 0.565 | 43.46 $\pm$ 46.75 | Discrete and regular-shape particles |
| W/O emulsion B (without Span80) | 104                    | 6.2                 | 343.6 $\pm$ 0.069 | 0.053 $\pm$ 0.053 | 42.53 $\pm$ 2.26 | Discrete and regular-shape particles |
| W/O emulsion B                | 26                     | 25.0                | 317.2 $\pm$ 0.075 | 0.075 $\pm$ 0.075 | 45.64 $\pm$ 2.65 | Discrete particles with narrow distribution and regular shape |
| W/O emulsion B                | 52                     | 12.5                | 304.3 $\pm$ 0.432 | 0.398 $\pm$ 0.398 | 47.26 $\pm$ 2.83 | Discrete particles with narrow distribution and regular shape |
| W/O emulsion B                | 104                    | 6.3                 | 312.8 $\pm$ 0.062 | 0.062 $\pm$ 0.062 | 47.26 $\pm$ 2.83 | Discrete particles with narrow distribution and regular shape |

Table 1 also shows that at the same addition time, e.g., at 25 min, the $P_r$ of HC-prepared chitosan NPs was the highest, e.g., at 42.53%, for the addition mode of W/O emulsion B (with Span 80) compared with the two other addition modes. This result was expected because W/O emulsion B (with Span 80) had finer droplets, a narrower distribution, and more stability, which could lead to an increase in the total surface area [30,31], thus greatly increasing the opportunities of collision between emulsion droplets A and B in the oil phase during HC. Likewise, for the three addition modes, $P_r$ increased with reducing the addition time. This finding could be mainly due to the comparatively longer crosslinking reaction time between emulsion droplets A and B, when the specified volume of Glu solution (i.e., 70 mL) was added at a comparatively faster rate. In addition, the surface morphology of chitosan NPs had a slight change with increasing the addition rate for all the addition modes (Fig. S1 and Table 1). Fig. S1 shows that the discrete chitosan NPs with a narrow distribution, a regular shape, and a smooth surface could be formed using the addition mode of W/O emulsion B (with Span 80), which as a result, was selected to prepare the chitosan NPs during all the following experimental runs.

**Table 1** reveals unexpectedly that as the addition time was reduced from 25 min to 6.3 min, MD decreased from 1489.7 nm to 712.9 nm, and PDI decreased from 0.968 to 0.852 for the drop-by-drop addition mode; MD decreased from 768.5 nm to 343.6 nm, and PDI decreased from 0.712 to 0.565 for the addition mode of W/O emulsion B (without Span 80). These results may be because as the addition rate increased, the cavity collapse event increased due to the sudden increase of the amount of aqueous phase, which resulted in increased turbulence and shear [16]. Thus, MD and PDI decreased with increasing the addition rate.

**3.2. Selection of the addition mode of glutaraldehyde (Glu) solution**

Table 1 shows the variations of the MD, PDI, $P_r$, and surface morphology of HC-prepared Chitosan NPs with the addition mode of Glu solution while $P_{in}$, $\phi_{in}$, and $\phi_i$ were kept at 3.0 bar, 12.5%, and 3.2% respectively. The addition mode of Glu solution had remarkable effects on the MD and PDI of HC-prepared chitosan NPs. More specifically, as the addition rate was reduced to ca. 25 min, MD and PDI were 1489.7 nm and 0.968 for the drop-by-drop addition mode, respectively; 768.5 nm and 0.712 for the addition mode of W/O emulsion B (without Span 80), respectively; and 317.2 nm and 0.367 for the addition mode of W/O emulsion B (with Span 80), respectively. These results indicate that the addition mode of Glu-loaded W/O emulsion (with Span 80) is more favorable for obtaining a narrow distribution of chitosan NPs. Similar changes in MD and PDI values were found while the addition time was 12.5 or 6.3 min mainly because compared with the two other addition modes, the initial droplet size of Glu solution in the emulsion for the drop-by-drop addition mode was larger, and at a low operating pressure (i.e., 3.0 bar), HC alone provided the poor disruptive force when the initial stage of HC treatment was applied, thereby supplying the initial nonuniform droplets in the Glu-loaded W/O emulsion [16], which were subsequently reduced to fine droplets as the treatment time increased [16,17]. Moreover, compared with W/O emulsion B (with Span 80), larger droplets with broad distribution of W/O emulsion B (without Span 80) can be easily formed due to the coalescence or flocculation before addition to W/O emulsion A [16]. Simultaneously, the sequential use of stirring and cavitation was favorable for obtaining the monodispersity of the W/O emulsion containing Glu [22]. Thus, smaller MD and PDI values for the addition mode of W/O emulsion B could be obtained after crosslinking between emulsion droplets A and B.

The variation in PDI values as the addition time was reduced from 25 min to 6.3 min is shown in Table 1. The PDI decreased from 0.968 to 0.852 for the drop-by-drop addition mode; MD decreased from 768.5 nm to 343.6 nm, and PDI decreased from 0.712 to 0.565 for the addition mode of W/O emulsion B (without Span 80). These results may be because as the addition rate increased, the cavity collapse event increased due to the sudden increase of the amount of aqueous phase, which resulted in increased turbulence and shear [16]. Thus, MD and PDI decreased with increasing the addition rate. By contrast, as the addition rate increased, MD and PDI slightly changed for the addition mode of W/O emulsion B (with Span 80). This result was expected, which can be ascribed to the fact that the droplet size and its distribution of W/O emulsion B (with Span 80) had a slight change with increasing the addition rate. This finding could be mainly due to the comparatively longer crosslinking reaction time between emulsion droplets A and B, when the specified volume of Glu solution (i.e., 70 mL) was added at a comparatively faster rate. In addition, the surface morphology of chitosan NPs had a slight change with increasing the addition rate for all the addition modes (Fig. S1 and Table 1). Fig. S1 shows that the discrete chitosan NPs with a narrow distribution, a regular shape, and a smooth surface could be formed using the addition mode of W/O emulsion B (with Span 80), which as a result, was selected to prepare the chitosan NPs during all the following experimental runs.

**3.3. Influence of operating inlet pressure ($P_{in}$) on chitosan nanoparticles**

$P_{in}$ and cavitation number ($C_c$) are two major parameters that affect the condition of cavitation inside the cavitation device and the efficiency of the HC system [14,38]. $C_c$ quantifies the cavitation intensity of the cavitation device, which is a dimensionless number and can be expressed as follows [14,17]:

$$C_c = \frac{2(P_r - P_\gamma)}{\rho V^2}$$

(2)
where $P_v$ is the fully recovered pressure downstream of the venturi; $P_s$ is the saturation vapor pressure of water, which is equal to 15.733 kPa at 55 °C; $\rho$ is the density of water; and $V$ is the velocity of the emulsion through the throat of the venturi. Under the ideal condition, cavitation occurs for $C_v \leq 1$ [14,15]. Table 2 presents the $C_v$, throat velocity, and flow rate through the circular venturi at varying $P_{in}$. The rise of $P_{in}$ from 1.0 bar to 3.5 bar could lead to the reduction in $C_v$ from 0.884 to 0.124. Fig. 4 illustrates the variations of the MD, PDI, and $P_y$ of chitosan NPs with $P_{in}$. The MD and PDI values of chitosan NPs substantially decreased with increasing $P_{in}$ from 1.0 bar to the optimum value of 3.0 bar (Fig. 4 and Table 3). This result was expected. Nevertheless, when $P_{in}$ was continually increased to greater than 3.0 bar, the MD and PDI of chitosan NPs presented a slow decreasing trend with $P_{in}$ varying from 3.0 bar to 3.5 bar ($C_v = 0.124$), indicating that as $P_{in}$ was beyond the optimum pressure, the higher $P_{in}$ had a weakened effect on the particle size and size distribution of chitosan NPs. The reduction in MD and PDI values with increasing $P_{in}$ can be because at a lower $C_v$, a higher cavitation intensity resulted in an increase in the cavity collapse event and an increased turbulence and shear, which would lead to a higher micro mixing efficiency among the water–palm oil–Span 80 emulsion system [16,17,39], and then a finer, more uniform CS-loaded W/O emulsion, i.e., emulsion A, could be formed, which was confirmed by DLS test (Fig. 5 and Table 3). Similarly, as the Glu-loaded W/O emulsion, i.e., emulsion B, was added to emulsion A, a finer, more uniform Glu-loaded W/O emulsion would be formed before dispersed droplet A approached droplet B in the oil phase during HC-induced emulsification. Simultaneously, the crosslinking reaction between the aldehyde (–CHO) groups of Glu and the –NH$_2$ groups of CS was triggered by the collision between emulsion droplets A and B, and then chitosan NPs could be produced during the HC-induced reaction. Such an HC-induced emulsion–droplet crosslinking was somewhat similar to the emulsion–droplet coalescence reported by Tokumitsu et al. [40], in which CS-loaded W/O emulsion droplet and sodium hydroxide-loaded W/O emulsion droplet were mixed and stirred to solidify CS due to the collision and coalescence among the droplets, and then the chitosan NPs with a MD of 452 nm were obtained.

Fig. 5 shows the droplet size distribution (DSD) of CS-loaded W/O emulsion at different $P_{in}$. The rise of $P_{in}$ could lead to the droplet size distribution curve shifting gradually to the region of small droplet diameter, and the size distribution became narrower. This trend agrees with the findings reported by Wu et al. [26]. However, Amin et al. [41] found no free radical produced at $P_{in}$ below 6.8 bar while using salicylic acid dosimetry to optimize the HC reactor, suggesting that the free radicals (e.g., -OH) cannot be produced under a $P_{in}$ of 1.0–3.5 bar in this work. From the aspect of DSD at various $P_{in}$, the physical effects contributed by this relatively low cavitation intensity was sufficient for the mixing requirement between the oil and aqueous phases. Furthermore, previous studies confirmed that the presence of nano aggregates in liquids does not exhibit any noticeable effects on the cavitation threshold through a cavitating device [28,42], suggesting that the sub-micron/nano size CS particles have a negligible effect on the occurrence of cavitation.

Fig. 4 also shows that the $P_y$ of HC-prepared chitosan NPs considerably increase with rising $P_{in}$, which is mainly due to the increase of cavitation intensity at rising $P_{in}$, thus leading to a higher mixing efficiency and mass transfer rate [15,43]. Table 3 shows an acceptable

- **Table 2**

| Pressure (bar) | Flow rate through cavitation device (L/h) | Throat velocity (m/s) | Cavitation number ($C_v$) |
|---------------|------------------------------------------|-----------------------|---------------------------|
| 1.0           | 403                                      | 13.92                 | 0.884                     |
| 2.0           | 705                                      | 24.35                 | 0.289                     |
| 3.0           | 962                                      | 33.23                 | 0.155                     |
| 3.5           | 1076                                     | 37.16                 | 0.124                     |

- **Table 3**

| $P_{in}$ (bar) | 1.0 | 2.0 | 3.0 | 3.5 | Linear regression model | $R^2$  |
|---------------|-----|-----|-----|-----|------------------------|-------|
| Droplets size of CS-loaded emulsion before crosslinking (nm) | 625.9 | 422.7 | 358.4 | 296.7 | $y = -1.256271x + 725.3644$ | 0.9494 |
| CS particle size after crosslinking (nm) | 656.8 | 398.1 | 317.2 | 286.5 | $y = -1.441322x + 757.0389$ | 0.9039 |

Fig. 4. Variations of MD, PDI and $P_y$ values of chitosan NPs with $P_{in}$ during HC-intensified emulsification cross-linking process.
linear relationship between the $P_{in}$ and droplet size of CS-loaded W/O emulsion ($R^2 = 0.9494$) and a similar linear relationship between the $P_{in}$ and MD of chitosan NPs ($R^2 = 0.9039$), suggesting a positive correlation between the MD of Chitosan NPs and the droplet size of CS-loaded W/O emulsion at rising $P_{in}$. Moreover, Fig. S2 shows that the chitosan NPs produced at $P_{in}$ of 3.0 bar are discrete with a uniform size, a regular shape, and a smooth surface. Wu et al. [43] found that a high inlet pressure is not conducive to energy utilization. Hence, $P_{in}$ of 3.0 bar was selected to prepare Chitosan NPs during all the following experimental runs.

### 3.4. Effect of the rheological properties of chitosan (CS) solution

#### 3.4.1. Effect of the type of acidic solvent

The rheological properties of CS solution are greatly dependent on the feature of acidic solvent (e.g., solvent type and pH value) [44,45], which probably have a close relationship with the characteristics of Chitosan NPs. Table 4 shows the variations of MD, PDI, $P_y$, and surface morphology of HC-prepared chitosan NPs with type of acidic solvents for CS at an initial pH of ca. 2.18. The acidic buffer was prepared with 0.1 mol/L citrate and 0.2 mol/L disodium hydrogen phosphate. **The products obtained from the given initial pH of the acidic solvent for CS were not the chitosan micro- or nano-particles.**

| Acidic solvent type                      | $c_{as}$ (vol %) | initial pH | MD (nm) | PDI   | $P_y$ (%) | General aspects observed                        |
|----------------------------------------|-----------------|------------|---------|-------|----------|-----------------------------------------------|
| Formic acid                            | 1.0             | 2.16       | ——***   | ——*** | 34.62 ± 3.35 | No stable chitosan NPs formed.                |
| Acetic acid                            | 10.0            | 2.18       | ——***   | ——*** | 35.57 ± 4.82 | No stable chitosan NPs formed.                |
| Citrate-disodium hydrogen phosphate buffer | ——***          | 2.20       | 1485.5 ± 26.7 | 0.936 ± 0.158 | 41.28 ± 3.67 | Discrete particles with non-uniform distribution, but with a good smooth surface |

$c_{as}$ represents the concentration of acidic solvent.

**The acidic buffer was prepared with 0.1 mol/L citrate and 0.2 mol/L disodium hydrogen phosphate.

***The products obtained from the given initial pH of the acidic solvent for CS were not the chitosan micro- or nano-particles.

Fig. 5. Droplet size distribution of HC-prepared W/O emulsion containing CS with $P_{in}$ when palm oil was used as oil phase: (a) 1.0 bar; (b) 2.0 bar; (c) 3.0 bar; (d) 3.5 bar.

Table 4

Variations of MD, PDI, $P_y$ and surface morphology of HC-prepared chitosan NPs with type of acidic solvents for CS at an initial pH of ca. 2.18.
broad particle-size PDI of 0.936 (Table 4). Accordingly, the MD and $P_y$ for using C-DHPB were measured at 1485.5 nm and 41.28%, respectively (Table 4).

Acetic acid is one of the most commonly used acidic media for CS, and its concentration ($c_{ac}$) used to prepare CS spheres during the conventional ECLK method is typical at 1%–5% [5,6,35,46–48]. The preparation was repeated thrice using the stirring-based method to ascertain whether HC-induced effects prevented the formation of CS spheres at a pH of ca. 2.18 for formic acid (i.e., $c_{af} = 1\%$) or acetic acid (i.e., $c_{ac} = 10\%$) while they were used as acidic solvents for CS. As expected, a certain amount of sediments was formed for 1% formic acid (Fig. S3.c) and 10% acetic acid (Fig. S3.d), but these sediments were also not Chitosan NPs. These results were confirmed by SEM test (Fig. 6.d and e), where the surface of the dried sediments also presented a rough, uneven, and fractured structure (Fig. 6.d and e). These results indicate that HC-induced effects have a minimal effect on the formation of CS spheres while acetic acid or formic acid is used as a solvent for CS with pH of ca. 2.18. These findings may be due to the differences of molecular conformations and physico–chemical behaviors for polysaccharide CS in various acidic solvents with a certain pH value [44,49]. Therefore, an important conclusion can be drawn, namely, the formation of Chitosan NPs is greatly dependent on the acidic solvent for CS with a certain pH value regardless of the existing HC effects.

### 3.4.2. Effect of the pH value of the acidic solvent

The rheological properties of CS solution greatly depend on the pH of an acidic solvent [44,45]. SA-ACBs are one of the commonly used acidic solvents for CS and have a wide range of pH [44]. Table 5 presents the variations in the MD, PDI, $P_y$, and surface morphology of HC-prepared Chitosan NPs with different pH values of SA-ACB. The MD and PDI values of Chitosan NPs increased when the pH of SA-ACB increased from 3.6 to 4.0, and the surface morphology of Chitosan NPs produced at pH 4.0 became irregular (Fig. S4). Thus, the increase in the pH of SA-ACB is not conducive to obtain fine Chitosan NPs with a narrow distribution (Fig. S4). Furthermore, the dried samples produced as the pH of SA-ACB continually increased from 4.6 to 5.0 were not Chitosan NPs but large...
The products obtained at the given pH value of SA-ACB were not chitosan. **SA-ACB** is the sodium acetate-acetic acid buffer with different pH values. Variations of MD, PDI, and surface morphology of HC-prepared Chitosan NPs could not be formed as $c_f$ increased from 1.0 g/L to 5.0 g/L (Fig. S5) even though the $P_y$ for each $c_f$ ranged between 3.29% and 16.08%. This result indicates that a low $c_f$ cannot support the formation of Chitosan NPs. Chitosan NPs were formed when $c_f$ was increased to 10.0 g/L (Fig. S5), and the MD and PDI values increased with the increased in $c_f$. $P_y$ also presented an increasing trend with the rise in $c_f$ from 1.0 g/L to 15.0 g/L during HC-intensified ECLK.

The results were expected because the non-Newtonian properties of the dilute polysaccharide CS solution, which had a low viscosity, were comparatively less at low $c_f$ (1.0–5.0 g/L), therefore, Newtonian behavior exhibited a dominant role [52]. Then, finer CS-loaded W/O emulsion droplet could be formed because of the lesser HC resistance in breaking up the liquid under the same $P_y$ [30]. We found that the net CS content in each emulsion droplet used to crosslink Glu was too small to form Chitosan NPs. This result is well in accordance with that reported by Varma and Sadasivan [53], who found that CS microspheres could not be produced using ECLK approach when $c_f$ is below 5.0 g/L. The same result was also given by Al-Helw et al. [47]. Nevertheless, non-Newtonian behavior exhibited a dominant role in concentrated, highly viscous CS solutions with high $c_f$ (10.0–15.0 g/L) [52], and the droplet size of the W/O emulsions increased because of the increase in HC resistance under the same $P_y$ [30]. As a result, the net CS content of each emulsion droplet used to crosslink Glu was enough to form Chitosan NPs. The same result was reported by Denkbas et al. [46] and Al-Helw et al. [47]. The increase in $P_y$ with the increase in $c_f$ could be ascribed to the fact that the amount of CS used to crosslink Glu increased with rising $c_f$, simultaneously, ECLK could be performed in a highly efficient way because of the intensified mass and heat transfer in the liquid during the HC process [15,43].

### 3.5. Effect of HC-induced effects on oil microstructure

Previous studies confirmed that HC-induced submicron or nanoscale emulsions are produced by the physical effects generated from violent cavity collapse in liquids, such as high-speed microjets, mechanical vibrations, microstreaming, and local high turbulence [14,17,21]. Moreover, chemical effects can be generated through the thermal breakdown of larger molecules into smaller molecules under local hot spots or through the formation of free radicals (–OH) from the dissociation of water molecules [14,25]. Thus, the FTIR spectrum of palm oil collected after HC-induced crosslinking was compared with that of pure palm oil as shown in Fig. S6 to find out the potential influence of HC-induced chemical effects on the structure of palm oil during the HC-intensified preparation of Chitosan NPs.

As illustrated in Fig. S6, the FTIR spectra between pure palm oil and HC-processed palm oil collected from the end of the crosslinking reaction process have no difference. Therefore, the molecular structure of palm oil was unaffected by HC. Furthermore, key functional groups, such as aldehydes ($C=O$ stretch at 1750–1625 cm$^{-1}$) and ketones ($C=O$ stretch at 1750–1625 cm$^{-1}$), and carboxylic acid ($C=O$ stretch at 1730–1650 cm$^{-1}$ and hydrogen bonded O–H stretch at 3400–2400 cm$^{-1}$), were not found in the HC-processed palm oil. This result indicates that no secondary oxidation products were produced during HC. This result agrees well with the findings reported by Ravidadaran et al. [31], who found that no oxidation products were formed.
### Table 7
Comparison results among HC process, acoustic cavitation, mechanical stirring (MS)-based and conventional drop-by-drop process for preparing the chitosan NPs.

| Parameters                        | HC process | Ultrasound | MS-1000 | MS-2000 | Conventional |
|-----------------------------------|------------|------------|---------|---------|--------------|
| Oil phase                         | Palm oil WH/O emulsion | Palm oil WH/O emulsion | Palm oil WH/O emulsion | Palm oil WH/O emulsion | Paraffin oil |
| Adding mode of Glu                |            |            |         |         |              |
| Acidic solvent                    | SA-ACB     | SA-ACB     | SA-ACB  | SA-ACB  | Acetic acid  |
| pH of acidic solvent              | 3.6        | 3.6        | 3.6     | 3.6     | 2.4 – 2.8    |
| c (g/L)                           | 10         | 10         | 10      | 10      | 10 – 20      |
| Total emulsification time (min)   | 60         | 60         | 60      | 60      | 150 – 360    |
| Cross-linking reaction time (min) |            |            |         |         |              |
| RPM                               | —          | —          | 1000    | 2000    | 250 – 2000   |
| Ultrasonic power (W)              | —          | 300        | —       | —       | —            |
| Inlet pressure (bar)              | 3.0        | —          | —       | —       | —            |
| MD (nm)                           | 317.2 ± 16.9 | 621.5 ± 20.7 | 654.8 ± 21.4 | 632.7 ± 26.5 | greater than 1000 |
| PDI (%)                           | 42.53 ± 2.26 | 41.62 ± 3.58 | 36.72 ± 4.23 | 40.36 ± 4.65 | ≤ 79.00 ± 4.60 |
| General aspects observed          | Discrete particles with uniform size, regular shape, a smooth surface and good morphological and good surface defect | Particles with obvious non-uniform size, irregularly-shaped agglomerate and partially surface defect | Particles with spherical geometry, but with non-uniform size, serious adhesion and surface-breaking defect | Particles with spherical geometry, but with non-uniform size, irregular fragments and surface-breaking defect | Stable CS microspheres formed with regular shape, and smooth surface, but with a micron-size and broad distribution |

The data of conventional process were obtained from the references at [5], [6], [35], [46], [47] and [48].

SA-ACB is the sodium acetate-acetic acid buffer that was used to dissolve CS.

The conventional process represents the Glu solution (25% in H2O) was added dropwise into the W/O emulsion containing CS, and both the emulsification and the crosslinking process are fulfilled with stirring.

---

**Fig. 7.** SEM images of chitosan NPs produced using different processes based on emulsion cross-linking (ECLK) approach: (a) HC-induced ECLK process; (b) ultrasound-induced ECLK process; (c) stirring-based ECLK process at 1000 rpm; (d) stirring-based ECLK process at 2000 rpm.
under ultrasonic assisted water-in-palm oil nanoemulsion. Similarly, Zhang et al. [30] reported that ultrasonic-induced chemical effects cannot affect the molecular structure of paraffin oil during the ultrasonic-assisted formation of CS-loaded W/O emulsion. This result indicates that from the point of view of engineering application, palm oil can be reused as a suspension medium for the synthesis of Chitosan NPs during HC-induced ECLK.

3.6. Comparison between HC, acoustic cavitation, stirring-based and conventional drop-by-drop processes

We investigated the extent of the intensification of Chitosan NP synthesis by comparing cavitation techniques (e.g., HC and ultrasound) and conventional stirring method. For ultrasound or stirring-based process, the volumes of palm oil used for preparing CS-loaded and Glu-loaded emulsions were 120 and 30 mL, respectively, and the W/O emulsions that contain CS or Glu were prepared using the same composition as in the HC-induced process, that is, 12.5% (v/v) of phase volume ratio (W/O) and 3.2% (v/v) of Span 80 vol fractions. Sonication was performed at an amplitude of 32% using the ultrasonic horn (SCI-ENTZ, China; 950 W and 25 kHz) as shown in our previous work [30]. The stirring rates were 1000 and 2000 rpm. The experimental runs were carried out at 55 °C, and the results are listed in Table 7. The surface morphology of the Chitosan NPs produced using HC, ultrasonic horn, and stirring-based processes are shown in Fig. 7.

Table 7 shows that the $P_y$ of HC was marginally higher compared with the other processes. However, the MD and PDI of Chitosan NPs were at 317.2 nm and 0.367 for HC and 621.5 nm and 0.722 for ultrasonic horn, respectively. This result indicates the formation of much smaller Chitosan NPs with narrower distribution by HC as compared with ultrasonic horn. This outcome is possible because of the local occurrence of cavitation events near the transducer surface of the ultrasonic horn, the non-uniform cavitation intensity in the liquid [19,31], and the easier occurrence of coalescence or flocculation among the newly disrupted droplets triggered by gravity in the limited space of the vessel [30]. This result was confirmed by SEM as shown in Fig. 7. The Chitosan NPs obtained from ultrasonic horn were non-uniform in size, irregularly shaped, agglomerated, and have partial surface defect. In mechanical stirring, the MD of Chitosan NPs using the addition mode of Glu-loaded W/O emulsion (i.e., stirring-based emulsion–droplet cross-linking) at the stirring rate of 1000 rpm was 654.8 nm. This MD is smaller (i.e., micron size) than that obtained by adding Glu solution dropwise at the stirring rate of 1500 rpm [5,46]. MD was slightly reduced to 632.7 nm and PDI was 0.758 when the stirring rate was increased to 2000 rpm; thus, the prepared Chitosan NPs still had a broad distribution (Table 7). However, as seen in Fig. 7, the Chitosan NPs prepared at the stirring rate of 2000 rpm have a spherical geometry but with irregular fragments and surface-breaking defect. This outcome is probably due to the fierce collision among the newly-formed Chitosan NPs at high stirring rate. A similar outcome was reported by Rai et al. [54], who found that increasing the agitation rate from 1000 rpm to 1500 rpm could lead to irregularly shaped microparticles and surface defect in the prepared CS hydrochloride microparticles.

The energy efficiencies of HC, ultrasonic horn, and stirring-based processes were also compared. Energy efficiency was calculated based on the procedure suggested by Carpenter et al. [39]. The results of the energy efficiency evaluation are shown in Appendix A. The energy utilized for the change in the dried weight of particles was $7.788 \times 10^8$ J/kg (m/kg) in HC, 5.831 $\times 10^8$ J/(m/kg) in ultrasonication, and 8.229 $\times 10^8$ J/(m/kg) in the stirring-based process. This result indicates that the energy required to treat a unit volume of emulsion liquid by obtaining the unit weight of Chitosan NPs was lower compared with ultrasonication or stirring-based process. The same result was also found for the energy required to treat a unit volume of emulsion liquid based on the unit reduction in the particle size of Chitosan NPs. HC also has a high energy efficiency in the formation of mustard oil-in-water nanoemulsion [39] and the interesterification process for the synthesis of biodiesel [55] compared with ultrasonication or stirring-based process.

Besides, in conventional drop-by-drop approach, wherein Glu solution (25% in H$_2$O) was added dropwise into the water-in-paraffin oil emulsion containing CS by stirring, the MD of the prepared CS particles is at a micron-size level and the size distribution of these CS microparticles are very broad [5,6,35,46–48]. The high $P_y$ (79.0%) obtained by conventional drop-by-drop approach is due to the long crosslinking reaction time, which lasted for 6.0 h [5]. Thus, the conventional drop-by-drop approach is an energy-intensive and time-consuming process for obtaining high $P_y$. In summary, compared with ultrasonic horn, stirring-based process, and conventional drop-by-drop process, the introduction of HC-induced effects to ECLK can produce finer Chitosan NPs with a narrower distribution in a more energy-efficient manner.

4. Conclusions

The present work established a novel route for the application of HC to intensify the emulsification and crosslinking processes in the preparation of Chitosan NPs based on ECLK. The newly developed route is superior compared with conventional drop-by-drop approach. The minimum MD of 286.5 nm and the PDI of 0.342 were obtained at a $P_y$ of 3.5 bar, a $\phi_w$ of 12.5%, a $\phi_f$ of 3.2%, a HC-induced emulsification time of 20 min, and a HC-induced crosslinking time of 60 min. $P_y$ was 46.62% at this operational condition. Vegetable oil, such as palm oil, soybean oil, and corn oil, was more appropriate than paraffin oil as the oil phase for the HC-intensified processes, and the addition mode of water-in-oil (W/O) emulsion containing Glu (with Span 80) was favorable to obtain narrowly distributed Chitosan NPs. The rise in $P_y$ was beneficial to obtain smaller and more uniform-size Chitosan NPs due to the formation of smaller W/O emulsion droplets containing CS or Glu with narrower distribution at higher $P_y$. The formation of Chitosan NPs could be controlled to produce Chitosan NPs with discrete, smooth, and regular shape features. The particle size, size distribution, and formation of Chitosan NPs were greatly dependent on the rheological properties of the CS solution. FTIR analysis indicated that the molecular structure of palm oil as a suspension medium was unaffected by HC-induced effects. Overall, the work clearly demonstrated the feasibility of HC as an energy-efficient approach for intensifying the ECLK synthesis of Chitosan NPs compared with ultrasonic horn and conventional drop-by-drop process. The novel approach can greatly reduce the particle size and distribution of synthesized Chitosan NPs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Energy efficiency evaluation of HC, ultrasonic horn and stirring-based processes

A.1. Energy efficiency evaluation of hydrodynamic cavitation process

(Pump power: 1300 W, operating pressure: 3 bar, processing time for emulsification: 20 min, volume: 3.240 L, and processing time for crosslinking: 60 min, volume: 3.888 L)

The mean diameter and the dried weight of particles obtained was respectively 317.2 nm and 9.42 g (i.e., crosslinking: 60 min, volume: 3.888 L, and processing time for 9.42 g obtained after crosslinking 3.0 bar-initial dried weight at 3.0 bar).

For emulsification, electrical energy supplied to pump in 20 min = 1300 J/s × 20 min × 60 s = 156000 J = 156 kJ

For crosslinking, the electrical energy supplied to pump in 60 min = 1300 J/s × 60 min × 60 s = 4680000 J = 468 kJ

Energy density for emulsification = Electrical energy supplied/volume = 1560 kJ/(3.240 L × 10^3 m^3) = 481481.48 (kJ/m^3)

Energy density for crosslinking = Electrical energy supplied/volume = 4680 kJ/(3.888 L × 10^3 m^3) = 1203703.70 (kJ/m^3)

Total Energy density = 481481.48 (kJ/m^3) + 1203703.70 (kJ/m^3) = 1685 × 10^3 (kJ/m^3)

Change in particle size—(Initial size-size obtained after crosslinking at 3.0 bar) = (1000 nm-317.2 nm) = 682.8 nm

Change in dried weight of particles—(dried weight of particles obtained after crosslinking 3.0 bar-initial dried weight) = (9.42 g-0 g) = 9.42 × 10^-3 kg

Energy utilized for the change in particle size—Total energy density/change in particle size = 1.685 × 10^6 (kJ/m^3)/ 682.8 nm = 2468.65 kJ/(m^3 nm)

Energy utilized for the change in dried weight of particles—Total energy density/change in dried weight of particles = 1.685 × 10^6 (kJ/m^3)/9.42 × 10^-3 (kg) = 1.788 × 10^8 kJ/(m^3 kg)

A.2. Energy efficiency evaluation of ultrasonic horn process

(Power of horn: 950 W, processing time for emulsification: 20 min, volume: 138.9 mL, and processing time for crosslinking: 60 min, volume: 173.7 mL)

The mean diameter and the dried weight of particles obtained was respectively 632.7 nm and 0.46 g (i.e., P_y = 42.53%) using HC at an approach of chitosan nanoparticle preparation via emulsion crosslinking as a carrier of protein drug, J. Controlled Release 106 (1-2) (2005) 62–75.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2021.105551.

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