International Journal of GEOMATE, Jan., 2020, Vol.18, Issue 65, pp. 124-131
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2020.65.73202
Geotechnique, Construction Materials and Environment

EFFECT OF COMBINED BETWEEN MICROBUBBLE AND ULTRASONIC OF C-PHYCOCYANIN EXTRACTION FROM *S. PLATENSIS*

Phirunrat Thaisamak, Somkiat Jaturonglumlert*, Jaturapatr Varith, Kanjana Narkprasom and Chanawat Nitatwichit

Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, Thailand

*Corresponding Author, Received: 06 Sept. 2019, Revised: 04 Oct. 2019, Accepted: 25 Oct. 2019

ABSTRACT: The aims of this work were to approve the performance of the combination between microbubble and ultrasonic with controlled temperature on CPC extraction from *S. platensis*. The influence of microbubble combined with ultrasonic and controlled temperature (MBUT) was investigated under various ultrasonic frequency (28, 45, 100 kHz), various of microbubble size (30, 40, 50 µm) and various of controlled temperature (308.15, 313.15, 318.15 K). This study suggested that MBUT employed reduce the extraction time, and improved extraction efficiency namely diffusion coefficient and enhancement factor. The diffusion coefficient obtained from experimental data was in the range of 1.959×10^{-11} to 2.415×10^{-11} $m^2.s^{-1}$. The modified correlation was established based on theoretical diffusion in liquid (Stoke & Einstein equation). The correlation of solution viscosity as functions of microbubble size, ultrasonic frequency, and temperature was also established as an empirical model for calculating the viscosity using in the modified model. A good agreement between experimental data and the modified correlation predicted was achieved lower than 15% as the reported of %Error. Thus, these combination techniques were found to be effective for the extraction of CPC from wet *S. platensis* and this technique can be employed for other bioactive compounds.

Keywords: *C-phycocyanin (CPC), Ultrasonic, Microbubble, S. platensis, Diffusion coefficient*

1. INTRODUCTION

Spirulina platensis (*S. platensis*) or Arthrospira platensis (*A. platensis*) is a microalga consisting of several valuable compounds. CPC is a major group of phycobiliproteins in *S. platensis*, which are brightly colored pigments and soluble in water. It is commonly used as a natural colorant in the food and cosmetic industries [1].

The extraction of CPC from *S. platensis* is a series of mass transfer processes. The mechanism is consists of two main steps: internal and external mass transfer. The internal transfer is diffusion of CPC from interior side to surface of solid particle. Then, the CPC penetrates to fluid, travels, and diffuses within until the CPC in fluid phase reaches equilibrium (external transfer). This mechanism can be represented by kinetic, mathematical model and can be solved to obtain the mass transfer parameter (diffusion coefficient, etc.). The appropriate model and mass transfer parameter can be utilized to facilitate the scale-up from laboratory data into industrial-scale design.

Molecular diffusion or diffusion coefficient (D_{AB}) is the most essential kinetic parameter which describes the mass transfer of solute to solvent during the extraction. The kinetic study cannot probably be pursued without information about the diffusion coefficient. However, in practical experimental system, it is preferable to measure diffusion coefficient because it remarkably varies depending on the experimental condition such as the viscosity of solvent, temperature, and size of solute molecule [2]. There are several experimental methods for measurement of diffusion coefficient, for example, the optical method, spectroscopic method. These methods are effective for accurate measurement. However, some of them need to use analytical instruments, which makes the measurement costly and some of them are tedious and complicated [2].

Many researchers have been conducted several extraction methods of CPC from *S. platensis* such as homogenization, repeat freeze-thaw (RFT), shaker, ultrasonic and ultrafine shearing. However, these work only focus on finding efficient methods, and a bit of kinetic study and modeling [1, 3, 4].

Ultrasonic assisted extraction (UAE) is a technique capable of enhancing its mass transfer mechanism in extraction process. UAE provides cavitation and thermal effect to extraction solvent, as well as structural effect to the solid particle. Among the advantages of sonication, the reduction of particle size, rupture of cell walls are also observed when cavitation occurs, this phenomenon may be highlighted for enhancement mass transfer. Nevertheless, the controlling extraction temperature should be observed in the case of the thermal sensitive bioactive compound such as CPC, the extraction temperature should maintain lower than 50 °C [1]. Ultrasonic assisted extraction with controlled temperature (UAET) is an alternative technique used to extract bioactive compounds [5, 6].

Microbubble (MB) is a tiny bubble with a respective diameter of 10-50 µm and have been explored for various application such as used in yeast industry, biomass production, also use in the separation process, etc. [7]. Using microbubble is not extensive in extraction process. Masuda et al. (2015) studied the combination between microbubble and various frequency of ultrasonic to suppressed free radical formation in aqueous solution [8].

To date, the publications on the establishment of microbubble combined with ultrasonic performance in CPC extraction and their kinetics are not extensive. Therefore, in this work, the experimental study of effect of microbubble combined with ultrasonic and controlled temperature (MBUT), and mass transfer parameters in finding diffusion coefficient, and enhancement factors were investigated. Furthermore, the modified correlation of diffusion coefficient would be presented.

2. MATERIALS AND METHODS

2.1 Raw Material and Chemical

2.1.1 Microorganism and culture medium

S. platensis was supplied by the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Thailand. The culture medium was supplemented with 16 $g.L^{-1}$ sodium bicarbonate ($NaHCO_3$), 2.5 $g.L^{-1}$ sodium nitrate ($NaNO_3$), 1 $g.L^{-1}$ sodium chloride (NaCl), 0.5 $g.L^{-1}$ di-potassium hydrogen phosphate (K_2HPO_4), and 0.2 $g.L^{-1}$ magnesium sulfate ($MgSO_4$) [9]

2.1.2 Culture and harvesting

All the experiments involved the utilization of *S. platensis* that was cultivated by a smart control algae system [10]. The growth of *S. platensis* was evaluated by measuring the optical density of biomass using a spectrophotometer at a wavelength of 680 nm. The initial optical density of the *S. platensis* was recorded, while harvesting was only carried out when the optical density of about 1.2 has been reached. Freshly harvested biomass was thoroughly washed twice using the ozone water, to remove all the culture media components. The moist *S. platensis* obtained was stored at -10°C up until it is being used for the experiment.

2.2 Extraction procedure

All experiments involving CPC extraction were conducted using 0.1 M sodium phosphate buffer (pH 7.0) as the solvent. All the chemicals used in this study were analytical grade.

2.2.1 Effect of microbubble

To ensure the performance of microbubble, the experiment was investigated that the extraction was conducted with microbubble (MBUT) and without microbubble which the method is ultrasonic-assisted extraction with controlled temperature (UAET) that Thaisamak et.al (2019) have been studied. The condition of the experiment was defined as the optimal condition found in their work that was controlled temperature of 45 ° C, 30 minutes, solid to liquid ratio of 1:5 w/v [6].

2.2.2 Effect of ultrasonic frequency

The effect of ultrasonic frequency has investigated. The experiments were performed at various ultrasonic frequency (28, 45, 100 kHz). The microbubble size and controlled temperature were fixed at 30 µm and 318.15 K, respectively.

2.2.3 Effect of microbubble size

The size of microbubble is a factor affect extraction, so the present of mass transfer under various microbubble size has investigated. Since the diameter of microbubble are usually considered to be 50 µm or less than.

The digital microscopic (1000x) was set to take a picture, the size of bubble was analyzed by image analysis (PortableCapture Pro, version 2.1). The effect of microbubble size has investigated by fixed the optimal ultrasonic frequency obtained from previous section, while the temperature was fixed at 318.15 K. The experiments were performed at various of microbubble size (30, 40, 50 µm).

2.2.4 Effect of controlled temperature

The effect of controlled temperature has investigated, while the ultrasonic frequency and microbubble size were fixed following the obtained from previous section. The experiments were performed at various of controlled temperature (308.15, 313.15, 318.15 K).

Fig.1 shows the schematic diagram of the MBUT experiment. The extraction process starts by making salt solvent after that turn on microbubble pump to making microbubble salt solvent. The heater is also turn on to maintain the temperature. After the microbubble salt is ready (around 20-40 minutes), the *S. platensis* is take into the extraction chamber then the ultrasonic transducer is turn on. During the experiment, the mixture was sampling to collect the data of CPC concentration.

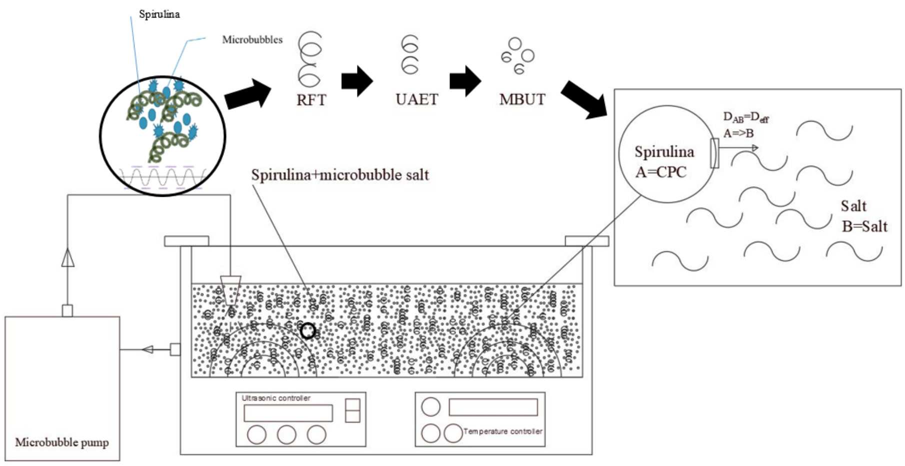

Fig. 1. Schematic sketch of experiment setup

2.2.5 Enhancement factor

In order to quantify the microbubble effect, the enhancement factor (EF) was used. It is defined by the ratio of CPC concentration obtained from various methods (CPC) and CPC concentration obtained from conventional method that is repeat freeze-thaw (RFT) (CPC_o).

$$EF = \frac{CPC}{CPC_o} \tag{1}$$

2.2.6 CPC analysis

All the extracted CPC samples were analyzed according to the procedure carried out by Boussiba & Richmond (1979). The supernatant obtained was analyzed at wavelengths of 620 and 652 nm, using a spectrophotometer (Model SPECTROSC, USA). The concentration and yield of CPC were estimated using Eq. (2) and Eq. (3) [11], respectively.

$$CPC = \left(A_{620} - 0.474A_{652}\right)/5.34 \tag{2}$$

$$Yield = \left(CPC \times V\right)/d \tag{3}$$

Where A_{620}, A_{652} are the absorbance wavelength at 620 and 652 nm, V is the volume (ml), and d is the dry weight (g dry) of the biomass.

2.2.7 Statistical Analysis

The results were expressed as the mean ± standard deviation (mean ± SD) of triplicate data set, from an independent experiment. Statistical analysis was performed using the analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). The differences were considered significant if $p < 0.05$ (95 % confidence interval).

2.3 Mass Transfer Parameters

2.3.1 Determination of diffusion coefficient

A one-dimension at unsteady state diffusion model, i.e. derived from the Fick's second law, was used to describe the mass transfer of solute from the spherical particles:

$$\frac{\partial CPC}{\partial t} = D_{AB}\left(\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial CPC}{\partial r}\right)\right) \tag{4}$$

where, CPC is the C-phycocyanin concentration (mg.ml^{-1}), D_{AB} is the diffusion coefficient (m^2.s^{-1}), r is the radius of solid particles (m), and t is the time (min). Based on the Fick's second law, the mathematical expression that linked between the CPC_t and diffusion coefficient of the solute can be described as follows:

$$\frac{CPC_t}{CPC_s} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{D_{AB}n^2\pi^2 t}{r^2}\right) \tag{5}$$

where, n is the positive root of Bessel function of the first kind of order zero. However, following the short extraction period, only the first term of the series solution is considered to be significant. Then, the linearized form is achieved by applying the natural logarithm function on both sides, as follows:

$$\ln\left(\frac{CPC_s}{CPC_s - CPC_t}\right) = \ln\frac{\pi^2}{6} + \frac{D_{AB}\pi^2 t}{r^2} \tag{6}$$

2.3.2 Correlation of diffusion coefficient

The starting point for many correlations is the Stoke & Einstein equation. This equation is derived from continuum fluid mechanic and classical thermodynamics for motion of large spherical particles in liquid. In this case, the need for a molecular theory is cleverly avoided.

The correlation used to prediction the protein diffusion coefficient of biological solute is several correlations. There are two equations usually used for prediction the diffusion coefficient for macromolecule and small molecule of protein that are Stoke & Einstein correlation, Wilke & Chang [12], the 10^{-9} to 10^{-11} are the order of magnitude of theirs. CPC is a protein that defines as phycobiliproteins in *S. platensis*. The correlation between Stoke & Einstein Eq. (7) and Wilke & Chang Eq. (8) were showed following equation:

$$D_{AB} = \frac{9.96 \times 10^{-16} \cdot T}{\mu \cdot V_A^{1/3}} \quad (7)$$

$$D_{AB} = \frac{1.173 \times 10^{-16} \left(\phi M_B\right)^{0.5} \cdot T}{\mu \cdot V_A^{0.6}} \quad (8)$$

where, μ is the solution viscosity (Pa.s). T is absolute temperature (K). M_B is the molecular weight of solvent. ϕ is an associate parameter of solvent. V_A is the molar volume of the solute at boiling point [12, 13]. The relative error calculated by the following equation,

$$\%Error = \left| \frac{D_{AB}^{pred} - D_{AB}^{\exp}}{D_{AB}^{\exp}} \right| \times 100 \quad (9)$$

Where, D_{AB}^{pred} is D_{AB} obtained from correlation, $D_{AB}^{\exp}$ is D_{AB} obtained from experimental.

If the diffusion coefficient obtained from these correlation showed %Error more than 20%. The interesting variation in viscosity is reassuring the correlation. Because the temperature variation suggested by Stoke & Einstein is apparently correct, but it much smaller than effect of solvent viscosity and solute radius. To investigate this assumption, the viscosity of liquid medium was measured to produce the correlation to predict the viscosity used in correlation. The viscosity correlation is an empirical model include functions of microbubble, ultrasonic and temperature. The viscosity correlation is produced by multiple linear regression model as:

$$\mu = \alpha_o \cdot UF^{\alpha_1} \cdot MB^{\alpha_2} \cdot T^{\alpha_3} \quad (10)$$

Where, $\alpha_0, \alpha_1, \alpha_2, \alpha_3$ are constant, UF is defined as ultrasonic frequency, MB is defined as microbubble size, and T is the controlled temperature. For the determination of the solution viscosities, the DV-III+ Rheometer is used by using the needle No. 27 (Brookfiled, USA) [14]. In this study, it was tried to propose the diffusion coefficient of CPC in liquid phase and demonstrate the possibility of the extensive application of correlation and also modified the correlation to predict the diffusion coefficient that includes applications of ultrasonic frequency and microbubble size.

3. RESULTS AND DISCUSSION

3.1 Influence Parameter of MBUT

3.1.1 Effect of microbubble

The CPC extracted from *S. platensis* has been presented in previous study, the extraction efficiency examined using various parameters such as ratio of solid to liquid, solvent type, extraction method. However, to investigate the effect of the main process parameter as microbubble over the CPC extraction, the experiments were performed with microbubble and without microbubble at fixed solid to liquid ratio. The CPC under condition of with microbubble was represented the highest among four extraction methods that are 3.50 mg.ml^{-1} and 173.10 mg.g^{-1} of CPC concentration and yield, respectively. The experimental extraction presented in Fig. 2, the results of RFT, UAE, and UAET were referenced from Thaisamak et al. (2019) who has been presented the utility of ultrasonic assisted extraction with controlled temperature in CPC extraction. They have been presented the influence of various extraction methods namely RFT, UAE, and UAET which was found the high CPC concentration in UAET method and they were proposed that the combination between ultrasonic and controlled temperature is suitable for thermal sensitive bioactive compound [6]. Additionally, the result was found that using microbubble can increased CPC concentration around 35% when compared with no using microbubble (UAET), and around 70% when compared with conventional method (RFT), respectively.

The result also conformed to Tomita et al. (2009) who reported that the microbubble destroyed quickly by ultrasonic wave and the cavitation can occur and sometime reveals in the form of a large number of bubble which can enhancement effect in drug delivery [15].

From this viewpoint, the microbubble is a significant parameter affect in CPC extraction. The mechanism of microbubble combined with ultrasonic is provide using of a large number of microbubble in solvent integrated effect with ultrasonic wave to increase efficiency in disruption cell wall, by the effect of microbubble exploding which can increase the CPC transfer to salt solvent. While general generation of microbubble is usually disappeared over time [17]. Using ultrasonic to explode the microbubble before it disappears is main idea use in this work.

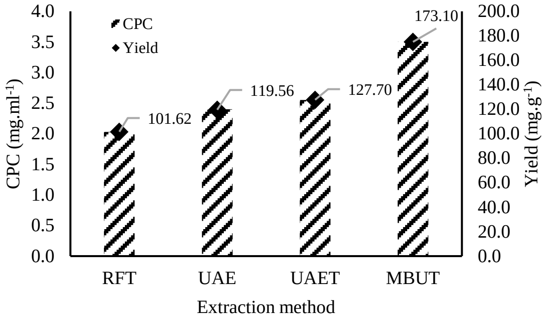

Fig. 2 The concentration and yield of the extracted CPC

The better describe the relative effect of various extraction method enhancement for particular compound normally uses enhancement factor. This value indicates how much more effective particular in each method would be for extraction relative to conventional method. The values are shown in Table 1, the values calculated based on the ratio of CPC concentration obtained from different extraction methods and the CPC obtained from conventional method (RFT) the value reported in previous work that is 2.03 mg.ml^{-1} [6]. The enhancement factor of MBUT was found better than UAE and UAET methods, respectively. Thus, microbubble combined with ultrasonic and controlled temperature (MBUT) is an extraction strategy for CPC extraction.

Table 1 Comparison of enhancement factor of various extraction method

Method	CPC	Yield	EF
RFT*	2.03±0.29^{a}	101.62±1.44^{a}	1.00
UAE*	2.40±0.18^{b}	119.56±8.99^{b}	1.18
UAET*	2.55±0.14^{b}	127.70±0.22^{b}	1.26
MBUT	3.50±0.11^{c}	175.10±0.79^{c}	1.72

* The data are represented in the previous work of Thaisamak et al. (2019) [6]. The data are represented as mean±SD of three replicate. $^{a-c}$ means in the same column with different letter are significantly different ($p<0.05$)

3.1.2 Effect of ultrasonic frequency

The influence of various ultrasonic frequency on CPC extraction shows in Fig. 3(a), the 45 kHz of ultrasonic frequency represents the highest CPC among three levels. Hence, the 45 kHz of ultrasonic frequency has been chosen as a suitable level for next studies of various microbubble sizes. It was accordance with the Makuta et al. (2013) who have been reported the bubble collapse by the ultrasonic followed by the microbubble generation, and the 45 kHz of ultrasonic frequency could interact with microbubble, new small cavitation bubbles are formed [18], from this viewpoint, this phenomena can increase the amount of microbubble which was enhanced cell wall disruption that can increase CPC transfer in liquid salt solvent. Nevertheless, the effect of various ultrasonic frequency in the present study is not clearly given, it is only relevant to their work that is 45 kHz of ultrasonic frequency can form new small microbubble and 28 kHz and 100 kHz can be reduced the hot-spot created by the collapse of cavitation bubble [8, 18].

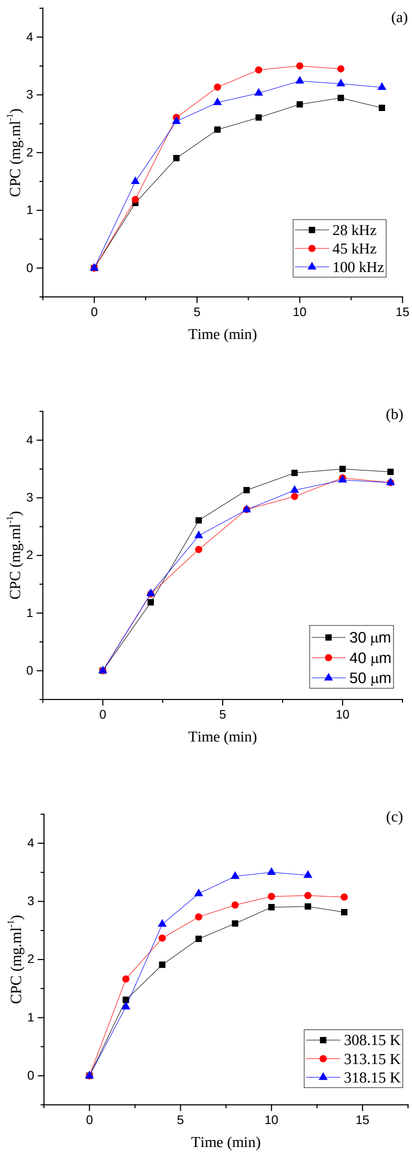

Fig. 3 The extraction profile of CPC under MBUT, (a) influence of ultrasonic frequency, (b) influence of microbubble size, and (c) influence of controlled temperature.

3.1.3 Effect of microbubble size

The influence of various microbubble on CPC extraction shows in Fig. 3(b), the 30 μm of microbubble size represents the highest CPC concentration among three different of microbubble size. So, the 45 kHz of ultrasonic frequency and 30 µm of microbubble size have been chosen as a suitable level for next experiment of various temperature controlled. The result might be explanation by a large number of microbubble contain in solution that is around 10^8 to 10^9 microbubbles.ml^{-1}[15, 16]. It can be meant to the number of microbubble in salt solvent which can effect to efficiency of cell disruption. Moreover, this work presented the effect of ultrasonic frequency combined with effect of microbubble size on the CPC enhancement which has not been reported before.

3.1.4 Effect of temperature controlled on MBUT

Temperature is an influential factor of mass transfer from solute to solvent. The influence of various controlled temperatures on CPC extraction under MBUT under suitable conditions obtained from previous section shows in Fig. 3(c), the 318.15 K of controlled temperature represents the highest CPC. The effect of microbubble combined with ultrasonic was assumed to be accelerating mass transfer CPC (A) to salt (B) (Fig.1).

3.2 Mass Transfer Parameters of MBUT

The diffusion coefficient was used to prove the performance of MBUT extraction. It is a factor directly influencing the change of solid-phase concentration and it is a complex function that depends on the structure of solid phase, pore size distribution, etc. During the extraction process, the solid phase changes its structure regularly or irregularly, thus diffusion coefficient will generally depend on the spatial coordinate and time. The effect of ultrasonic frequency and microbubble size on diffusion coefficient were investigated. Microbubble combined with ultrasonic frequency was found as the significant parameter, 30 µm microbubble size and 45 kHz of ultrasonic frequency show the highest values.

In this study, Fick's second law solution was present for spherical shape. The numeral solution was obtained for solid particle radius (radius of *S. platensis*) r = 2.5×10^{-5} m. The results were presented in range of 2.030×10^{-11} to 2.415×10^{-11} m^2.s^{-1} as shown in Table 2. These results were better than those found in the literature for other extraction method that might be depending on the mechanism of each method. These values also varied on influence parameters such as solvent, solid-liquid ratio, particle size, and also depend on extraction method.

3.3 Correlation of Protein Diffusion Coefficient

For the Stoke & Einstein correlation, the molar volume of the CPC at boiling point was calculated as the chemical formula of CPC that is $C_{165}H_{185}N_{20}O_{30}$, the value obtained is 3,512.50 cm^3.mol^{-1} [13, 19]. The Wilke & Chang correlation, the molecular weight of solvent is 514.16 g.mol^{-1}. The diffusion coefficient of CPC in salt solvent was determined from the proposed correlation, the values obtained was accordance with reported diffusion coefficient of protein with similar molecular weight such as Bovine serum albumin (67,500 MW, 6.81×10^{-11} m^2.s^{-1}), and α-amylase (51,000 MW, 6.75×10^{-11} m^2.s^{-1}), while the calculation state of both correlation given and order of magnitude higher value [20]. Thus, the modified correlation for predict CPC diffusion should be developed.

The interesting in the variation in viscosity can reassure the accuracy of correlation. Because of the correlation based on the Stoke & Einstein is not only effect by variation of temperature. Furthermore, the viscosity and radius of solute are also affected [21]. The variation of viscosity was obtained, the value was in range of 0.00204 to 0.00354 Pa.s, The effect of ultrasonic frequency combined with effect of microbubble was influenced by the viscosity of solution. The phenomena of the collision and collapsing might be the main reason that effect to mass transfer of CPC. Makuta et al. (2013) have been reported the 45 kHz of ultrasonic frequency could interact with microbubble, new small cavitation bubbles are formed [18]. This reason might be the main effect of the cell disrupt which can reduce the size of cell through the solution can become homogeneous. The regression model of viscosity was established as Eq. (11) (R^2=0.979), and used to predict the viscosity of solution.

Table 2 Experimental extraction and diffusion coefficient of MBUT.

MBUT Condition	CPC	Yield	D_{AB}
UF, 28 kHz; MB, 30 µm; and T, 318.15 K	2.95	147.30	2.029×10^{-11}
UF, 45 kHz; MB, 30 µm; and T, 318.15 K	3.50	175.10	2.415×10^{-11}
UF, 100 kHz; MB, 30 µm; and T, 318.15 K	3.24	162.00	2.378×10^{-11}
UF, 45 kHz; MB, 40 µm; and T, 318.15 K	3.35	167.30	2.407×10^{-11}
UF, 45 kHz; MB, 50 µm; and T, 318.15 K	3.31	165.50	2.247×10^{-11}
UF, 45 kHz; MB, 30 µm; and T, 308.15 K	2.91	145.64	1.959×10^{-11}
UF, 45 kHz; MB, 30 µm; and T, 313.15 K	3.10	155.02	2.227×10^{-11}

Note: UF is the ultrasonic frequency, MB is the size of microbubble, and T has controlled the temperature.

$$\mu = 6.773\times10^{3}\cdot UF^{0.302}\cdot MB^{0.871}\cdot T^{-3.705} \quad (11)$$

For, the modified correlation for predicting the CPC diffusion coefficient is proposed by adapting the Stoke & Einstein equation to a model for the equivalent hydrodynamic sphere. The radius of gyration and molecular weight, which accounts for the size and shape of protein molecule in solution, was used in deriving the modified correlation. This correlation successful predicted the diffusion coefficient of protein, for which experimental data were available [22]. In this work, the modified correlation was proposed by employ the molecular weight and radius gyration. The modified correlation was established as following Eq. (12) (R^2=0.936). This modified correlation is suitable for prediction of CPC diffusion in salt solvent under MBUT extraction, the boundary condition include: 308.15-318.15 K of the controlled temperature, 30-50 µm of microbubble size, and suitable for *S. platensis*. The comparative between correlations and experimental values under the condition of 30 µm of microbubble size, 45 kHz of ultrasonic frequency and 318.15 K controlled temperature shown in Table 3, together with %Error.

$$D_{AB} = \frac{6.243\times10^{-15}\cdot T}{\mu\cdot\sqrt{M_A^{1/3}\cdot R_A}} \quad (12)$$

For the estimation of the diffusion coefficient of CPC, the CPC molecular weight (M_A) and radius of CPC (R_A) are 18,500 Da (Enzmart biotech, Bangkok) and 54.1 A° [23], respectively.

Table 3 The comparison of diffusion coefficient by various correlation

Correlation of	D_{AB}	%Error
Experimental	2.415×10^{-11}	-
Stoke and Einstein	4.604×10^{-12}	81
Wilke and Chang	1.002×10^{-11}	51
Modified	2.370×10^{-11}	2

The %Error values were presented the error of estimation between modified correlation and experimental under the suitable condition. The D_{AB} value obtained from correlation of Stoke & Einstein and Wilke & Chang was found high %Error. The result might be attributed to the fact that the molar volume of CPC which was calculated with the data of atomic volume at the normal boiling point may not accurate, these relations should be used with the caution, and the temperature of MBUT is over the range of outside temperature (278.15 to 313.15 K) [19]. The modified correlation shows similar to the experimental, the values lower than other correlation.

The comparative between the D_{AB} value obtained from experimental and predicted by the modified correlation shows in Fig. 4, the straight line with rectangular symbol is D_{AB} obtained from all of the experiment, the circle symbols are the D_{AB} obtained from modified correlation. Note that the modified correlation gives a satisfactory agreement between the experimental and predicted values with a maximum %Error of 13% and the average %Error of 8%.

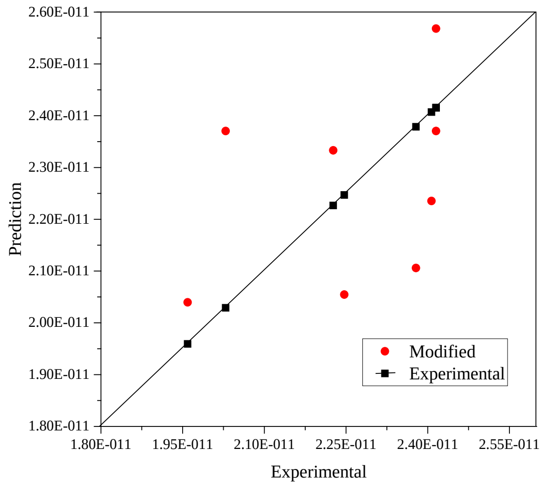

Fig. 4 Comparison of diffusion coefficient values of various conditions under MBUT extraction by modified correlation and experimental measured.

To the best of our knowledge, such results about the possible of microbubble combined with ultrasonic and controlled temperatures have never been yet reported in the literature. This explanation leads to the new finding of CPC extraction which can propose the microbubble combined with ultrasonic and controlled temperature technique is an effective extraction method for CPC extraction.

4. CONCLUSIONS

The work reported about the test of the CPC extraction by MBUT extraction. This combination of two technologies can present the phenomena of the microbubble exploding by ultrasonic that can enhance interaction force that ability to enhance the cell disruption or ultrasonic cavitation which can increase the diffusion of CPC to salt solvent. The 30 µm of microbubble size, 45 kHz of ultrasonic frequency, and controlled temperature at 318.15 K show the highest CPC concentration, yield, diffusion coefficient that are 3.50 $mg.ml^{-1}$, 175.10 $mg.g^{-1}$, and 2.415×10^{-11} $m^2.s^{-1}$, respectively. The modified correlation to predict the diffusion coefficient was developed, while the correlation of viscosity was also established to predict the viscosity used in the modified correlation.

5. ACKNOWLEDGMENTS

The authors express their appreciation to the Thailand Research Fund (TRF) and the Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, Thailand.

6. REFERENCES

1. Su, C.-H., C.-S. Liu, P.-C. Yang, K.-S. Syu, and C.-C. Chiuh, Solid–liquid extraction of phycocyanin from Spirulina platensis: Kinetic modeling of influential factors. Separation and Purification Technology, 123, 2014, pp. 64-68.
2. Miyabe, K. and R. Isogai, Estimation of molecular diffusivity in liquid phase systems by the Wilke–Chang equation. Journal of Chromatography A, Vol. 1218, Issue 38, 2011, pp. 6639-6645.
3. Tavanandi, H.A., R. Mittal, J. Chandrasekhar, and K.S.M.S. Raghavarao, Simple and efficient method for extraction of C-Phycocyanin from dry biomass of Arthospira platensis. Algal Research, Vol. 31, 2018, pp. 239-251.
4. Mittal, R., H.A. Tavanandi, V.A. Mantri, and K. Raghavarao, Ultrasound assisted methods for enhanced extraction of phycobiliproteins from marine macro-algae, Gelidium pusillum (Rhodophyta). Ultrason Sonochem, Vol. 38, 2017, pp. 92-103.
5. Dias, A.L.B., et al., Ultrasound-assisted extraction of bioactive compounds from dedo de moca pepper (Capsicum baccatum L.): Effects on the vegetable matrix and mathematical modeling. Journal of Food Engineering, Vol. 198, 2017, pp. 36-44.
6. Thaisamak, P., S. Jaturonglumlert, J. Varith, F.S. Taip, and C. Nitatwichit, Kinetic model of ultrasonic-assisted extraction with controlled temperature of C-phycocyanin from S. Platensis. International Journal of GEOMATE, Vol. 16, Issue 55, 2019, pp. 176-183.
7. Hanotu, J., D. Kong, and W.B. Zimmerman, Intensification of yeast production with microbubbles. Food and Bioproducts Processing, Vol. 100, 2016, pp. 424-431.
8. Masuda, N., A. Maruyama, T. Eguchi, T. Hirakawa, and Y. Murakami, Influence of Microbubbles on Free Radical Generation by Ultrasound in Aqueous Solution: Dependence of Ultrasound Frequency. J Phys Chem B, Vol. 119, Issue 40, 2015, pp. 12887-93.
9. Kaewdam, S., S. Jaturonglumlert, J. Varith, C. Nitatwichit, and K. Narkprasom, Kinetic models for phycocyanin production by fed-batch cultivation of the spirulina platensis. International Journal of GEOMATE, Vol. 17, Issue 61, 2019, pp. 187-194.
10. Jaturonglumlert, S., J. Promya, and J. Varith, Modeling of Spirulina growth rate with led illmination and applications. Engng.J.CMU., Vol. 24, Issue 1, 2017, pp. 142-151.
11. Ruangyot, T., S. Jaturonglumlert, C. Nitatwichit, and J. Varith, Factors affecting phycocyanin extraction from Spirulina platensis by using freezing and thawing combined with ultrasonic method. Journal of fisheries technoligy research, Vol. 10, Issue 4, 2016, pp. 78-87.
12. Ghosh, R., Principles of Bioseparations Engineering. 2006, Singapore: World Scientific Publishing Co. Pte. Ltd. 284.
13. Demirel, Y., Nonequilibrium Thermodynamics (Third Edition). 2014, Amsterdam: Elsevier. 758.
14. Varith, J., Physical properties of Agricultural and food product. 2015, Chiangmai: Maejopress. 283.
15. Tomita, Y., R. Uchikoshi, T. Inaba, and T. Kodama. Microbubble disruption by ultrasound and induced cavitation phenomena. in 7th International Symposium on Cavitation. 2009. Ann Arbor, Michigan, USA.
16. Krehbiel, J.D., L.C. Schideman, D.A. King, and J.B. Freund, Algal cell disruption using microbubbles to localize ultrasonic energy. Bioresource technology, 173, 2014, pp. 448-451.
17. Jeon, S.-Y., J.-Y. Yoon, and C.-M. Jang, Bubble Size and Bubble Concentration of a Microbubble Pump with Respect to Operating Conditions. Energies, 11(7), 2018, pp. 1864.
18. Makuta, T., Y. Aizawa, and R. Suzuki, Sonochemical reaction with microbubbles generated by hollow ultrasonic horn. Ultrasonics Sonochemistry, Vol. 20, Issue 4, 2013, pp. 997-1001.
19. Bas, G.L., The molecular volumes of liquid chemical compounds. 1915, New York: David McKay Co. 275.
20. Geankoplis, C.J., Transport processes and separation process principles. 2003, Upper Saddle River: Prentice Hall Press, NJ. 804.
21. Cussler, E.L., Diffusion: Mass Transfer in Fluid Systems. 3 ed. Cambridge Series in Chemical Engineering. 2009, Cambridge: Cambridge University Press. 647.
22. Tyn, M.T. and T.W. Gusek, Prediction of diffusion coefficients of proteins. Biotechnology and Bioengineering, Vol. 35, Issue 4, 1990, pp. 327-338.
23. Saxena, A.M., Phycocyanin aggregation: A small angle neutron scattering and size exclusion chromatographic study. Journal of Molecular Biology, 200(3), 1988, pp. 579-591.