Jurnal Teknologi

Full Paper

OPTIMISATION OF MICROENCAPSULATION OF *CITRUS HYSTRIX L.* OIL SUB AND SUPERCRITICAL CO_2 USING RESPONSE SURFACE METHODOLOGY

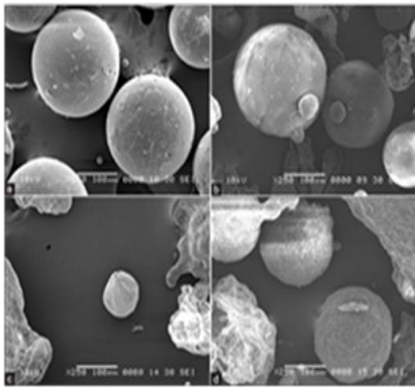
Noorrasyidah Mohd Sarmin[a,b], Shazana Azfar Razali[a], Masturah Markom[a*]

[a]Department of Engineering and Build Environment, National University of Malaysia, 43600 UKM, Bangi Selangor, Malaysia
[b]Industrial Biotechnology Research Centre, SIRIM Berhad, 1 Persiaran Dato' Menteri, P.O Box 7035, 40700 Shah Alam, Selangor, Malaysia

Article history
Received
16 October 2015
Received in revised form
4 February 2016
Accepted
20 February 2016

*Corresponding author
masturahmarkom@ukm.edu.my

Graphical abstract



Abstract

Response surface methodology (RSM) was used to optimise the microencapsulation process *of Citrus Hystrix L.* Oil (CHO) by depressurization of an expanded liquid organic solution (DELOS) sub and supercritical CO2. The particle size and yield (%) was studied with considered to three key factors variables including pressure (30-80 bar), temperature (40-60˚C) and resident time (10-60 minutes) on the microencapsulation process. The optimum result from RSM is 549.4 nanometer for the particle size and 38.193% yield at optimum pressure 54.14bar, temperature 59.65˚C and resident time 58 minutes. The results clearly show that the interactions between pressure, temperature and time have a significant effect on the microencapsulation process. Thus, the microencapsulated formulation has potential to be applied to other volatile compounds.

Keywords: Expanded Liquid Organic Solution (DELOS), Supercritical CO_2, Citrus Hystrix L. Oil (CHO), Response surface methodology (RSM), microencapsulation

1.0 INTRODUCTION

Essential oils are obtained from plant extracts. In numerous years, many have reported regarding the use of essential oils as preservatives, flavour, antioxidant, for applications like cosmetics and personal care, paints for textile, and additives [1]. *Citrus Hystrix L.*, commonly known as citrus oils are very susceptible to oxidative deterioration. Citrus oils produced from the extraction process, will experience very seriously changes on off-flavours that known as "terpy", "anise", and loss "from the top" if stored for two months at room temperature. Many researchers believed that these changes attributable off- flavours arise from oxidative decomposition of terpenese especially limonene, into various aldehydes and ketones [2].

Microencapsulation is a method to encapsulate essential oils [3, 4] purposely to prolong its shelf life and protect from light, moisture, oxidation and degradation processes, and heat [5]. This process coats small particles of solids/droplets of liquids and dispersion using polymeric material to produce small particles 1- 5000 µm in size [6]. The encapsulation technique by using supercritical fluid (SF) (Tc = 31.1 ° C, Pc = 7:38 MPa), specifically supercritical carbon dioxide ($SCCO_2$), that usually applied in many fields such as particulation for essential oils, chromatography, chemical extraction, reaction engineering, organic and inorganic synthesis, waste

management, processing materials, porous materials, and also pharmaceutical applications of materials due to its low viscosity (penetration of the matrix as a gas-like), a comparatively high density and dispersion, also near-zero surface tension [7,8]. Additionally, the advantage of SF such as very high diffusivity in polymer [9], high in solubility and plasticising action, will promising new latest discovery for microencapsulation technique. Recently, the combination of compressed CO_2 and the properties of water –in- oil was attracted researchers to form the particles that can be tuned easily by the pressure and temperature of CO_2 [10]. Thus, DELOS or depressurization of an expanded liquid organic solution of compressed CO_2 is viewed as a terrific drug carrier into biocompatible polymers, where In this processing, supercritical CO_2 is used as a co-solvent in addition to an organic solvent. The overall process summarizing as substances are dissolved in an organic solvent and then the solution is mixed with supercritical CO_2 at a given temperature, and pressure with taking into account the time specified in a high-pressure vessel [11]. The CO_2 gasses was diffuses rapidly out of the polymer, deplastacising it and warranting the complete removal of solvent, without exposing polymers and drugs to high temperatures, which may degrade them [12,13] in the depressurization process. In addition, this very effective "smart" method can be substituted the conventional methods that used in industry to prepare the fine powder particles for medicine with significantly improved its performance. Thus, with many benefit of compressed CO_2 technique that poses as a feasible alternative, it can be seen as new alternative to produce biological for drug delivery with easy to scale-up process, efficient manufacturing costs, technology platform for a variety of therapeutic areas, better performing drugs, and lower risk of product recalls, reduced wastage and an easier, faster process. In this paper, we used the DELOS process with some modification.

Even though variety types of polymers that used in microencapsulation preparation, cellulose derivatives are among the most frequently used [14]. The nature of ethyl cellulose (EC) which is non-biodegradable and very biocompatibility polymer, is one of the reasons why it was chosen, and extensively used especially in the pharmaceutical industry for controlled released drug [15]. In addition, EC as a hydrophobic material also chosen for a variety of applications such as sustained release and taste making, microencapsulation, and preparing controlled delivery systems of water-soluble materials [16].

In the current study, we were report the optimisation of microencapsulation of CHO using response surface methodology (RSM). RSM was widely used in various fields of food chemistry such as optimisation of the extraction of phenolic compounds from wheat [17], preparation of β-carotene nanoemulsions [18], and microencapsulation of seed oil [19]. In such situations where multiple variables may influence the output, RSM is an effective technique for exploring the relationships between the independent variables and response and optimizing the process of products [20]. There are many reports [21] about the use of RSM as a powerful statistical and mathematical tool. RSM has a major advantage over the one-factor-a time approach in their interactions on the output variables with reduced number of trials. The effect of three variables such as pressure, temperature, and time towards the particle size and percent yield were investigated and analysed systematically with introducing a quadratic polynomial model to correlate both and at the same time determine the optimal conditions for microencapsulation of CHO.

2.0 METHODOLOGY

2.1 Materials

Citrus Hystrix L. oil was extracted in Applied Chemistry Laboratory in SIRIM Berhad. Sodium Lauryl Sulphate (SLS) (Texapon OC-N) were purchased from Chemical solution -Malaysia, and Ethyl Acetate and Ethyl cellulose from SIGMA ALDRICH - Malaysia. Carbon dioxide (CO_2) was purchased from NIG Gases Sdn. Bhd. (Malaysia) and deionised water (model: Favorit W4L) was used in the preparation of CHO particles.

2.2 Preparation of *Citrus hystrix* Oil-in-water (o/w) Emulsion

Oil-in-water (O/W) emulsions were prepared using Solvent Removal technique with some modification from U.S. Patent No. 7,838,037, B2 [22]. There are two phases to produce O/W emulsion; aqueous phase and organic phase. An aqueous phase was prepared as follows: 0.5 g of sodium lauryl sulphate (SLS) was dissolved in 50 ml of deionised water with 6 ml of ethyl acetate. For organic phase, 0.7 g of CHO and 0.3 g of ethyl cellulose was dissolved in 5 ml ethyl acetate. The resulting organic phase was poured into the aqueous phase, followed by adding 100 ml of deionized water while stirring at high speed.

The O/W emulsion was then loaded into a standard steel vessel. The vessel was then sealed and pressurised. The pressure range of CO_2 used in this study was 30- 80 bar, with the effect of temperature 40-60˚C, and time 10-60 minutes. The solution and CO_2 were released slowly after processing was completed. The precipitated CHO particles were collected, filtered using Whatman filter paper and washed thrice with deionized water. The particles

were then dried at room temperature for about 24 hours.

2.3 Characterization

2.3.1 Particle Size and Size Distribution

The particle size and size distribution of particles CHO were measured using a laser light diffraction instrument, Zetasizer Nano zs (Malvern instrument, Germany) at measurement position 4.65 mm and temperature 25˚C combined with Malverns DTS software (v.5.02).

2.3.2 Yield %

The prepared microencapsulated particles were collected from precipitation vessel, drained and weighed. The yield % of CHO particles was calculated using the method described below [35]. Eq. (1)

Yield% = [weight of microcapsules/ (weight of polymer+ weight of CHO)] X 100% (1)

2.4 Morphology (FESEM Analysis)

The particle size, shape and surface morphology of the microcapsules were examined by Field Emission Scanning Electron Microscope (FESEM) model LEO SUPRA 55VP. A small amount of microencapsulated particles was spread on glass stub. The stub was placed in the scanning electron chamber and coated with gold. The scanning electron photomicrographs were taken at the acceleration voltage 5.00 kV, chamber pressure of 0.01mm Hg and original magnification X 500.

2.5 Experimental Design for Response Surface Methodology (RSM)

Response surface methodology (RSM) was applied to investigate the variation of CHO with respect to specified operating parameters. The central composite design (CCD) was design to approach the composition of two variables. CCD is a 2k factorial design with star points and central points. All influence parameters as temperature (T), pressure (P) and resident time (t) of the CO_2 with, on the particle size (Y_1) and yield (Y_2) were selected to synthesize of CHO particles and summarized in Table 1. However, by run the CCD with two factors, about 20 of experimental runs were given with respective condition as in Table 2. All of these experiments were carried out in 1000 ml of standard steel cylinder to produce CHO microencapsulated particles. The individual experiments were carried out in random order.

Table 1 Selected factors and level for CHO particles optimization

Independent variable parameter	Factor	Range and level		
		-1	0	1
Temperature (°C)	A	40	50	60
Pressure (bar)	B	30	40	80
Time (minutes)	C	10	35	60

A second-order polynomial equation was used to express the particle size (Y1), and the yield% (Y2) of the CHO particles as a function of the independent variables as follows in Eq. (2)

$$Y_i = a_o + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X^2_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{14}X_1X_4 + a_{23}X_2X_3 + a_{24}X_2X_4 \quad (2)$$

where Y_i represents the response variables, a_o is a constant, a_i, a_{ii}, and a_{ij} are the linear, quadratic and interactive coefficients, respectively. By using Eq. (1), the particle size and yield% value of CHO nanoparticles was evaluated. Magnitude of the interaction between the independent variables (factors) and the response was obtained via the ANOVA statistical technique.

The correlation of fit for the polynomial model was expressed using the values from the normal regression (R^2) and the adjusted regression (R^2adj) coefficients.

3.0 RESULTS AND DISCUSSION

3.1 Statistical Analysis

The outcome interaction between the factors on the particle size and yield % of CHO particles was determined by RSM. Based on the central composite design (CCD), the interaction effect as indicated in the experimental runs can be explained through the analysis of variance (ANOVA) of the model. Furthermore, the most important thing is to check the adequacy of the model using the diagnostic graphs and to validate the model by confirming the optimum experimental conditions. The surface response and contour plots of the fitted polynomial regression generated from Statgraphics Centurion XV was used to visualise the relationships between response and the independent variables.

3.2 Model Fitting of Central Composite Design (CCD)

The effects of all factors on the value of particle size and yield % for CHO particles were investigated using the quadratic polynomial model. The experimental

data was used to calculate the coefficient of quadratic polynomial equation, which was used to predict values of particle size and yield % of the emulsions (Table 2). The predicted values agreed with the experimental ones obtained from the RSM design. ANOVA showed that the resultant quadratic polynomial models sufficiently represented the experimental data with the coefficients of multiple determination (R_2) for the response of particle size and yield % values being 0.8706 and 0.8767 respectively. This indicates that the quadratic polynomials models obtained were adequate to describe the influence of the independent variables studied on the particle size and yield % of the microemulsion.

Table 2 Experimental and predicted values of Particle size and yield based on design of experiment

	Particle Size					Yield%				
Source	**Sum of squares**	**Degree of freedom**	**Mean square**	**F-Value**	**P-value**	**Sum of squares**	**Degree of freedom**	**Mean square**	**F-Value**	**P-value**
Model	$1.537X10^5$	9	17075.47	76.36	0.0001	1517.01	**9**	168.56	7.98	0.0016
A	5970.69	1	5970.69	26.70	0.0004	162.73	1	162.73	7.71	0.0196
B	4846.60	1	4846.60	21.67	0.0009	426.54	1	426.54	20.20	0.0012
C	19604.53	1	19604.53	87.67	0.0001	297.57	1	297.57	14.09	0.0038
A^2	36015.98	1	36015.98	161.06	0.0001	302.37	1	302.37	14.32	0.0036
B^2	13354.43	1	13352.43	59.71	0.0001	13.18	1	13.18	0.62	0.4478
C^2	8642.21	1	8642.21	38.65	0.0001	1.94	1	1.94	0.092	0.7682
AB	2833.16	1	2833.16	12.67	0.0052	50.55	1	50.55	2.39	0.1528
AC	130.01	1	130.01	0.58	0.4634	0.12	1	0.12	$5.802 X10^{-3}$	0.9408
BC	28904.49	1	28904.49	129.26	0.0001	181.74	1	181.74	8.61	0.0149
Residua	2236.22	10	223.62			211.15	10	21.11		
Lack of fit	1477.86	5	295.57	1.95	0.2408	173.78	5	34.76	4.65	0.0585
Pure error	758.36	5	151.67			37.37	5	7.47		
Total	$1.559X10^5$	19				1728.15	19			

ANOVA was used to evaluate the significant coefficients of the quadric polynomial models (Table 3). For any terms in the models, to indicate a more significant effect on the respective response variables, the F-value shall be large, though P- value shall be small [23]. Therefore, the variable with the largest effect on the particle size of the particles was the linear term of pressure, followed by the linear term of temperature and resident time. The final models was estimated based on the experimental results with the respective coefficients as given in Eq. (2).

$$Y_{PS} = 551.28 – 24.43A – 22.01B – 44.28C +114.44A2 + 69.68B2 – 56.06C2 - 18.82AB – 4.03AC +60.11BC \quad (3)$$

$$Y_{y\%} = 19.72 – 4.03A + 6.53B +5.45C -10.49A2 + 2.19B2 + 0.84C2 - 2.51AB – 0.12AC +4.77BC \quad (4)$$

The model equation for the CHO particles preparation was well described within the range of the factors. The F-value of 76.36 and 7.98 for particle size and yield % respectively indicated that model is significant for CHO particles synthesis with an adequate precision of 33.704 for particle size and 10.322 for yield % indicating an adequate signal to noise ratio of which a value greater than 4 is considerable desirable. The p-value of the model is considered to be significant when it falls below 0.0500 with values greater than 0.100 are considered as not significant.

Table 3 Analysis of variance (ANOVA) results for quadratic model of CHO particles preparation of the fitted quadratic equations for the particle size (Y1) and yield % (Y2)

Experiment No	A Pressure (bar)	B Temperature (˚C)	C Resident Time (minute)	Particle Size (P_S) (nanometer, nm)		Yield (%)	
				Experimental	Predicted	Experimental	Predicted
1	30	60	60	803.67	800.83	4.05	3.23
2	30	40	60	807.63	804.79	3.19	2.63
3	80	40	10	675.49	672.65	15.20	14.77
4	80	60	10	592.10	589.26	1.02	2.29
5	55	50	60	606.20	603.18	5.45	4.84
6	30	50	10	584.93	582.09	3.62	4.71
7	80	40	35	727.39	724.55	30.48	31.70
8	55	50	35	618.95	616.11	18.22	19.70
9	30	40	10	690.53	701.88	6.70	7.34
10	55	50	35	638.29	649.64	4.32	1.03
11	55	50	35	622.95	634.30	9.34	10.23
12	55	40	35	575.87	587.22	27.04	23.50
13	80	40	60	539.11	550.46	10.23	1076
14	55	50	35	453.71	465.06	23.45	20.27
15	30	50	35	540.60	560.05	10.09	13.04
16	55	50	35	544.52	560.05	10.48	13.04
17	55	60	35	535.58	560.05	10.15	13.04
18	80	60	60	546.00	560.05	10.00	13.04
19	55	50	10	542.60	560.05	10.20	13.04
20	55	50	30	696.40	560.05	22.03	13.04
1	30	60	60	803.67	800.83	4.05	3.23
2	30	40	60	807.63	804.79	3.19	2.63
3	80	40	10	675.49	672.65	15.20	14.77
4	80	60	10	592.10	589.26	1.02	2.29
5	55	50	60	606.20	603.18	5.45	4.84
6	30	50	10	584.93	582.09	3.62	4.71
7	80	40	35	727.39	724.55	30.48	31.70
8	55	50	35	618.95	616.11	18.22	19.70
9	30	40	10	690.53	701.88	6.70	7.34
10	55	50	35	638.29	649.64	4.32	1.03
11	55	50	35	622.95	634.30	9.34	10.23
12	55	40	35	575.87	587.22	27.04	23.50

13	80	40	60	539.11	550.46	10.23	1076
14	55	50	35	453.71	465.06	23.45	20.27
15	30	50	35	540.60	560.05	10.09	13.04
16	55	50	35	544.52	560.05	10.48	13.04
17	55	60	35	535.58	560.05	10.15	13.04
18	80	60	60	546.00	560.05	10.00	13.04
19	55	50	10	542.60	560.05	10.20	13.04
20	55	50	30	696.40	560.05	22.03	13.04

The results of the model indicated that all terms A,B and C are significant according to the p-values of 0.0004, 0.0009 and 0.0001(particle size) and 0.0196, 0.0012 and 0.0038 (yield%), respectively, as shown in Table 3. In addition, the model's lack of fit with F-value of 0.2408 and 0.0585 for particle size and yield% correspondingly, appears to be insignificant relative to the pure error. The correlation coefficients of R_2 and R_{2adj} for both particle size and yield %, models were found to be 0.9857 and 0.9727, and 0.8778 and 0.7679 in that order, indicating a good fit between the regression model and the experimental values.

3.3 Analysis of Response Surface Plotting and Optimization of CHO Particle

To express the effect of any independent variable on dependent ones, surface response and contour plots of the quadratic polynomial models were created. Thus, Fig. 1 (a) and 1(b) was generated by interaction between CO_2 pressure and temperature, Fig. 2 (a) and 2 (b) was between resident time and pressure, though Fig. 3 (a) and 3 (b) showed the interactive between time and temperature for particle size and yield (%) respectively.

For interaction between CO_2 pressure and temperature, the value of particle size at the center point (pressure 55 bar, and temperature 50°C) were given smaller particle size with perfect circle shape compared with the result at pressure 80 and 40 bar with temperature 40°C. It is because, when applying the high pressure (80 bar), the particles will tend to burst while at lower pressure (40 bar), the particles were stick and adhere each other as not enough pressure to separate it, that affect bigger size of particles due to the limitation capability of the vessel [24]. In terms of yield (%), the results showed that lowest pressure (30 bar) was gave higher yield (%) compared than higher pressure (80 bar) at the same temperature (60˚C) because at the higher pressure, the liquid inside the vessel will scattered and stick at the wall and trapped at the bottom of the vessel. Thus, the yield collected at the end of the process was not as much abundant as yield for lower pressure and temperature.

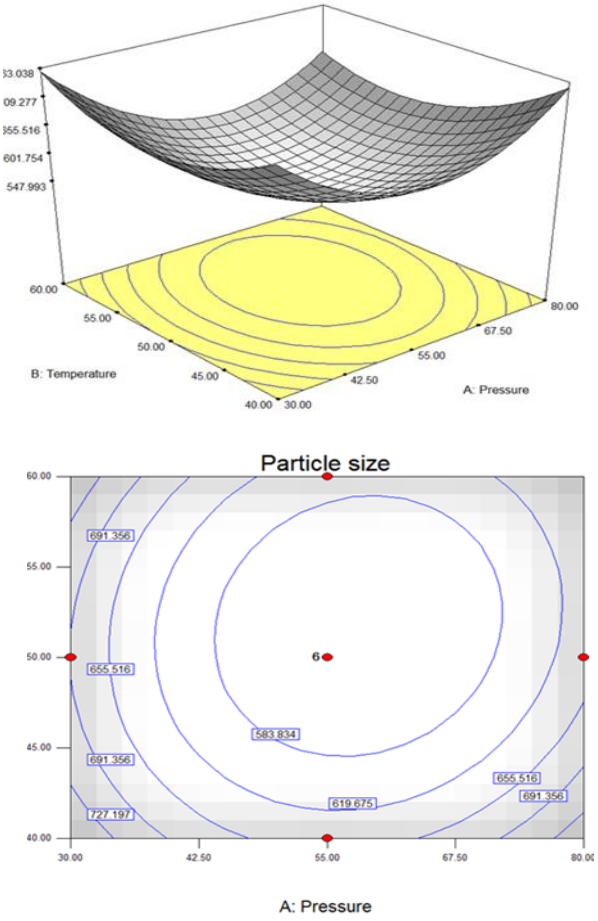


Figure 1 (a) Interactive effects of CO_2 pressure and temperature on particle size CHO particles

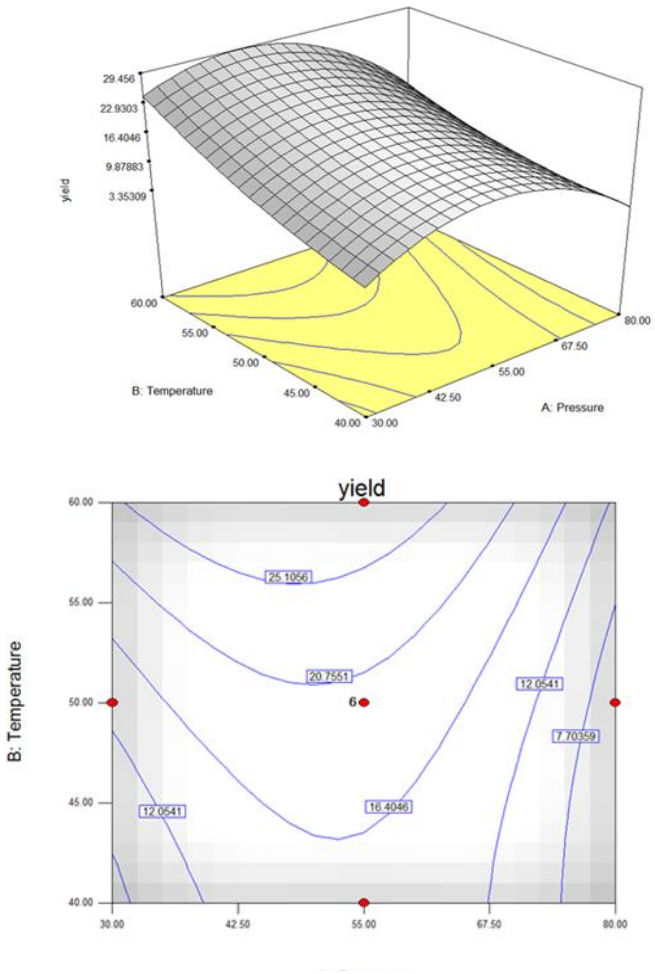


Figure1 (b) Interactive effects of CO2 pressure and temperature on yield (%) CHO particles

The result for interaction between resident time and pressure was discussed as follows that showed in Figure 2 (a) and 2 (b). The combination of highest pressure and shortest time (80 bar, 10 minutes) will produced biggest particle size and lowest yield (%).Teresa et all [25] reported that the reaction time has a strongly effect for the morphology and yield (%) of the particles where, when the reaction time increased up to 60min, the particles with high degree of aggregation were formed. The combination of lowest pressure at longest time (pressure 30 bar, 60 minutes) was produce higher yield (%) which is 37.59% and particle size 707.39nm, while the combination of pressure and time at center point (pressure 55 bar and time 10 minutes) was produced lowest particle size which is 539.11nm.

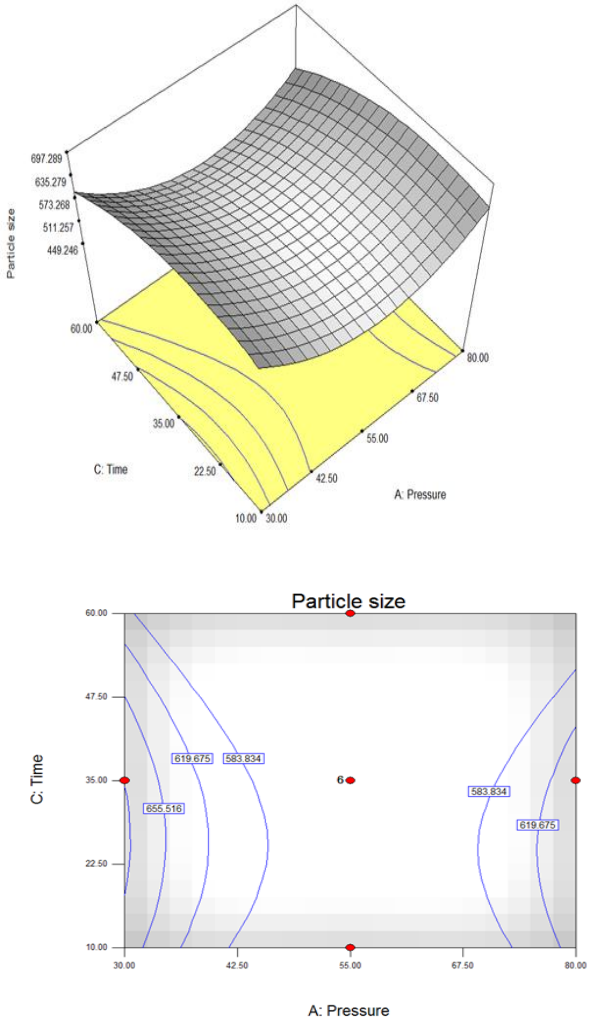


Figure 2 (a) Interactive effects of resident time and pressure on particle size of CHO particles

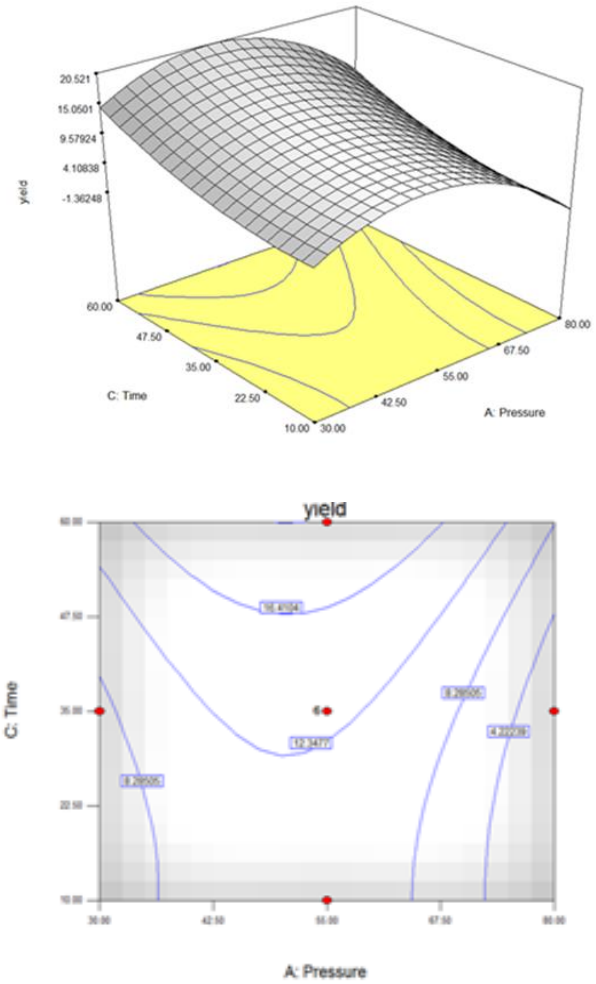


Figure 2 (b) Interactive effects of resident time and pressure on yield (%) of CHO particles

Interaction between resident time and temperature also been studied where the result showed highest temperature and longest time (60˚C, 60 minutes) was produced highest yield (%) with smaller particle size (37.59%, 707.39nm) compared than result at 40˚C and 50˚C with 10 minutes resident time, that gave results (816.63nm, 3.19%) and (539.11nm, 10.23%). The expected result is reinforced by Sheryl at el [26]. Figure 3(a) and 3 (b) showed the interaction for both particle size and yield % towards resident time and temperature.

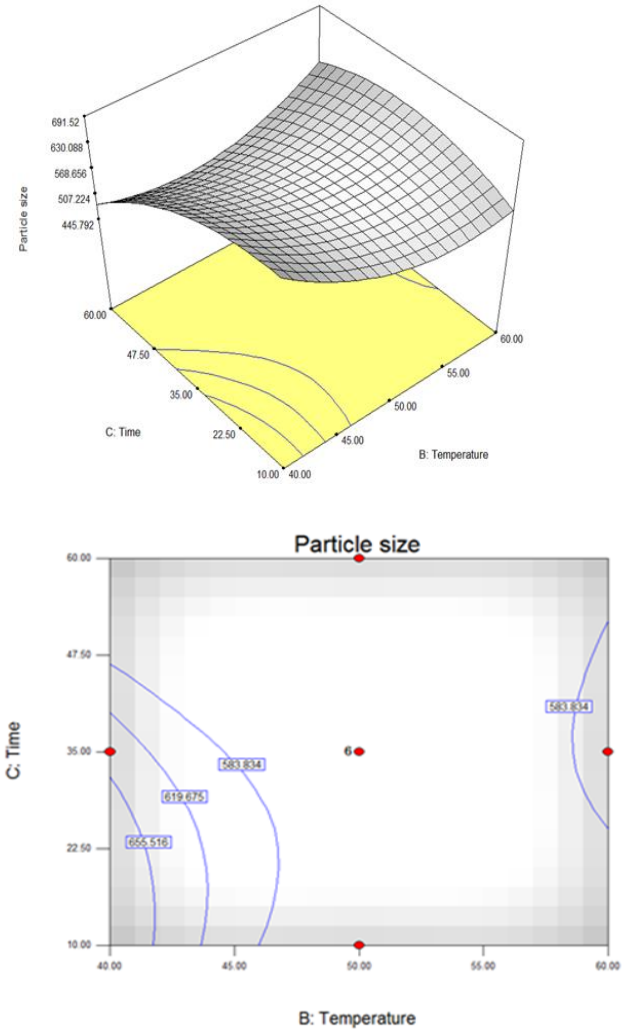


Figure 3 (a) Interactive effects of resident time and temperature on particle size of CHO particle

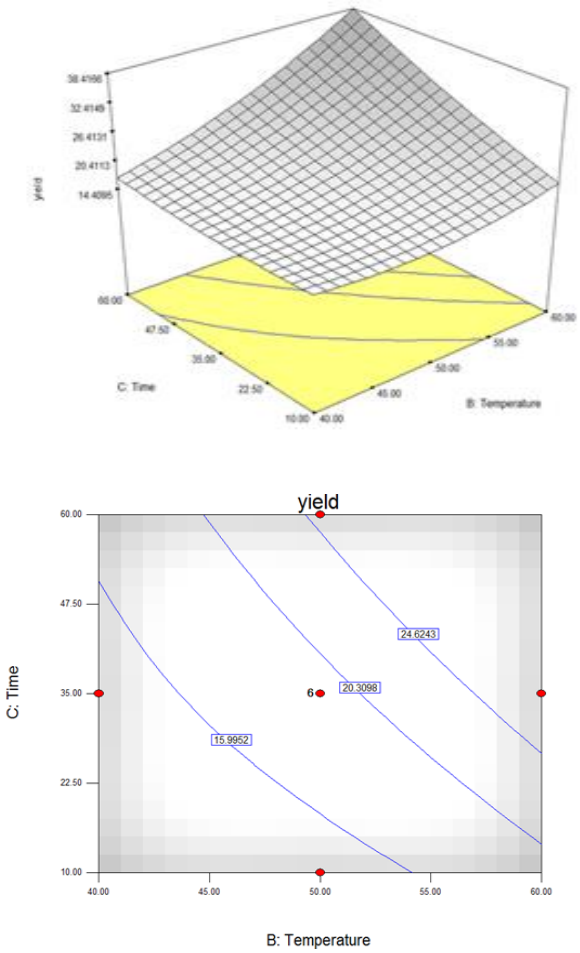


Figure 3 (b) Interactive effects of resident time and pressure on yield (%) of CHO particles

3.4 Optimization and Model Validation

A CCD was applied to develop a correlation process of homogeneous particle growth as a function of the process operating conditions among temperature, pressure and resident time of compressed CO2 in DELOS process. The condition for the preparation of CHO particles were considered optimum condition if the particle size and yield (%) attained the smallest and largest values respectively, according to the standard of ANOVA. Successively, twelve solutions for the optimal conditions were generated by the DOE software and the solution with highest desirability (≈ 1).

Triplicate experiment was done for validation. The results show that the optimum condition for CHO particle encapsulation process can be reached by following process conditions: Pressure (54.14 bar), temperature (59.65 ˚C), and time 58.84 minutes. In order to verify the sufficiency of the model, triplicate experiment have done for CHO particle formation. The parameter was as follow as the suggested optimal condition by the DOE. The result show for the particle size and yield (%) for triplicate experiment was slightly same as the result before optimum which is 549.4 nm, 598.2nm, and 639.4 nm, while yield (%) is 38.19%, 37.24%, and 31.24% respectively.

3.5 Characterization of CHO Particles

A good created method in producing CHO particles would not only controlling its size, nevertheless the yield (%) too. Figure 4 (a) was showed the FESEM result that demonstrate the shape of particles at the optimum condition, while Figure 4 (b) represented the comparison result with other encapsulation with same polymer. This spherical shape morphology was similar

to microcapsule manufactured by mix solvent system of oil-in-oil emulsion solvent evaporation by Satit *et al.* [27].

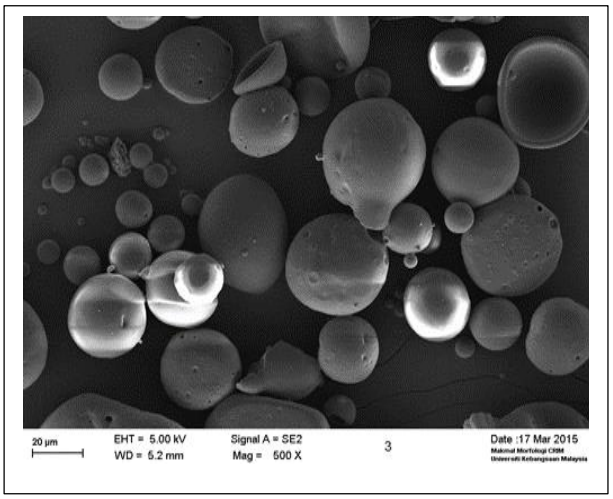


Figure 4 (a) Scanning electron micrographs of CHO particles at optimum condition

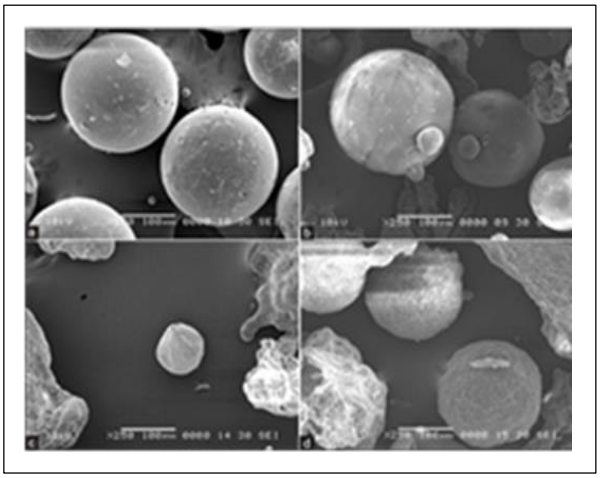


Figure 4 (b) Folic acid microcapsule by ethyl cellulose produced using mix solvent system by Satit *et al.* [27].

FTIR was studied the chemical composition of the CHO microencapsulated particles prepared by the DELOS process. Figure 5 (a), (b) and (c) showed the FTIR spectra in the region 4000- 650 cm^{-1} of CHO (crude), ethyl cellulose (EC) and CHO microencapsulated particles anti-solvent. The broad peak (3200-3600 cm-1) was appear for CHO, EC and CHO microencapsulated particles that belongs to the OH bond which is strong and broad [28]. The same peak appear both at the CHO and CHO microencapsulated particles which is 1643 cm^{-1} for Amide C=O stretch strong and 2920 cm^{-1} that indicate as Alkane C-H stretch strong, that showed the existing of CHO in capsulate particles [29]. The absorption peaks of EC at 1052 cm–1 due to C–O–C stretching appeared at EC and CHO microencapsulate particles that indicate EC was used in the encapsulation process [30].

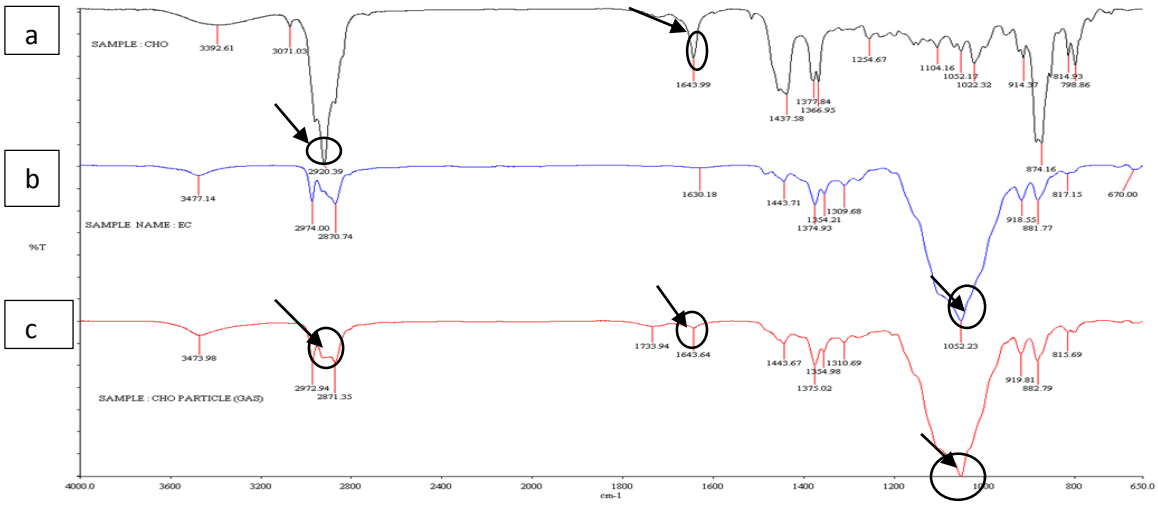


Figure 5 FTIR spectrum for: (a) CHO, (b) EC, and (c) CHO microencapsulated particles at optimise condition

4.0 CONCLUSION

From the study, it was concluded that CHO was successfully encapsulated into Ethyl cellulose microencapsulation particles using modification of DELOS process. According to the above result, we have to demonstrate the optimization of microencapsulation condition for CHO by using response surface methodology (RSM) where the particle size and yield (%) of CHO microencapsulation particles is significantly affected by pressure, temperature and time. According to the RSM result, the overall conclusion were highlighted where the particle size increased when pressure, temperature and time decreased, and at the same time, yield (%) increased when pressure decreased, but temperature and time increased. In addition, the approach presented in this study can provide a very useful guideline to the researchers in how to optimize other oil system very efficiently.

Acknowledgement

We are grateful for the the financially supported by Universiti Kebangsaan Malaysia which under Project FRGS/2/2013/TK05/UKM/02/2 and Seed Fund from SIRIM Berhad.

References

[1] Angel, M., Salima, V., Alexandra, N., and Maria, J.C. 2010. Encapsulation and Co-Precipitation Processes with supercritical Fluids: Applications with Essential Oils. *Chem. Egineering. Journal*. 4: 31-41.

[2] Bakkali, F., Averbeck, S., Averback, D, and Idaomar, M. 2008.Biological Effects of Essential Oils- A Review. *Food & Chem Toxicology*. 446-475.

[3] Irving, I. Rusoff, Orang,e E., and Lincoln. 1953. U.S Patent [2.657997]

[4] Rosenberg, M., and Lee, S. L. 1993, Microstructure of Whey Protein/ Anhydrous Milkfat Emulsions. *Food Structure*. 12: 267-274.

[5] Rosenberg, M., & Young S.L. 1993. Whey Proteins as Microencapsulating Agents. Microencapsulation of Anhydrous Milk-fat Structure Evaluation. *Food Structure*. 12: 31-41.

[6] Shahidi, F., and Han, X. Q. 1993. Encapsulation of Food Ingredients. *Critical Reviews in Food Science and Nutrition*. 33: 501-547.

[7] Leon, L., Herbet, A. L., and Joseph, L.K. 1987. The Theory and Practice of Industrial Pharmacy, Sustained Release Dosage Form. Vaghese publishing House (Bombay).

[8] Nalawade, S. P., Picchion, F., Marsman, J. H., and Janssen L. P. B. M. 2006. The FT-IR Studies of the Interactions of CO2 and Polymers Having Different Chain Groups. *J. Supercritical Fluids.* 36: 236.

[9] Kikic, I., and Vecchione, F. 2003. Supercritical Impregnation of Polymers. *Curr. Opin. Solid State Mater*. 7: 399.

[10] Kikic, I., and Sist, P. Applications of Supercritical Fluids to Pharmaceuticals, Controlled Drugs Delivery Systems. Supercritical Fluids: Fundamentals and Applications. Proceedings.

[11] Rahizana, M. I., Mohd I., Masturah, M., Muneer, M. B., Syazana, A. R., and Kamal, F. 2014. Optimization of Process Parameters for Synthesis of ZnS:Mn2+ Nanoparticles via Reverse Micelle Method Modified Using Sub and Supercritical CO2.

[12] Yukiya, H., Hiromichi, H., and Kunio, A. Supercritical Fluid Research Center. National Institute of Advanced Industrial Science and Technology (AIST), Nigatake 4-2-1, Miyagino-ku, Sendai 983-8551, Japan.

[13] Berens, A. R., Huvard, G. S., Korsmeyer, R. W., and Kuning, F. W. 1992. Application of Compressed, Carbon dioxide in the Incorporation of Additives into Polymers. *J. Appl. Polym. Sci.* 46: 231-242.

[14] Parsetmanakit, S., Prappharaksit, N., Chiang Thong W., and Muangsin, N. 2009. Ethyl Cellulose Microcapsules for Protecting and Control Release of Folic Acid AAPS. *Pharm Sci Tech*. 10(4): 1104-1112.

[15] Ana Rita, C. D., Mariana, S. C., Ana Luisa, S., Maria, M. C., and Catarina M. M. D. 2006. Preparation of Controlled Release Microspheres using Supercritical Fluid Technology for Delivery of Anti-inflammatory Drugs. *Int. Journ. of Pharm*. 168-174.

[16] Liyana-Pathirana, C., & Shahidi, F. 2005. Optimization of Extraction of Phenolic Compounds from Wheat Using Response Surface Methodology. *Food Chemistry*.93: 47-56.

[17] Yuan, Y., Yanxing, G., Like, M., and Jian, Z. 2008. Optimisation of Conditions for the Preparation of β-carotene Nanoemulsions using Response Surface Methodology. *Food Chemistry*. 107: 1300-1306.

[18] Jang-Hyuk, A., Young-Pil, K., Eun-Mi, S., Ki-Woong, L., and Hak-Sung, K. 2008. Optimization of Microencapsulation of Seed oil by Response Surface Methodology. *Food Chemistry*. 107: 98-105.

[19] Giovanni, M. 1983. Response Surface Methodology and Product Optimization. *Food Technology*. 37: 41-45.

[20] Bas, D., and Boyac, I. H. 2007. Modelling and optimization I: Usability of Response Surface Methodology. *Journal of Food Engineering*. 78: 836-845.

[21] Porretta, A., Birzi, A. C., and Vicini, E. 1995. Effects of Ultra-high Hydrostatic Pressure Treatments on the Quality of Tomato Juice. *Food Chemistry*. 52: 35-41.

[22] Emma, K., Kiryat, S., Yury, S., Givat S., Olga, P., Kiryat, Y., Irena, O., Maalot, Igor, P., and Kiryat, H. 2010. Method of Microencapsulation. U. S Pat. No. US 7,838, 037, B2.

[23] Quanhong, L., and Caili, F. 2005. Application of Response Surface Methodology for Extraction Optimization of Germinant Pumpkin Seeds Protein. *Food Chemistry*. 92: 701-706.

[24] E. Reverchon, R. Adami, G. Caputo, I., and De Marco. 2008. Spherical Microparticles Production by Supercritical Antisolvent Precipitation: Interpretation Results. *J. of Sup. Fluids.* 47: 70-84.

[25] Teresa, C., Anna, M. B., Ana, M. R., Manuel, N. P., and Ana, A. 2005. Synthesis of Highly Cross-linked poly (diethylene glycol dimethacrylate) Microparticles in Supercritical Carbon Dioxide. *European Polymer Journal.* 41: 1947-1953.

[26] Sheryl, H. E., Maria, I. A., and Michae, I. R. Z. 1999. Effect on Temperature and Vapor-phase Encapsulation on Particle Growth and Marphology. *Journal of Materials Research*. 1664-1671.

[27] Satit, P., Nalena, P., Worawadee, C. and Nongnuj, M. 2012. Ethyl Cellulose Microcapsules for Protecting and Controlled Release of Folic Acid. *Indian Journal of Pharm. Science*. 74(6): 512-520.

[28] Marino, M., Silva, L., Duran, N., and Jasic, L. 2015. Enhanced Materials from Nature: Nanocellulose from Citrus Waste. *Journal of Moleculus.* 20(4): 5908

[29] Mittal, V. 2011. *Nanocomposites with Biodegradabl Polymers: Synthesis, Properties, and Future Prospective*. Oxford University Press, Oxford, New York.

[30] Vaishal Suthar, Pratap, A., and Raval, H. 2000. Studies on Poly (hydroxy alkanoates)/(ethylcellulose) Blends *Bull. Mater. Sci.* 23(3): 215-219.