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OPTIMIZATION OF ENCAPSULATED CLOVE OIL PARTICLE SIZE WITH BIODEGRADABLE SHELL USING DESIGN EXPERT METHODOLOGY

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ABSTRACT

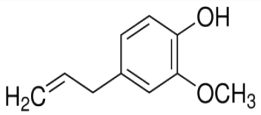
To protect clove oil from volatilization and oxidation, encapsulation in nano scale by biocompatible polymers via ultrasonication emulsification was performed. In order to define an optimal particles size of encapsulated clove oil, a novel crossed experimental design, including mixture design along with the clove oil concentration as process parameter was employed. For this purpose, 28 different formulas by combination of both mixture and process factors were prepared. The encapsulated shell having fixed composition of mixture (20 %): Arabic gum (0-20 %, w/w), whey protein concentrate (0-20 %, w/w) and Tween 80 (0-4 %, w/w) was used. Moreover, the effect of variable content of clove oil (2 to 10 %, w/w) as a core of particles and the remaining of blending is Milli-Q water was investigated. Particles size of all samples, were characterized by dynamic light scattering. Analysis of variance was preformed to analyze experimental data while using stepwise regression analysis. The developed models from the Based on experimental design necessary models were developed; the model having high coefficient of determination (R^2=0.995) value; also predicted and fitted well with the experimental data. Finally, from the optimal encapsulating condition obtained particles size of encapsulated of clove oil (128.2 ± 4.3 nm) that was close to predicted value (135.7 nm).

KEYWORDS: Arabic gum; Clove oil; Design expert; Nanoencapsulation; Particles size

INTRODUCTION

Nature has an ability to create materials in nanoscale dimensions such as the building blocks of biomolecules like DNA, hormones, amino acids, proteins and polysaccharides (Weiss et al., 2006). In fact, science and technology with inspiration from nature creation, man has created new nanomaterials for the excellence and welfare of the people (Liu and Jiang 2011). In past decades, nanotechnology has merged number of research fields and created an avenue as promising field to respond our needs (De et al., 2014).

Table- 1: Specification of eugenol

Molecular Formula	Structural Formula	Specific gravity
$C_{10}H_{12}O_2$		1.06 g /cm³

Clove oil is an essential oil which contained significant quantity of eugenol. Eugenol is an organic phenol compound (El Gharras 2009) (Table 1); which is antipyretic, analgesic, anti-inflammatory and anesthetic effects. Based on latest conducted research, clove oil (eugenol) possesses antibacterial, antioxidant, antifungal, insecticide and anticancer activities. Minimizing eugenol side effects, with low toxicity and non-metabolized residue, the green pest (eugenol) has wide applications in foods, agriculture, pharmaceuticals, pesticides, cosmetics and dentistry (Kong et al. 2014; Shahavi et al. 2014; Shahavi et al., 2015a; d'Avila et al., 2014)

Eugenol is highly sensitive to light and easily oxidized in air; such phenomena caused its limited application and bioavailability. Thus, encapsulation techniques of eugenol are investigated for the benefit of shelf life extension and storage in different environments. Encapsulation of eugenol in nano scale is rapidly expanding technology that is expected to offer protection against oxidization and volatilization and also easily in usage (Shah et al., 2012; Shahavi et al., 2015c; Shahavi et al., 2015b). In addition, nano-emulsion of oil in water is not easily separated; new approaches like application of certain char-

ges in an electric field may require (Hosseini and Shahavi 2012; Hosseini et al. 2012)

Nanoencapsulation is a technique whereby solids, liquids or gases are enclosed within a thin film of wall material to generate nanoscale particles (Esser-Kahn et al. 2011). The particles have functional core and a polymeric continuous outer coating and the size of particle is less than 1 μm. Encapsulation methods are employed to protect bioactive compounds such as polyphenols (Fang and Bhandari, 2010), microntrients (Han et al. 2008), antioxidants (Souto et al. 2013), and nutraceuticals (Mourtzinos et al. 2008). Finally, application of encapsulated active biomolecules are protected from adverse environment and also well control released at targeted sites (Onwulata 2012).

Biocompatible polymers are usually divided into two categories such as polysaccharides and proteins. As natural biomaterials, polysaccharides are stable, safe, nontoxic, hydrophilic, and biodegradable. Polysaccharides include compounds originated from plant (such as: pectin, cellulose, starch, Arabic gum, carrageenan, and alginate) and polysaccharides originated from microbial or animal, the well known compounds are xanthan gum and chitosan (Zong et al., 2012; Lin et al., 2012). Proteins such as bovine serum albumin, ovalbumin, whey proteins, gelatin, soy and wheat gluten are often underutilized as emulifying agents, which are the most important components acting as mediator in polymer–organism interactions (Chen et al., 2008; Lam and Nickerson 2013). In past decade, protein-polysaccharide complex received full attention. There are many applications for complex protein polysaccharides in food, pharmaceutical, and cosmetic industries; as the macromolecules are oppositely charged biopolymers through electrostatic attractions. In addition, development of bioactive complex protein-polysaccharide are used for replacement of fat, meat analogues, gels, emulsions, edible films, coatings and food texturization (Ye 2008; Zhang et al., 2011; Bouyer et al., 2012; Li et al., 2012; Durán and Marcato 2013; Soazo et al., 2015). Recently, adsorptions and purifications of biomolecules were carried out through specific adsorbent into chromategraphy column such as expanded bed adsorption and fluidized bed (Ebrahimpour et al., 2009; Shahavi et al., 2008; Jahanshahi et al., 2009; Jahanshahi and Shahavi 2015; Shahavi et al., 2011).

Arabic gum is one of the most common wall materials used in encapsulation. This is attributed to its high solubility with low viscosity, good emulsifying agent, and it is used in drug delivery, sensor, tumour imaging (Patel and Goyal 2015). Whey is a major co-product of cheese industries. These proteins are globular and amphiphilic. The major whey proteins are α-lactalbumin and β-lactoglobulin. These proteins are about 70% of the total whey proteins which are responsible for many of the functional properties of whey (Rullier et al., 2010).

Recently, different essential oil has been encapsulated by protein polysaccharide complex, such as formation of heat treating electrostatic (Jones and McClements 2011), oil-in-water emulsions bases such as encapsulation in whey protein isolate (WPI)- maltodextrins (Shah et al., 2012) whey protein concentrate (WPC) with mesquite gum (MG) or Arabic gum (Rodea-González et al., 2012) WPC, Arabic gum and lecithin (Luo et al., 2014). Also, encapsulated essential oil carried out by host molecules, like beta-cyclodextrin (Chun et al. 2012; Cevallos et al., 2010), and encapsulation into liposomes (Sherry et al., 2013). In addition, other complex mixture of polysaccharides was used, such as fish gelatins and Arabic gum complex (Piacentini et al. 2013), alginate/cashew gum (de Oliveira et al., 2014), alginate/pectin (Koo et al., 2014) and chitosan/gum (Abreu et al., 2012).

The constituent of wall and the ratio of components of the wall may have significant influence on particle size. Implementation of a suitable method used for optimization and formulation of particles are quite interesting process. In this research, combination method with application of proteins and polysaccharides is the first time used for the development of nanoencapsulation of essential oil. In this research crossed design methodology (CDM) a part of response surface methodology (RSM) was used to optimize the simultaneous effects of the type of wall (formulation) and levels of loaded oil (process). CDM was used to investtigate the reciprocal influence of the protein, polysaccharide and surfactant concentrations with different percentage of clove oil; then responses were evaluate by dynamic light scattering. The conclusion of encapsulated particles is based on the mean particle size. The purpose of this research was to determine the optimal conditions for clove oil loaded micro and nanoencapsulation with different percenttages of oil in three different biomaterials of shell walls (Arabic gum, WPC and Tween80) via nano-emulsion method.

MATERIALS AND METHODS

Materials: Clove oil and Arabic gum were purchased from Sigma–Aldrich (St. Louis,MO, USA), whey protein concentrate (WPC) with 80% protein on dry basis was purchased from Bodylab Nutrition (Hadsund, Denmark); and Tween 80 (polyethylene glycol sorbitan monooleate) is a hydrophilic polymer for synthetic grade was purchased from Merck Millipore (Darmstadt,Germany). In addition, water used in all experiments was purified using a Milli-Q system (Millipore Co., Bedford, MA, USA).

Crossed mixture-process experimental design: The term experimental design is described in three steps. The first step is to identify factors that affect experimental data; the next step is to design the experiment in order to minimize the effects of parameters cannot be controlled. The final step, statistical analysis was used, to separate the effects of the various factors (Margaritelis et al., 2013). Mixture design is a modified experimental design while optimal formulation for mixtures is allowed. Crossed design is combination of the mixture design and process factors. In the present research the experimental design and statistical analysis were performed using Design-Expert software (Version 6.0.2, State-Ease Minneapolis, MN, USA) and a crossed D-optimal design which combine both mixture and process factor was selected. For optimization and prefect formulation, the comprehensive crossed design is able to project complex interactions between compositional variables (mixtures) and process factors. In order to minimize the model regression coefficient, the design points from the source points were selected by D-optimal criterion (Myers et al., 2009; Lee and Gilmore 2005). The independent factors (for mixture part of combined D-optimal design) were Arabic gum content (A), WPC content (B), Tween 80 content (C) which forms the wall material with a total amount of 20 % (A + B + C = 20 %) and the independent numeric factor for process section of crossed design was clove oil concentration (D) and remaining added to Milli-Q water at 25 ◦C. The coded and actual levels of variables are summarized in Table 2.

Table- 2: Selected independent factors and process parameters in the experimental design

Component	Compound	Type	Low actual	High actual	Low coded	High coded
A	Arabic gum	Mixture	0.0	20.0	0.0	1.0
B	WPC	Mixture	0.0	20.0	0.0	1.0
C	Tween 80	Mixture	0.0	4.0	0.0	0.2
D	Clove oil	Numeric	2.0	10.0	−1.0	1.0

A + B + C = 20 %

All of the emulsion components (A: Arabic gum, B: WPC, C: Tween 80 and D: clove oil) were prepared for the optimization procedure based on a crossed experiment design. In this method for defining D-Optimal point we had 28 different formulas via combined mixture methodology and process factors methodology together (Table 3). The complete design consists of 28 runs. In each experiment, size of particles (nm) was evaluated as a response to our test run.

Combination set of candidate point for mixture section and selected candidate point are related to process section. The variance for each factor assessed was partitioned into (a) linear model, (b) quadratic model (linear points plus), (c) special cubic model (quadratic points plus), and (d) full cubic model (special cubic points plus).

ANOVA: Tables were generated for analysis of variance (ANOVA). The effects of individual linear, quadratic and other terms were determined. The significance of all the terms in the method was statistically evaluated and computed by the prob>F at a confidence level of <0.0001. The model was recalculated based on stepwise deletion of terms that were insignificant.

In optimization research, R^2, Adj-R^2 and lack of fit should also be evaluated in order to study the accuracy of the final model. R^2 is used to determine the model's power in explaining the variation in experimental data. The R^2 values provide measurement of how much of variability in the observed response value can be explained by the experimental factors and their interactions. A good model (R^2 values above 90% are considered very well) explains most of the variation in the response. Lack of fit shows the adequacy of models. However, in crossed design the software output for these parameters were R^2, Adj-R^2 and lack of fit that make the final decision. Finally, optimum conditions were determined using a "Crossed D-Optimal" design by means of mathematical and graphical overlay contour plots for global optimization approach.

Table- 3: Crossed design (mixtures and process) layout and results

ID	Run	Mixtures			Process	Response
		A: Arabic gum (%)	B: Whey protein concentrate (%)	C: Tween 80 (%)	D: Clove oil (%)	Average of particles size (nm)
9	1	0.0	16.0	4.0	2.0	1905.0
13	2	12.5	4.5	3.0	4.0	1199.0
7	3	16.0	0.0	4.0	2.0	284.2
1	4	10.0	10.0	0.0	2.0	1496.0
0	5	0.0	20.0	0.0	6.0	835.4
4	6	20.0	0.0	0.0	2.0	312.2
0	7	0.0	16.0	4.0	6.0	1448.4
17	8	20.0	0.0	0.0	4.0	475.3
10	9	10.0	10.0	0.0	10.0	983.8
2	10	16.0	0.0	4.0	10.0	231.2
5	11	0.0	16.0	4.0	10.0	1172.0
16	12	4.5	14.5	1.0	8.0	663.1
14	13	12.5	4.5	3.0	8.0	764.0
0	14	0.0	16.0	4.0	6.0	1365.0
2	15	16.0	0.0	4.0	10.0	199.3
11	16	0.0	18.0	2.0	2.0	924.8
0	17	20.0	0.0	0.0	6.0	549.3
0	18	18.0	0.0	2.0	6.0	129.8
3	19	0.0	20.0	0.0	10.0	839.2
12	20	18.0	0.0	2.0	10.0	230.0
8	21	20.0	0.0	0.0	10.0	688.6
0	22	16.0	0.0	4.0	6.0	237.2
6	23	0.0	20.0	0.0	2.0	1097.0
0	24	10.0	10.0	0.0	6.0	671.7
15	25	4.5	14.5	1.0	4.0	1044.0
5	26	0.0	16.0	4.0	10.0	1267.6
3	27	0.0	20.0	0.0	10.0	813.6
9	28	0.0	16.0	4.0	2.0	1701.7

Preparation of clove oil encapsulated samples: Based on designed formula presented in Table 3; for every formula, Arabic gum and WPC were added to Milli-Q water and mixture was separately blended by vortex on a Vortex-2 Genie (Scientific Industries, USA) for 2 min. Then, they were kept for 24 hours at cold room (4 °C) to deserve a full hydration of the biopolymer molecules. In order to obtain oil in water emulsions (O/W) variations with different core to wall material ratios; based on the experimental design (Table 3), content of Tween 80 and clove oil were added to the solution and was used a vortex mixer IKA®-MS2 Minishaker (IKA Labortechnik, Staufen, Germany) to mix and homogenize samples and then further emulsified sonication was performed for 2 min using an ultrasonic homogenizer with the power of 70 W at 20 kHz using a Sonopuls HD 2070 (Bandelin, Germany) equipped with a 3 mm diameter Sonotrode probe made of titanium. The emulsions were maintained in an iced water bath in order to keep the temperature below 30 °C. The emulsions were stored at 4°C, and re-equilibrated to room temperature just before analysis.

Particle size measurement: For all the samples, Z-average particle diameters were determined by dynamic light scattering (DLS) using a Zetasizer® Nano Series (Nano ZS model ZEN 3600, Malvern, UK) at a fixed scattered angle of 173°. The concentrated emulsions were diluted with Milli-Q water (1:1000) before size measurement. The software used to collect and analyze the data was the Zetasizer Software version 7.03 from Malvern. These parameters were obtained from the autocorrelation function using the "general purpose mode" analysis model. Measurements were controlled at 25 °C and each measurement was performed three times.

Optimization and validation of optimized condition: In this study, numerical optimization technique was adapted to optimize the process

conditions. This optimization can be defined by a general non-linear algorithm. In this optimization, the desired goals known as constraints for each response and factors were selected along with weight and importance allocated for each goal. For process variables optimization with high desirability, the stepwise regression model was developed. In this study, the optimal condition such as minimum particles size of encapsulated clove oil was determined. In addition, for the validity of optimized condition, triplicate experiments were performed. In order to define highly accurate and suitable optimal conditions; average values of experimental data were compared to predicted values.

RESULTS AND DISCUSSION

Results of average particles size for each experimental crossed design are shown in Table 3.

Fitting the response surface models: Results of the regression analysis and ANOVA for all the models are summarized in Tables 4 and 5. The coefficients of the model for constant term, cubic effects, quadratic effects and interaction effects were presented in Table 4. It is customary to add or remove quite complex terms due to crossing the mixture model terms with the process model ones for crossed mixture-process designs by stepwise regression with alpha to enter = 0.1, alpha to exit = 0.1. Besides, Hierarchical terms (AB, BC, CD, ABC, BCD, and AD^2) added after stepwise regression.

Table- 4: ANOVA and regression of selected model for particles size of encapsulated clove oilusing crossed design

Term	Coefficient Estimate	t for H_0 coefficient= 0	Prob > |t|	R-squared	M.S.E.
Added					
AC(A-C)	-9860.25	-4.50	0.0001	0.7531	66107.40
ABD^2	2296.68	3.51	0.0019	0.8392	44915.03
BD	-205.16	-2.98	0.0069	0.8855	33445.90
BC(B-C)	-8193.43	-2.59	0.0170	0.9133	26538.89
AC	-18860.00	-3.55	0.0020	0.9468	17106.10
ABCD	-18384.53	-2.57	0.0184	0.9598	12900.63
BCD(B-C)	-1510.58	-2.08	0.0512	0.9673	11058.82
ABD	-768.04	-2.81	0.0116	0.9773	8111.99
AD	76.66	1.97	0.0657	0.9815	6996.60
ACD	-1098.36	-3.27	0.0048	0.9889	4451.43
BD^2	103.22	2.52	0.0237	0.9922	3338.07
AC(A-C)	-4025.51	-1.88	0.0813	0.9938	2856.57
Removed					
AC(A-C)	-1138.99	-0.22	0.8253	0.9466	16332.26

ANOVA for combined reduced cubic (mixture: A, B, and C) × quadratic model (process: D) is shown in Table 5. The high R^2 and adjusted R^2 indicate a good explanation of the variability by the selected model for particles size of encapsulated clove oil (0.995 and 0.983). In addition, the model F-value and Prob>F are 84.47 and <0.0001, respectively. That implies the model is significant. Furthermore, the lack of fit and F-value were 0.9377, and 0.13, respectively that implies the lack of fit is not significant and relative to the pure error. Inasmuch as, when we want the model to fit, non-significant lack of fit is good. Therefore, the crossed model (mixtures (cubic) × process (quadratic)) appears to be a reliable model for particles size of encapsulated of clove oil from the crossed design.

The purpose of predicted equation is to fit the data into the model for desired optimization. The following equation as final regression functions for particles size of encapsulated clove oil in terms of pseudo-components and coded factors were applied for generating various graphical models.

$$\text{Particles size} = 547.18 \times A + 833.52 \times B + 72499.68 \times C - 74.60 \times A \times B - 113100 \times A \times C + 181.47 \times A \times D - 96876.59 \times B \times C - 135.54 \times B \times D - 5200.74 \times C \times D + 82973.03 \times A \times B \times C - 1116.27 \times A \times B \times D + 5399.88 \times A \times C \times D + 7402.31 \times B \times C \times D - 32.27 \times A \times D^2 + 128.89 \times B \times D^2 + 35594.72 \times A \times C \times (A\text{-}C) + 18139.39 \times B \times C \times (B\text{-}C) - 15562.31 \times A \times B \times C \times D + 2079.56 \times A \times B \times D^2 - 3412.17 \times B \times C \times D \times (B\text{-}C) \quad (1)$$

Table -5: ANOVA and regression of selected model for particles size of encapsulated clove oil using crossed design

Variable Component	Sum of squares	Degree of freedom (d.f.)	Mean square	F value	Prob > F
Model	6.4E6	19	3.4E5	84.47	< 0.0001
Linear Mixture	3.5E6	2	1.7E6	439.54	< 0.0001
AB	271.5	1	271.5	0.07	0.8007
AC	689.9	1	689.9	0.17	0.6882
AD	71400.8	1	71400.8	17.92	0.0029
BC	529.1	1	529.1	0.13	0.7250
BD	49800.9	1	49800.9	12.50	0.0077
CD	1534.5	1	1534.5	0.39	0.5521
ABC	555.4	1	555.4	0.14	0.7186
ABD	1.1E5	1	1.1E5	27.63	0.0008
ACD	1059.1	1	1059.1	0.27	0.6201
BCD	978.6	1	978.6	0.25	0.6335
AD^2	1302.6	1	1302.6	0.33	0.5831
BD^2	26257.2	1	26257.2	6.59	0.0333
AC(A-C)	392.9	1	392.9	0.099	0.7615
BC(B-C)	122.0	1	122.0	0.03	0.8654
ABCD	7081.6	1	7081.6	1.78	0.2191
ABD^2	1.4E5	1	1.4E5	36.52	0.0003
BCD(B-C)	347.5	1	347.5	0.087	0.7753
Residual	31868.5	8	3983.6		
Lack of Fit	2319.1	3	773.0	0.13	0.9377
Pure Error	29549.4	5	5909.9		
Corrected Total	6.4E6	27			
Total					
S.D.	63.12		R-Squared	0.9950	
Mean	840.30		Adj R-Squared	0.9833	
C.V.	7.51		Adeq Precision	31.363	

Besides, in Table 5, the values Prob>F of all regression model terms were less than 0.05; which indicate models terms are significant at a confidence interval of 95%.Also, values greater than 0.1 indicate the model terms are not significant. So that, in this research linear mixture components, AD, BD, ABD, BD^2, ABD^2 are significant model terms. Hence, equation 1 is rearranged and simplified as equation 2.

$$\text{Particles size} = 547.18 \times A + 833.52 \times B + 72499.68 \times C + 181.47 \times A \times D - 135.54 \times B \times D - 1116.27 \times A \times B \times D + 128.89 \times B \times D^2 + 2079.56 \times A \times B \times D^2 \quad (2)$$

The equation 2 can explain the significant effect of components and process factors on particle size of encapsulated clove oil. Accordingly, this equation shows that the wall types A, B and C have significant effects on particle size and with the amount of oil content having higher effects than the wall types.

Diagnostic Model: Before accepting any model, the satisfactoriness of the adopted model must be checked by an appropriate statistical analysis. The major diagnostic analysis as shown in Figs. 1a to1d may be given for diagnostics for residual behavior. There are several residuals graphs to test the model assumptions. The primary analysis is to examine a normal probability plot of the residuals, that is, the number of standard deviations of actual values based on predicted values (Fig. 1a). The normal probability plot determines the residuals follow a normal distribution. This is the most important assumption for checking sufficiency of statistical model.

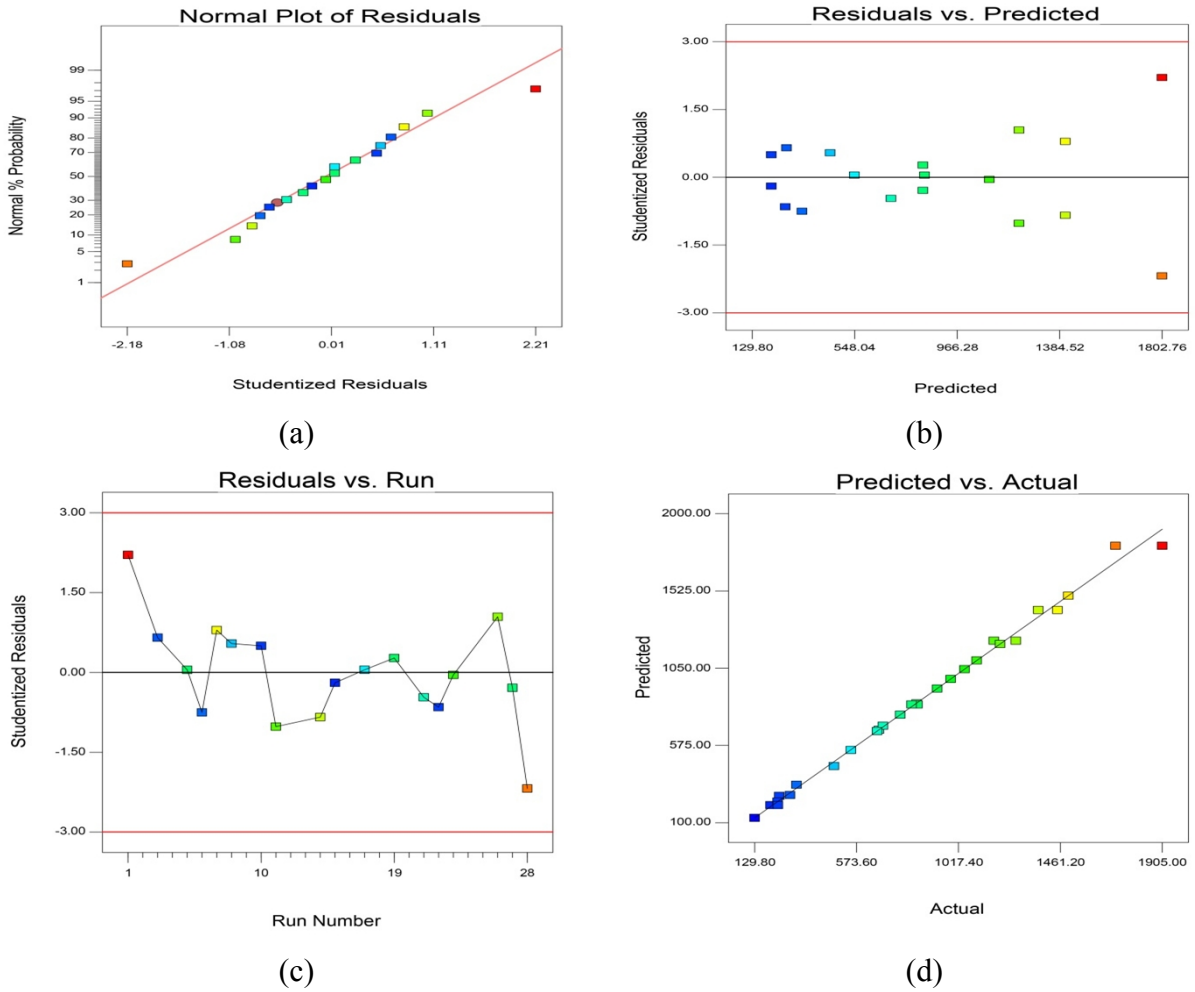


Fig. -1: Residual diagnostics of crossed model for particles size: (a) normality; (b) residuals vs. predicted; (c) residuals vs. run order; (d) predicted vs. actual.

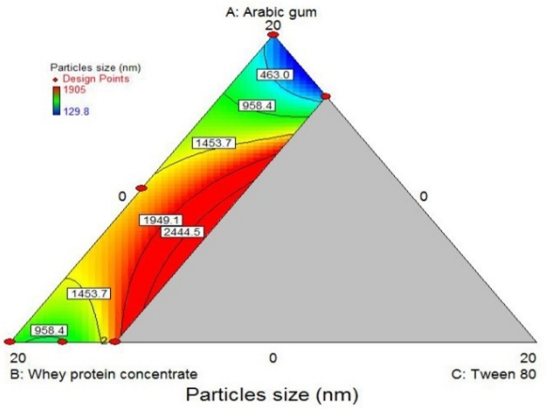
The residuals plotted versus the predicted responses are shown in Fig. 1b which is residuals analysis. Fig. 1c presents residuals versus run number graphs that reveal any time-based effects or sequential component. Actual data versus predicted values displays the real response data plotted against the predicted responses are shown in Fig. 1d.

Modeling: Models are used for prediction in order to generate response surface graphs and contour plots. There are significant interactions between mixture and process factors; the response surface graphs and contour plots as variation of the process conditions are shown in Figs. 2a to 2j. The unique characteristic of experimental design and modeling which is combination of response surface method (RSM) and process factors able to show statistical effects and the dynamic nature of the process knows mixture factors (Arabic gum, whey protein concentration, and Tween 80) along with a process factor (clove oil concentration). Figs. 2a to 2j depict models with contour and 3D formation of particles size of encapsulated clove oil, representing testing model for the define particles size of encapsulated clove oil with biodegradable shell separated by various percentage of clove oil (2, 4, 6, 8 and 10 %).

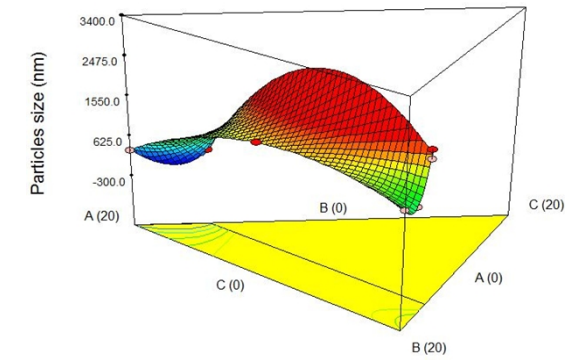
As illustrated in Fig. 2, contour and 3D surface plots of particles size change by the core level (clove oil) and orientation of the response curve of particle size has shifted. With increasing of core level oil concentration from 2 to 10 %; it can be seen by increasing wall A to 16 % and simultaneously reduction in wall type B content to 0 % and increasing wall type C to 4 % in relation the wall A, the smallest particle size was obtained (199.3 nm). Meanwhile, at the concentrations of 2 and 4 % oil (Figs. 2a to 2d), intermediate values in formulations of A and B walls and with increasing values of C wall have the effect of increasing the particle size.

At a concentration of 6 % oil (Figs. 2e and 2f), particle size contours dispersion is reduced, but the overall trend is similar to the previous oil level. At 6 % oil concentration, the minimum

particle size is achieved in the presence of higher walls content (more than 16 %). The similar trends are observed for 8 and 10 % oil content (Figs. 2g to 2j). In general, it can be concluded that the lowest particle size is achieved using 16 % wall type A and 4 % wall type C to by encapsulating 2 or 6 % of oil.



(a)



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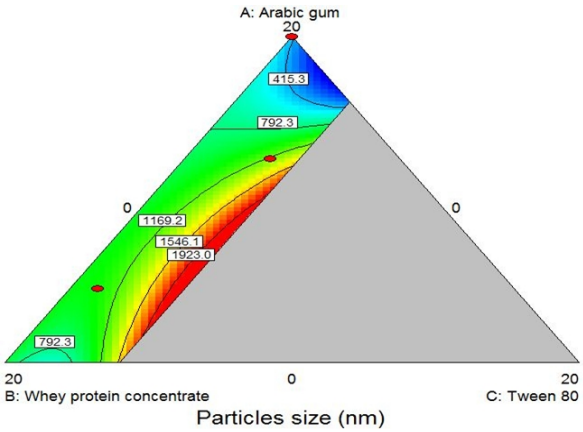

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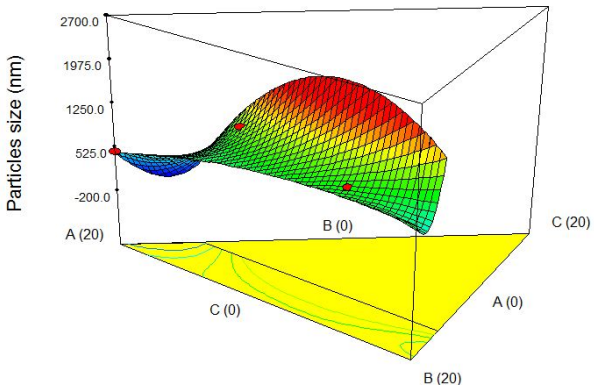
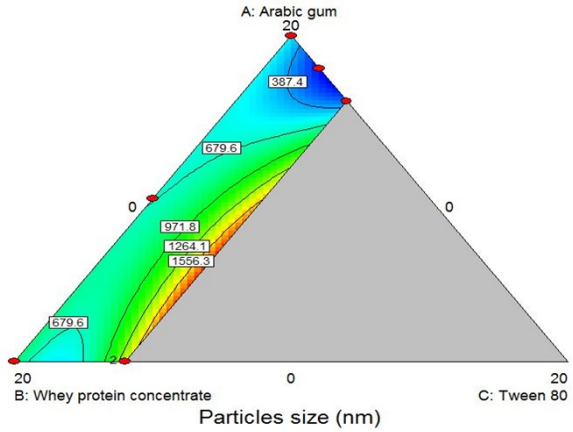
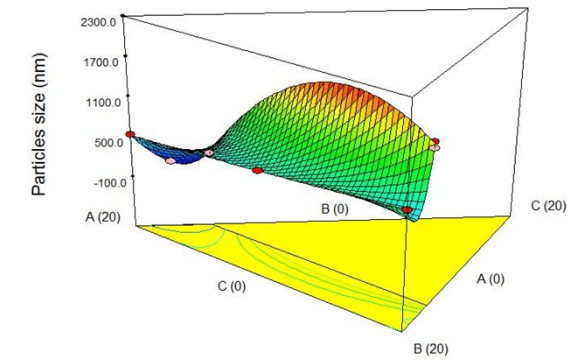


(d)



(e)



(f)

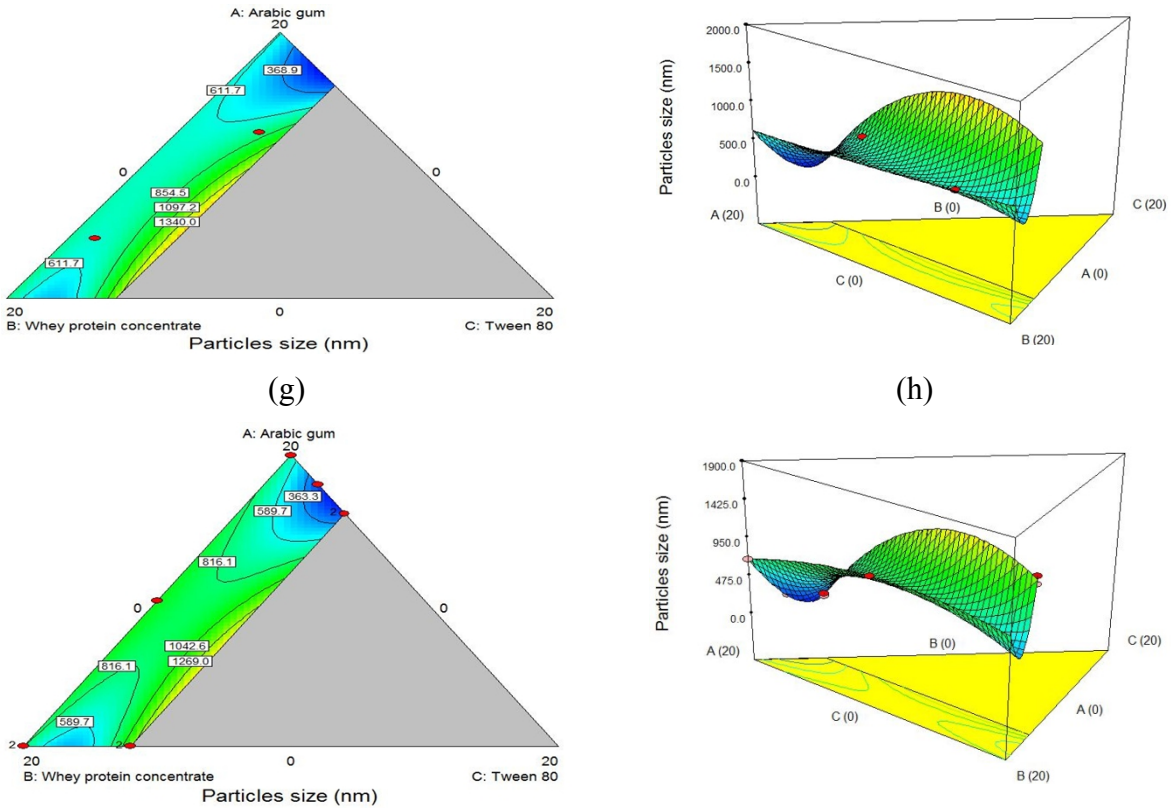


Fig. -2: Model graph of crossed design for encapsulated of clove oil with various clove oil concentration: (a, b) 2%; (c, d) 4%; (e, f) 6%; (g, h) 8%, and (i, j) 10%.

Optimization and formulation: The numerical optimization finds a point that maximizes the desirability function. Table 6 presents the specific optimum conditions for the define particles size of encapsulated clove oil with biodegradable shell. In fact, our goal is at optimum conditions to obtain minimum particles size with encapsulated maximize clove oil concentration with biodegradable shell.

Table- 6: Desirability specifications of numerical optimization for crossed design

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Arabic gum (wt. %)	is in range	0	20	1	1	3
WPC (wt. %)	is in range	0	20	1	1	3
Tween 80 (wt. %)	is in range	0	4	1	1	3
Clove oil (wt. %)	maximize	2	10	1	1	3
Particles size (nm)	minimize	129.8	1905	1	1	5

Table 7 shows that all goals are joined into one desirability function which are based on various responses and factors. The suitable optimum formulation (Arabic gum of 16.9 %, WPC of 0.0, Tween 80 of 3.1 wt. %, and clove oil concentration of 10.0 %) with high desirability of 0.998 was selected.

Table -7: Optimization conditions, prediction and desirability of model

Arabic gum (%, w/w)	WPC (%, w/w)	Tween 80 (%, w/w)	Clove oil (%, w/w)	Particles size (nm)	Desirability	Selection
16.9	0.0	3.1	10.0	135.6896	0.997925	Selected
0.0	18.3	1.7	10.0	468.8053	0.875949	
20.0	0.0	0.0	10.0	696.3877	0.786411	
10.0	10.0	0.0	8.4	727.8441	0.713479	

Optimized condition validation: Applying defined information in final step of optimization, experimental data were compared to theoretical expected values defined and projected by crossed design. In order to compare the exact predicted results with experimental value, additional experiments (triplicates) were carried at these optimal conditions. Fig. 3 shows particles size distribution of optimized condition used as follows: Arabic gum of 16.9 %, WPC of 0.0, Tween 80 of 3.1 wt. %, clove oil concentration of 10.0 % and Milli-Q water of 70 %. Under these conditions, the particles size of encapsulated clove oil (128.2 ± 4.3 nm) was close to predicted value (135.7 nm). Thus, for predicting the minimum particles size of encapsulated of clove of using ultrasonication emulsification method with crossed design was considered an accurate technique.

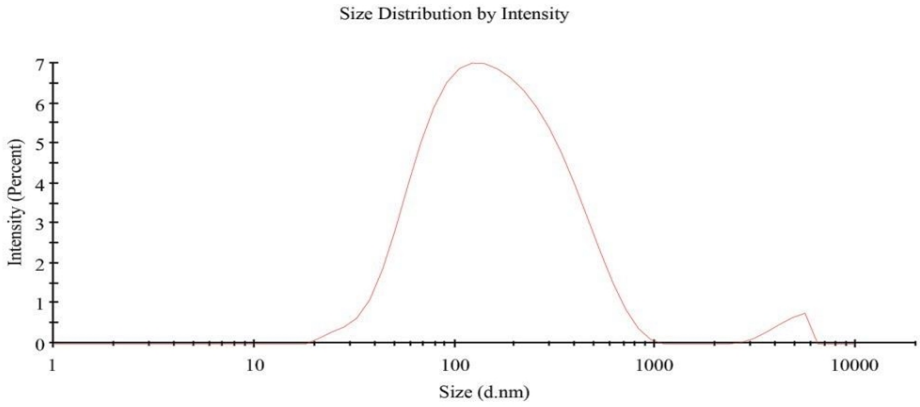


Fig. -3: Particles size distribution for final optimal formulation

CONCLUSION

In this research, the crossed D-optimal design which combine both mixture and process factor, for the minimum particles size of encapsulated clove oil using ultrasonication emulsification process was optimized. Checking the validity of the model, various relevant statistical indexes, such as F-value, coefficient of determination (R^2), Adj-R^2 and lack of fit and coefficient of variation (C.V.) were determined to be statistically adequate. Based on projected model, a highly suitable correlation as quadratic polynomial equation was developed. The desirability of obtained model was investigated. The findings of this study suggested that the among different mixture of shell and oil formulations obtained and the optimal conditions were selected: Arabic gum of 16.9 % w/w, WPC of 0.0, Tween 80 of 3.1 % w/w, clove oil concentration of 10.0 % w/w, and Milli-Q water of 70 % w/w. There was an optimum level of interactions between the component of encapsulated formulation that affect minimum size of these particles with encapsulated maximize clove oil concentration at biodegradable shell. Under these optimal conditions, experimental particles size of encapsulated clove oil was very close to the predicted values.

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