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Microencapsulation of vitamin E: Characterization of Complex Coacervation Conditions Using Response Surface Methodology

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Abstract

In this study, high efficiency vitamin E microencapsulation was aimed with the complex coacervation method. Response surface methodology (RSM) was used to optimize the microencapsulation efficiency of vitamin E. The microencapsulation efficiency of microencapsulated vitamin E was investigated in terms of two variables, including the amount of core material and surfactant concentration (SDS). According to the RSM results, the experimental condition with the highest efficiency (93.42%) was found in 4.00 g of core material and 0.50% surfactant in the experiment set. Morphological and chemical analyzes of microcapsules were characterized by optical microscopy and scanning electron microscopy (SEM) and Fourier transformation infrared spectroscopy (FT-IR).

Keywords: Vitamin E, microencapsulation efficiency, response surface methodology, complex coacervation, micro technology.

1. INTRODUCTION

Vitamin E is a member of fat-soluble vitamins and its chemical term is alpha-tocopherol [1-5]. Vitamin E has a significant role in the protection of fatty molecules in cell membranes and blood. It is referred to as an antioxidant because of its ability to quench or stabilize time-saving free radicals in degenerative diseases. Vitamin E can rapidly break down in the presence of free radical and oxygen induced oxidative processes [2-7]. It is suggested that it must be protected from its close surroundings before its application. The most commonly used technology to develop durability and safety of functional materials is microencapsulation [8]. Microencapsulation is a technique which is particles of liquid or solid materials or droplets are covered in a film of polymeric material [9-12]. Encapsulation preserves the secured active ingredient from the outer surrounding, then releases active material, as soon as interacting with exact stimulus, at a point when its functional features are required [13]. Encapsulation can also be described as the process of storing active ingredients in a carrier material to increase the distributing of active compounds to sustenance products. Various nutrient compounds, like enzymes, polyphenols, vitamins, essential oils and carotenoids are held into biopolymer micro particles and nanoparticles for retain their basic properties without changing them [14]. Microencapsulation is used for other several aims such as increasing the shelf life of

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foods, in particular, raising the nutritional value, providing digestibility, and shortening the duration of ripening [15,16]. In addition, microencapsulation has various implementation in cosmetics, pharmaceutical, pesticides and medical applications, catalysis, biology and many other fields [17]. Various techniques are available for encapsulating core materials [18]. In general, spray drying or solvent evaporation techniques have been observed in the literature as microencapsulation of vitamins. Unlike in the study, vitamin E containing microcapsules were developed using complex coacervation (physicochemical method). It has been reported that complex coacervation is based primarily on pH and occurs in systems including two dispersed colloids of the opposite electric charge. Optimum circumstances for complex coacervation are obtained when the pH is arranged to a point where two colloids are present [19, 20]. Microencapsulation with complex coacervation has many advantages. Complex coacervation is known for its simplicity, low cost, reproducibility and scalability, which provides high encapsulation efficiency even at very high transport loads [19]. The wall of the microcapsules does not dissolve in water when the cross-linked chemical is present. This is an important advantage over microcapsules such as spray drying or solvent evaporation. Microcapsules prepared with complex coacervation which has excellent oxidation stability and low moisture content such as pH change, diffusion, temperature, osmotic pressure, dissolution and wall deterioration [19-21]. Complex coacervation in active ingredient encapsulation basically involves the use of two mutually loaded biopolymers which can form complex shell surrounding the core material [19]. Present study, gelatin and gum Arabic with biocompatible properties were used as coating materials to produce microcapsules. Despite the protective effect of microencapsulation, serious oxidation may occur on the top of microcapsules because of high temperature exposure during the process. Residues on the top of microcapsules will have a damaging effect on the oxidation of microencapsulated active component. In the context, microencapsulation efficiency was used as a significant parameter to evaluate the quality of microencapsulated active components [22]. The purpose of the study is to research the effect of two different variables on microencapsulation efficiency using RSM, to prepare microcapsules and to perform the characterization of the microcapsules obtained.

2. MATERIAL AND METHODS

2.1. Materials

Vitamin E was purchased from medicine in Isparta/Turkey. Gelatin and gum Arabic (Merck), sodium dodecyl sulfate (Merck), sodium hydroxide (Merck), n-hexane (Merck), acetic acid (Merck) and glutaraldehyde (Merck) were used during all experiments.

2.2. Experimental design

The optimization of vitamin E encapsulation was planned with central composite design (CCD). CCD is a 2^k factorial design with central point and star points [23]. The response surface methodology (RSM) was performed to optimize the microencapsulation efficiency of vitamin E through two independent variables; amount of core material (g) and surfactant concentration (%w/v). To facilitate multiple regression analysis, independent variables are encoded (Table 1). The experimental design was generated using MINITAB 16 (Licensing: lifetime) software. The square polynomial regression model was presumed to predict the *Y* variable (microencapsulation efficiency). The model aimed to the response of *Y* fitted Eq. (1) as follows [22-25]:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon \quad (1)$$

Where *Y* is response (efficiency of microencapsulated vitamin E, %). β_0, β_1, β_2, β_{11} and β_{12} are coefficients of intercept, linear, square and interaction terms, respectively. X_1 and X_2 are uncoded independent variables (amount of core material and concentration of surfactant).

Table 1 It is used to show coded levels for independent variables in experimental design for microencapsulation of vitamin E

Variables	Coaded level	
	-1	+1
Amount of core material (g)	2.00	6.00
Concentration of surfactant (w/v%)	0.30	0.70

2.3. Preparation of microcapsules

Vitamin E particles covered by gelatin (GE) and gum Arabic (GA) have been prepared with complex coacervation method. Wall materials, aqueous solutions of GE and GA (2%, w/v) solutions were prepared separately. Then, 100 mL of an aqueous gelatin solution was warmed at 50 °C – 55 °C with stirring for vitamin E (2.00 g – 6.00 g) 15 min. For providing emulsifying, SDS solution (0.30% w/v – 0.70% w/v) was prepared and added. Finally, 100 mL of gum Arabic solution (2%, w/v) were added to the material and stirred for 30 min. Next, pH value of emulsion was adjusted to 4-4.5 with acetic acid (10%, v/v) which is the isoelectric point of gelatin and gum Arabic (at 1500 rpm, 45 °C, 90 min.). This mixture was transferred into 300 mL cooled deionized water. The temperature of the system was progresively decrease to 10 °C in an ice bath during the coacervation process. Glutaraldehyde solution was slowly added drop wise for cross-linking the microcapsules and was stirred 2.5 hours. Then pH system was adjusted to 9-9.5 with sodium hydroxide (10%, v/v). The mixture was allowed to stand for 1 day. The next day, the retiform microcapsules were splited by centrifugation for 5 mins at 1500 rpm and 25 °C, washed two times with deionized water and oven dried at 40 °C [26].

2.4. Microencapsulation efficiency

Microencapsulation efficiency was calculated from the next equation based on similar studies Eq. (2) [22, 27, 28].

$$MEE\% = \frac{Total\ amount\ vit.E - surface\ vit.E\ amount}{\text{Total amount vit. E}}\ x100 \quad (2)$$

The total vitamin E amount in this equation represents the amount of vitamin E that is known to be used based on the experimental set, while the surface extract amount represents the amount of non-encapsulated extract that remains between the products and the surface. Capsule sample was put in an erlenmeyer flask containing 50 mL of n-hexane and gently shaken for 5.00 minutes without capsule destruction while measuring the volume of surface extract. The solution was then filtered onto filter paper. A rotary evaporator was used to evaporate the n-hexane in the solution. After removing the n-hexane, the amount of vitamin E left was weighed and recorded [22, 27, 28].

2.5. Characterization of microcapsules

Morphological structure of the prepared microcapsules was examined by optical microscopy and scanning electron microscope (SEM). Optical images were taken with device of Boeco brand microscope. Microcapsules shape and morphology were measured from SEM images using Quanta FEG250 (Thermo Fisher Scientific). The chemical structures of samples were analyzed by a fourier-transform spectrometer. Samples were ground and mixed with KBr to make pellets, and FT-IR studies were performed on a Perkin Elmer Spectrum BX device.

3. RESULTS AND DISCUSSIONS

3.1. Experimental design and ANOVA results

The experimental studies for optimization of microencapsulation conditions of vitamin E, a two factor CCD was adjusted on the principle of coded from two independent variables (Table 1) and thirteen simplified experimental sets were obtained (Table 2). The amount of core material vitamin E and surfactant concentration were researched in the ranges of 2.00 g – 6.00 g and 0.30% w/v – 0.70% w/v, respectively.

Table 2 Central composite design for the optimization of vitamin E microencapsulation

NO	Core Material (A)	Concentration of Surfactant (B)	Experimental Efficiency (%EE)	Predicted Efficiency (%)
1	4.00	0.50	89.75	91.40
2	4.00	0.50	90.12	91.40
3	4.00	0.50	91.38	91.40
4	1.17	0.50	68.20	69.99
5	2.00	0.70	69.27	68.65
6	6.83	0.50	67.29	67.58
7	4.00	0.50	93.42	91.40
8	6.00	0.30	68.18	66.68
9	2.00	0.30	72.28	69.76
10	4.00	0.78	68.40	68.12
11	4.00	0.50	90.57	91.40
12	6.00	0.70	67.92	68.33
13	4.00	0.22	65.37	67.73

The response surface graphs for microencapsulation efficiency as a function of two selected parameters using important factors for microencapsulation efficiency are demonstrated in Fig.1. Each of the two parameters was observed to be effective on efficiency. Microencapsulation of vitamin E with the amount of core material concentration of 4.00 g and surfactant concentration 0.50% gave rise to the highest microencapsulation efficiency (93.42%) (Fig.1).

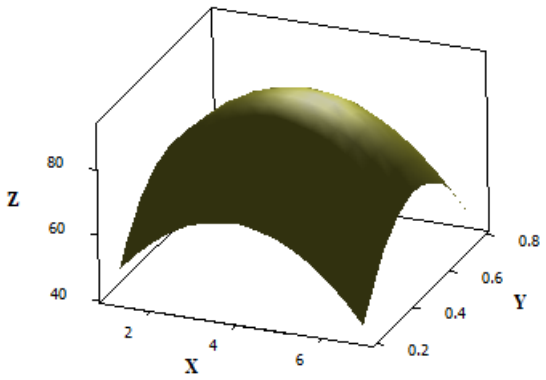

Figure 1 Microencapsulation efficiency (Z), amount of core material (X), surfactant concentration (Y).

According to RSM results, the ideal circumstances for microencapsulation of vitamin E aimed to be 4.00 g core material and 0.50% surfactant concentration where the microencapsulation efficiency was 91.38%. Response efficiency was determined under thirteen experimental circumstances; the regression coefficients were calculated. The model equation is shown in next Eq. (3):

$$\textbf{MEE\%} = -20.89 + 20.97*A + 282.69*B - 2.78*A*A - 288.92*B*B + 1.72*A*B \quad (3)$$

To determine the optimal state of microencapsulated vitamin E and the important variables, statistical analysis of ANOVA was carried out by the common test of two parameters (Table 3).

Table 3 Regression coefficient values are calculated for the microencapsulation of vitamin E

	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	1591.74	318.348	83.74	0.000
Linear	2	5.80	2.901	0.76	0.501
A	1	5.67	5.673	1.49	0.261
B	1	0.13	0.129	0.03	0.859
Square	2	1584.05	792.024	208.34	0.000
A*A	1	861.31	861.307	226.56	0.000
B*B	1	929.16	929.163	244.41	0.000
2-Way Interaction	1	1.89	1.891	0.50	0.503
A*B	1	1.89	1.891	0.50	0.503
Error	7	26.61	3.802		
Lack-of-Fit	3	18.10	6.033	2.84	0.170
Pure Error	4	8.51	2.128		
Total	12	1618.35			

R^2	R^2(adj)	R^2(pred)
98.36	97.18	91.22

The model is significant ($p<0.05$). The model does not show linearity ($p>0.05$). Quadratic part of model is significant ($p<0.05$). Two-way interaction is not significant in the model ($p> 0.05$). Lack-of-fit p value was found as 0.170. Hence the model matches the data. R^2 value was found 98.36. The probability plot of residuals chart is given in Figure 2.

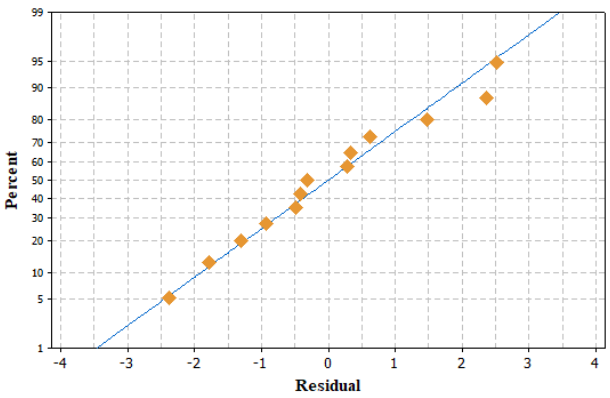

Figure 2 Probability plot of residuals graph

In the analysis of the graph, the mean and standard deviation of the residuals were 0.0±1.489 (n = 13). According to normality AD test, p=0.838. Residuals show normality (p=0.838).

3.2. Morphological analysis of microcapsules

The morphological characterizations of the microcapsules produced by considering the optimum conditions by RSM were performed with optic microscope and SEM images. Images of SEM analysis and optical microscopy showed that the microcapsules have a smooth shape and a flat shell structure. The optic microscope image which is taken from the highly efficient sample is shown in Fig.3.

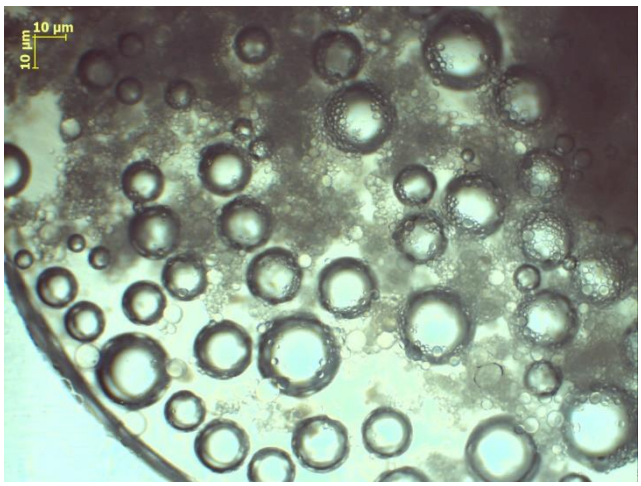

Figure 3 Optical microscope image of microencapsulated vitamin E

The SEM image which is taken from the highly efficient sample is shown in Fig.4.

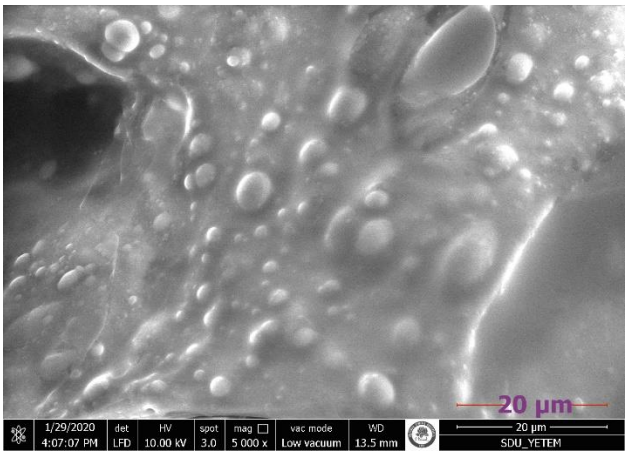

Figure 4 SEM images of microencapsulated vitamin E

The sphericity of the vitamin E microcapsules prepared by complex coacervation was good, and the particle size ranged from ~4 to ~80 µm.

3.3. FT-IR analysis of microcapsules

FT-IR spectrum of microcapsule produced was obtained. The comments were inferred utilizing the FT-IR analysis results of similar studies [29-31]. Specific bands of core material and polymers (vitamin E) used in microencapsulation were observed in the FT-IR spectrum of microcapsules also (Fig.5). FT-IR results show that the values of some groups deviate when complex is formed. When the FT-IR spectra of gelatin and gum Arabic are examined, it is seen that some bands in these spectra form a are lost complexes. Esterification was formed as a result of the reaction of alcohol in the functional group of gelatin with the acid in the medium. This peak is seen in the FT-IR spectrum of the microcapsule at 1600 cm^{-1}. The peak between 2340 cm^{-1} and 2300 cm^{-1} in the spectrum of microcapsules is the combination of C-N (amide I) stress peaks in gelatin and O-H stress peaks in gum Arabic. As seen from the microcapsule spectrum, ~1028 cm^{-1} band is the characteristic band of Arabic gum. This shows that the gum Arabic was successfully put into the structure of microcapsule. The notable FT-IR bands for gelatin came out at 3001.2 cm^{-1}, 2340 cm^{-1}, 1530 cm^{-1}, and 1480 cm^{-1}. From the spectrum of gum Arabic, OH stretching at ~2900 cm^{-1}, C-H stretching at ~2350 cm^{-1}, and C=O stretching at ~1665 cm^{-1} were observed (Fig.5). From the spectrum of gelatin, O-H bonds at

~3500 cm^{-1}, and C=O bonds at ~1678 cm^{-1} were observed. In the spectra, we confirmed the presence of -OH and -C-O-C- functional groups in the chromane ring of vitamin E at 1300-1750 cm^{-1}. FT-IR spectrum showed C=O streching vibration at around 700-1100 cm^{-1}, C-O formation at 1220 cm^{-1}, C=C formation at 1780 cm^{-1} and C-H alkanes group at 2945 cm^{-1} [31]. According to FT-IR spectrum results, there were electrostatic interactions between gelatin and gum Arabic, and the vitamin E was in the microcapsule.

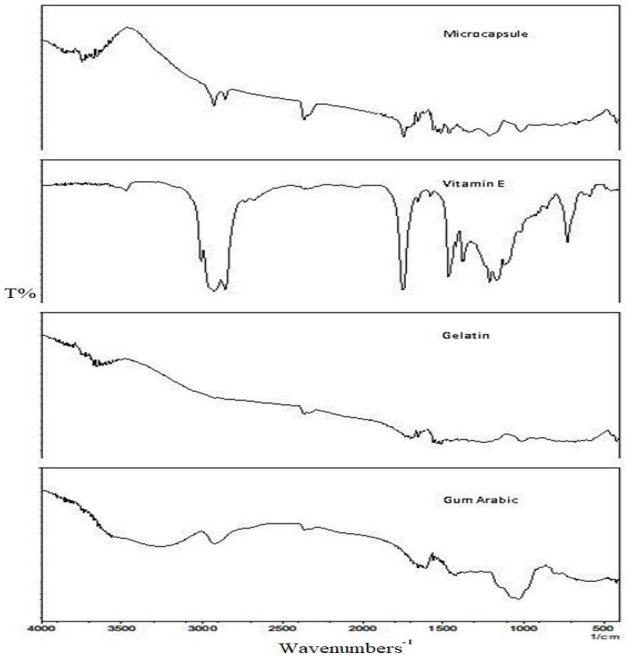

Figure 5 Results of FT-IR spectrum analysis (spectrum of microcapsule; spectrum of vitamin E; spectrum of gelatin, spectrum of gum Arabic).

4. CONCLUSION

In this study, we have aimed at optimizing microencapsulation conditions for vitamin E by using response surface methodology (RSM). The efficiency of microencapsulated vitamin E was remarkably affected by amount of core material surfactant concentration. As a result of RSM, the best conditions for this experiment set, microencapsulation of vitamin E were found to be 4.00 g core material and 0.50% surfactant concentration (%w/v). Microencapsulated vitamin E under optimized conditions showed 93.42% efficiency. The microcapsules were prepared at the optimum conditions in RSM. It was found from the morphological analysis of microcapsules with optical microscope and SEM that microcapsules generally have a regular and similar size structure. The FT-IR spectrum showed that there were electrostatic interactions between gelatin and gum Arabic and that vitamin E was in microcapsules. In a similar different study [32], α-TP was encapsulated in gelatin/pectin wall material using tween 80 as surfactant. In the study, nano-sized capsules were produced with the help of an experimental set created by RSM and capsule size was used as response to response. In our previous study, we encapsulated vitamin E in micro size with the help of different variables. In this study, with the help of RSM, it is investigated the effect of core matter amount and surfactant substance concentration on the efficient obtained at the end of the experiment. The FT-IR results show similarity to our previous study [33]. With this study, vitamin E was successfully microencapsulated with complex coacervation method in an experiment set with RSM.

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