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Microencapsulation of Hemp Oil in Gelatin and Gum Arabic and Investigation of Cumulative Release Properties in n-Hexane Medium

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Abstract: In this study, hemp oil was microencapsulated with gelatin (GE) and gum arabic (GA) polymers by complex coacervation method. The effects of three parameters (stirring speed 1000.0–1500.0 rpm, temperature 50–60 °C, surfactant concentration 0.3–0.7 w/v%) selected in the response surface methodology (RSM) on the encapsulation efficiency were investigated. The obtained results were maximized by multiple response prediction, and the release characteristics were investigated in n-hexane at different times (1 min, 3 min, 5 min, 7 min, 10 min, 30 min, 60 min, 120 min, 240 min, 360 min, 720 min, 1440 min, 2160 min, 2880 min). When the release results were examined, it was observed that the microcapsules started with a rapid release, and the release value remained constant as time progressed. Obtained microcapsules were examined under optical microscope and scanning electron microscope (SEM) devices under special conditions. The microcapsules were observed to be smooth and round in shape under the optical microscope.

Keywords: Gelatin, gum arabic, hemp oil, microencapsulation, n-hexane.

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1. INTRODUCTION

The active component is shielded from the environment using microencapsulation technology, which involves encasing the active ingredient (core material) in a second material or materials. Hence, the core component is the active ingredient, while the surrounding component is the shell (wall). This technique is employed in a wide range of industries, including textiles, cosmetics, and pharmaceuticals (1). Complex coacervation is one of the chemical microencapsulation techniques. Complex coacervation is one of the easiest, least expensive, scalable, repeatable, and most efficient ways to microencapsulate hydrophobic materials (2). This technique provides the core with strong oxidative stability, little surface oil, and good encapsulation efficiency (3,4). Since ancient times, people in Asia and Europe have utilized hemp (*Cannabis sativa L.*), an annual herbaceous plant with several uses, as a source of food, dietary oil, medicine, and fiber. Moreover, notably in North America, the hemp plant is now favored and utilized in significant sectors, including textiles, animal feed, and fiber manufacturing (5). *Cannabis sativa L.* seeds are used to make hemp seed oil, which is prized for its wholesome properties. High levels of polyunsaturated fatty acids and other bioactive minor components can be found in hemp oil. It is a particularly rich source of linoleic acid and alpha-linolenic acid when compared to other vegetable oils. Furthermore, significant are the moderate to high concentrations of tocopherols and tocotrienols, phytosterols, phospholipids, carotenes, and minerals found in hemp seed oil (6). Hemp seed oil is nutritionally better than similar seed oils since it contains gamma-linolenic acid (GLA) (7). In this study, hemp oil was microencapsulated in gelatin and gum Arabic polymers in an experimental set formed with the help of RSM. The obtained results were optimized, reproduced with optimized values, and characterized in SEM and optic microscope devices. In addition, their cumulative release in n-hexane medium was investigated.

2. EXPERIMENTAL SECTION

2.1. Response Surface Methodology and Parameters

Three parameters were chosen for the experimental design of the microencapsulation of hemp oil with gelatin and gum arabic polymers. These parameters are; stirring speed (A, 1000.0–1500.0 rpm), temperature (B, 50–60 °C) and surfactant concentration (C, 0.3–0.7 w/v%). Input responses were analyzed according to the percent yield response. The minimum and maximum points of the selected parameters were determined with the help of preliminary experiments and similar studies in the literature.

2.2. Microencapsulation of Hemp Oil by Complex Coacervation Method

4.0 g of hemp oil (core material) was used for each experiment. In all experiments, the stirring speed was adjusted between 1000.0-1500.0 rpm in the first stage, 400.0 rpm in the second stage, and the temperature (50–60 °C) was adjusted in the heating magnetic stirrer (Weightlab WNH550). GE/GA (1.25 w/v%) was used at a 1:1 ratio. In the first stage, hemp oil was mixed with the prepared GE solution for 20 minutes in the experimental environment and brought to the desired temperature (50-60 °C) from the experimental set. 0.25 mL of sodium dodecyl sulphate (SDS), determined in the experimental sets, was added to the mixture as a surfactant and mixed for another 20 minutes. GA (1.25 w/v%) was added, and mixing was continued for another 20 minutes. Then the pH value of the mixture was adjusted with acetic acid solution (10 v/v%) and stirred at 400 rpm for a further 90 minutes. At the end of the period, the heater was turned off, and 300 mL of cold deionized water and 2 mL of glutaraldehyde (10 v/v%) were added to the mixture. The temperature drop was checked with a digital thermometer and the probe of the heater. Stirring was continued at 400 rpm at this stage. Total stirring time was 4.5 hours. Afterward, the obtained mixture was kept for one day and washed, filtered, and processed (8–11). The diagram of the experiments is shown in Figure 1.

2.3. Microencapsulation Efficiency and Cumulative Release Calculation

While calculating the encapsulation efficiency and cumulative release, Equations 1 and 2, which were created with the help of similar studies in the literature, were used. The encapsulation efficiency was initially calculated with the help of the known amount of cannabis oil and the amount of cannabis oil taken from the surface. For cumulative release, firstly, a calibration set was prepared by mixing hemp oil and n-hexane. 1 mL of sample solutions from the medium at predetermined time intervals (1 min, 3 min, 5 min, 7 min, 10 min, 30 min, 60 min, 120 min, 240 min, 360 min, 720 min, 1440 min, 2160 min, and 2880 min) was withdrawn and replaced with fresh medium to maintain a constant volume and absorbance values were measured (12). UV readings were made at 209 nm.

$$\mathrm{EE\%} = \frac{\text{Total Hemp Oil Amount} - \text{Surface Hemp Oil Amount}}{\text{Total Hemp Oil Amount}} \times 100 \quad (1)$$

$$\mathrm{CR\%} = \frac{\text{Hemp Oil Released Amount}}{\text{Initial Hemp Oil Amount}} \times 100 \quad (2)$$

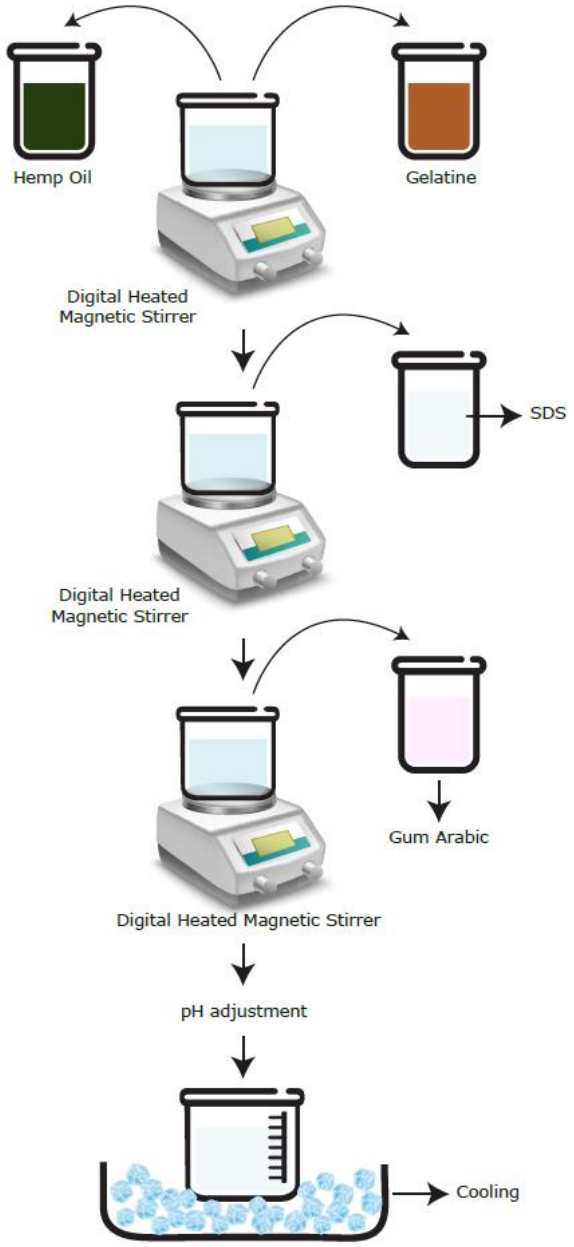

Figure 1: Diagram of the experiment with the complex coacervation method.

2.4. SEM and Optic Microscope Analysis

The obtained microcapsules were imaged with a Bueco BM-2000 optical microscope and Quanta™ 250 FEG brand SEM device to evaluate their size, shape, and color features.

3. RESULTS AND DISCUSSION

The experimental design, experimental yield, and calculated yield values for microcapsules containing hemp oil in gelatin and gum Arabic wall material are shown in Table 1.

ANOVA values calculated over the results obtained are shown in Table 2.

Table 1: Hemp oil-GE/GA experimental design results.

A (rpm)	B (°C)	C (%w/v)	Experimental Efficiency	Calculated Efficiency
1000.0	50.0	0.3	50.610	48.029
1250.0	55.0	0.5	59.360	59.857
1250.0	55.0	0.5	59.050	59.857
1500.0	60.0	0.3	62.780	62.779
1500.0	50.0	0.7	63.850	63.284
1000.0	60.0	0.7	51.850	52.944
1250.0	55.0	0.5	58.720	59.857
1250.0	46.8	0.5	52.520	56.687
1250.0	55.0	0.5	59.890	59.857
1250.0	55.0	0.8	66.850	66.481
1658.3	55.0	0.5	58.550	59.797
1250.0	55.0	0.2	58.950	62.557
1250.0	63.2	0.5	61.480	60.533
841.8	55.0	0.5	34.580	36.584
1000.0	60.0	0.3	49.820	49.024
1000.0	50.0	0.7	49.960	48.609
1250.0	55.0	0.5	59.660	59.857
1500.0	60.0	0.7	65.780	66.999
1500.0	50.0	0.3	64.850	62.404
1250.0	55.0	0.5	61.980	59.857
		R-square	96.070%	
		Lack-of-fit (p>0.050)	0.072	

Table 2: ANOVA results for hemp oil-GE/GA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	11	1037.940	94.358	17.800	0.000
Blocks	2	18.210	9.106	1.720	0.240
Linear	3	703.110	234.370	44.210	0.000
A	1	665.000	664.998	125.450	0.000
B	1	18.230	18.232	3.440	0.101
C	1	19.880	19.880	3.750	0.089
Square	3	310.800	103.599	19.540	0.000
A*A	1	253.460	253.456	47.810	0.000
B*B	1	2.890	2.889	0.550	0.481
C*C	1	40.200	40.202	7.580	0.025
2-Way Interaction	3	5.820	1.939	0.370	0.780
A*B	1	0.190	0.192	0.040	0.854
A*C	1	0.050	0.048	0.010	0.926
B*C	1	5.580	5.578	1.050	0.335
Error	8	42.410	5.301		
Lack-of-Fit	5	38.980	7.797	6.830	0.072
Pure Error	3	3.420	1.141		
Total	19	1080.340			

The model reached as a result of the analysis is significant (p<0.050). The model (p<0.050) shows linearity. A (p<0.050) shows linearity. In addition, B and C do not show linearity (p>0.050). Square is significant in the model (p<0.050). B*B and C*C squares are not significant (p>0.050). 2-Way Interaction is not significant (p>0.050). The p-value of the lack-of-fit was found to be 0.072 (p>0.050). A lack of fit value of p>0.050 indicates that the model fits the data. The R^2 value was found to be 96.070%. The equation showing the calculated encapsulation efficiency obtained as a result of the analysis is given in Equation 3.

$$EE\% = -131.3 + 0.2095\,A + 2.03\,B - 85.4\,C - 0.000070\,A^*A - 0.0187\,B^*B + 43.6\,C^*C - 0.000124\,A^*B + 0.0015\,A^*C + 0.835\,B^*C \quad (3)$$

Three-dimensional surface plots of how the three parameters selected for the encapsulation process with the complex coacervation method (stirring speed, surfactant concentration, and temperature) relate to the independent variables for hemp oil-GE/GA encapsulation efficiency are shown in Figure 2.

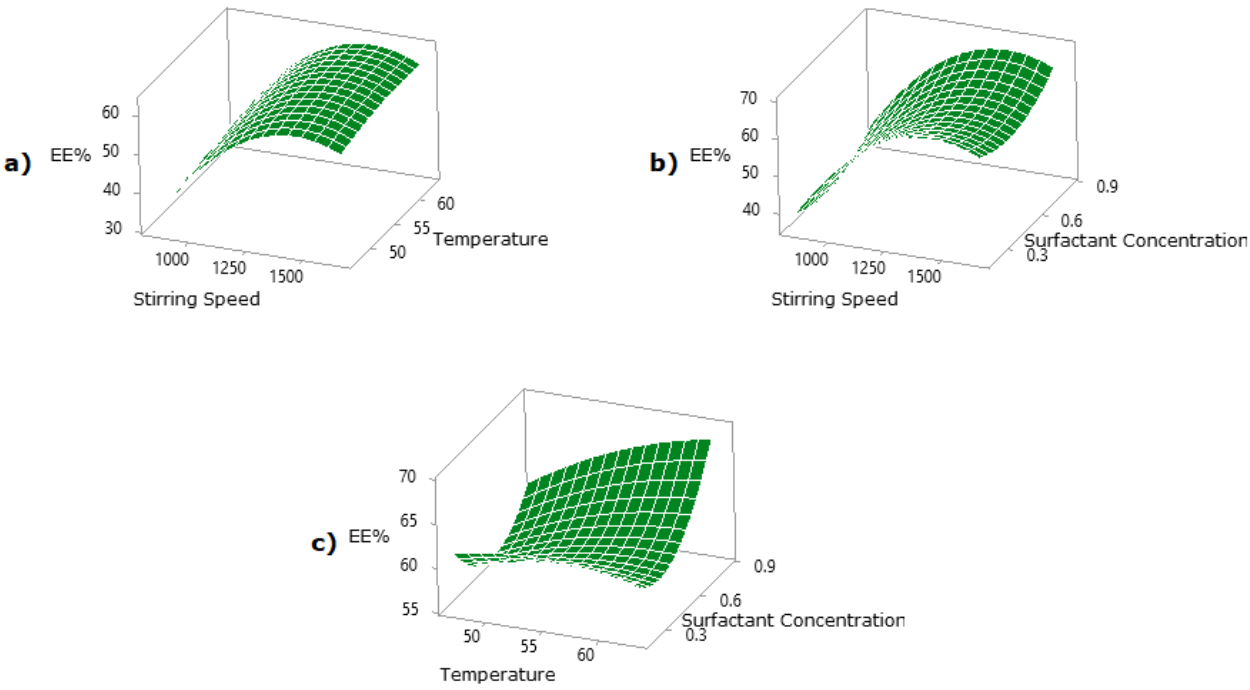

Figure 2: Hemp oil-GE/GA 3D surface plot for temperature, surfactant concentration, stirring speed.

When Figure 2a is examined, the EE% reaches its maximum value and then decreases with the increase in stirring speed and temperature together. When Figure 2b is examined, the EE% reaches its maximum value and then decreases with the increase in stirring speed and surfactant concentration. When Figure 2c is examined, the EE% value reaches its maximum, and a concave structure is observed as the temperature and surfactant concentration increase together up to a certain point.

The encapsulation efficiency relationship as a function of the three parameters (stirring speed, temperature, and surfactant concentration) selected for the microencapsulation process is shown in the contour plots in Figure 3.

The distribution of the residuals was examined and the normality test graph performed is shown in Figure 4.

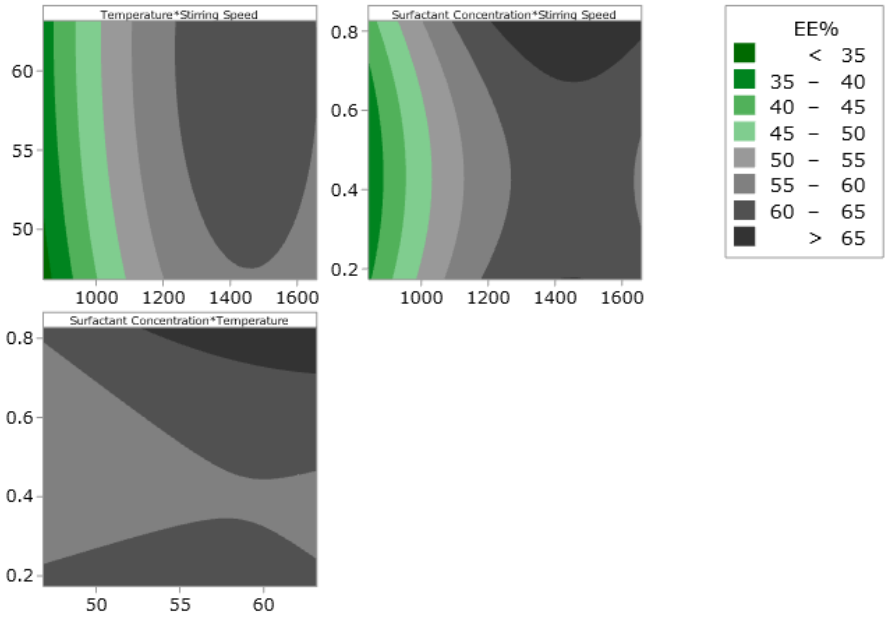

Figure 3: Temperature, stirring speed, and surfactant concentration contour plots for hemp oil-GE/GA.

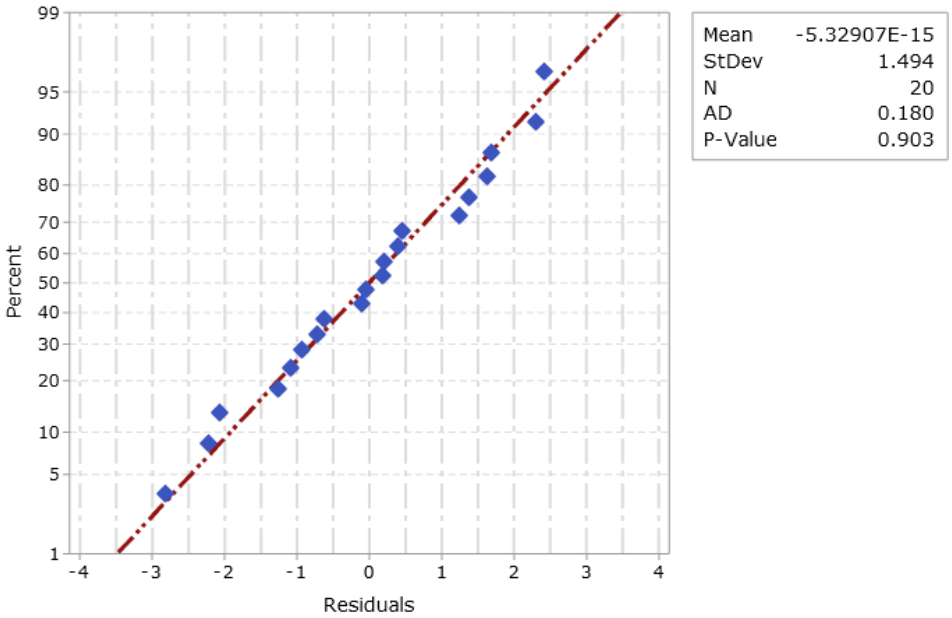

Figure 4: Graph of the distribution of residues for hemp oil-GE/GA.

In the analysis of the graph, the mean and standard deviation of the residuals were found to be 0.000±1.494 (n=20). According to the AD test, which is one of the normality tests, p=0.903. The residuals show a normal distribution. Multiple response predictions for hemp oil-GE/GA as a result of the maximization optimization are given in Table 3 (13,14).

The multiple response prediction graph obtained as a result of the optimization is shown in Figure 5.

The theoretical and experimental %EE results of hemp oil-GE/GA microcapsules produced with optimized parameters are given in Table 4.

Images obtained from the hemp oil-GE/GA optical microscope analysis are shown in Figures 6 and 7.

Table 3: Multiple response prediction for hemp oil-GE/GA.

Variable				
A (rpm)	B (°C)		C (%w/v)	
1443.820	63.165		0.827	
Response	Fit	SE Fit	95% CI	95% PI
EE%	72.026	3.590	(63.750; 80.310)	(62.190; 81.860)

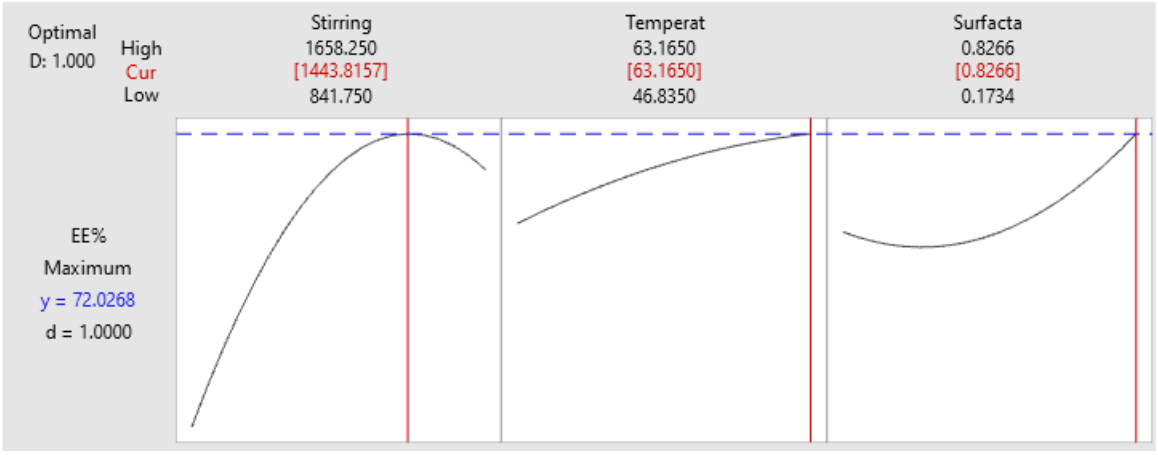

Figure 5: Hemp oil-GE/GA optimization graph.

Table 4: EE% result of hemp oil-GE/GA optimized parameters.

Core Material-Wall Material:	Hemp Oil-GE/GA
Maximized Theoretical EE%:	72.026%
Experimental EE%:	71.351%

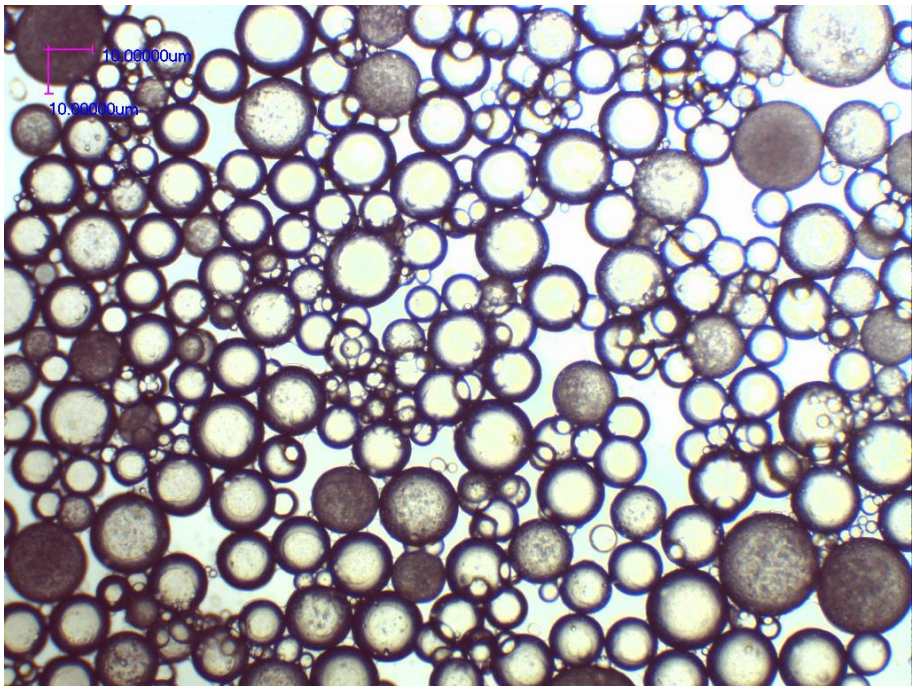

Figure 6: Hemp oil-GE/GA optical microscope image.

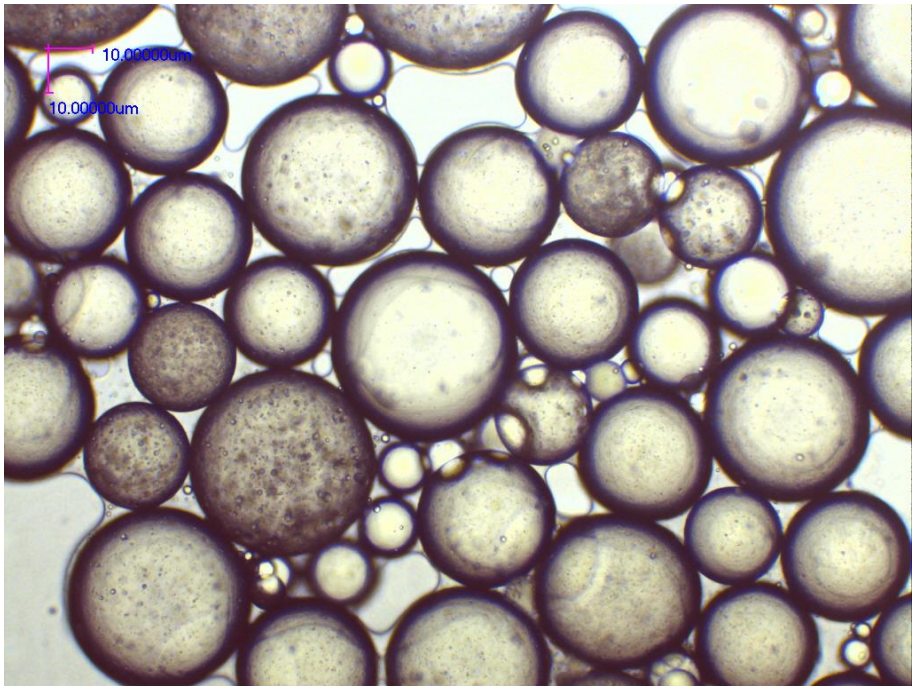

Figure 7: Hemp oil-GE/GA optical microscope image.

Images from the hemp oil-GE/GA SEM analysis are shown in Figures 8 and 9.

The investigated cumulative release properties of hemp oil-GE/GA microcapsules in n-hexane medium are shown in Figure 10. The cumulative release value, which was calculated as 0.289% at the 1st minute, was 20.140% at the 30th minute, 26.128% at the 60th minute, 49.319% at the 360th minute, 58.689% at the 720th minute, and 67.282% at the 2160th minute.

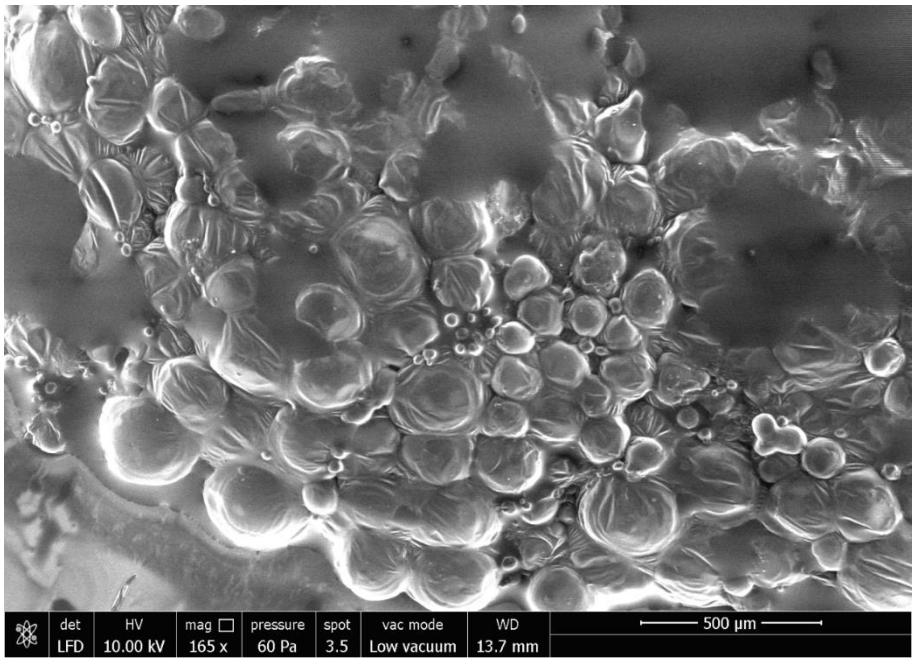

Figure 8: Hemp oil-GE/GA SEM image.

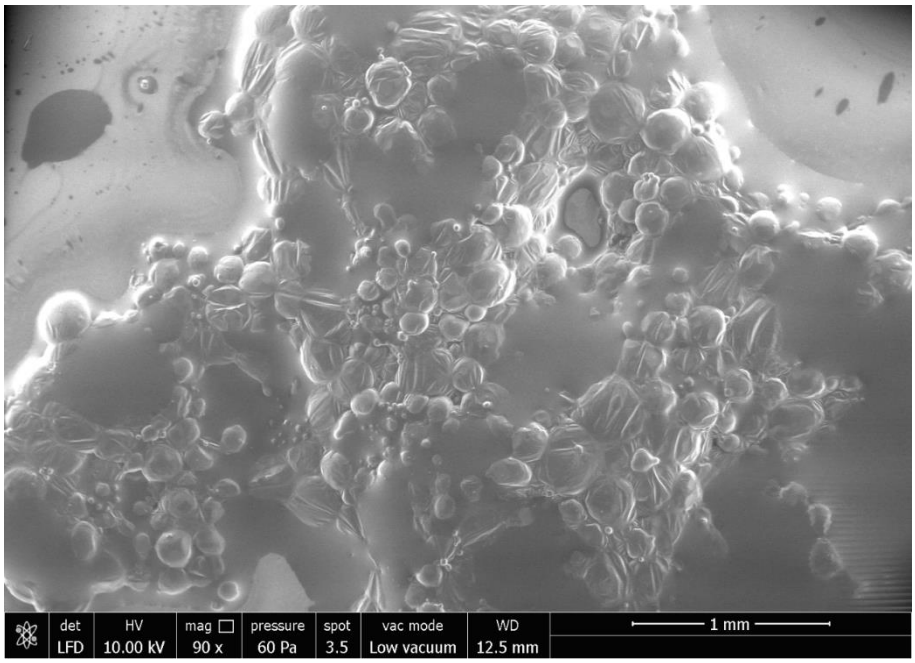

Figure 9: Hemp oil-GE/GA SEM microscope image.

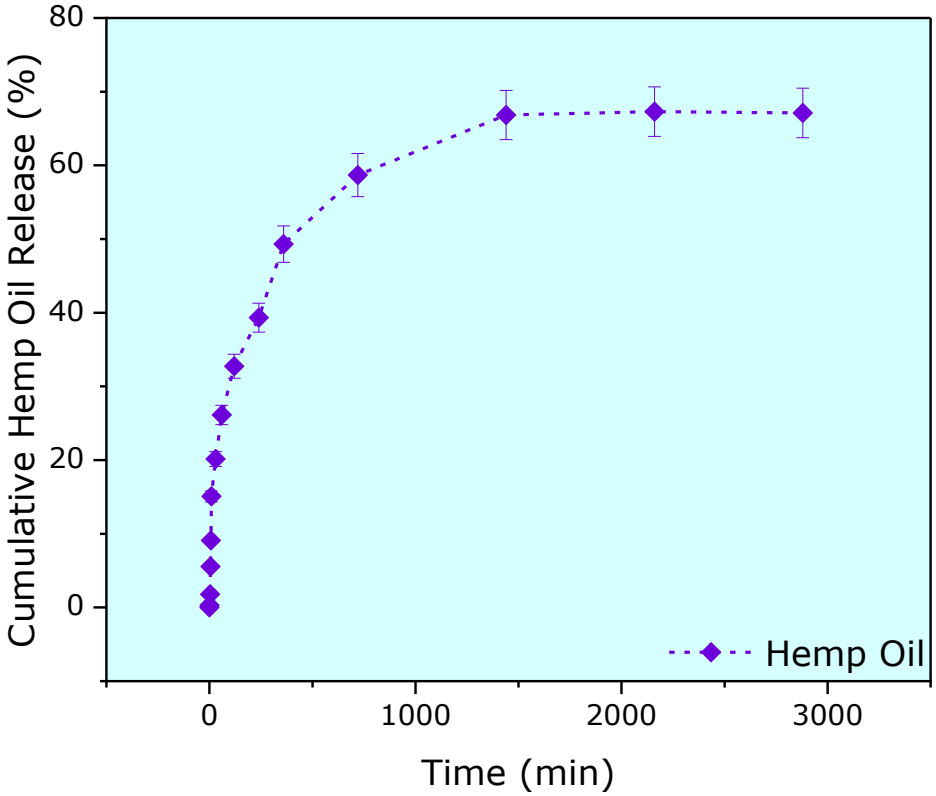

Figure 10: Hemp oil-GE/GA cumulative release graph.

4. CONCLUSION

In our study, we aimed to use hemp oil for different purposes by microencapsulating it with gelatin and gum Arabic polymers. In order to bring the process to the most optimal values, an experimental design was first created with RSM. RSM parameters were determined with the help of preliminary experiments and similar studies in the literature (A: 1000.0–1500.0 rpm, B: 50–60 °C, C: 0.3–0.7 w/v%). Microcapsules produced according to efficiency were examined in terms of efficiency and applied to RSM as a response. When the results were optimized to achieve maximum efficiency (EE% = 72.026%), the following conditions were found: stirring speed of 1443.820 rpm, temperature of 63.165 °C, and surfactant concentration of 0.827 w/v%.". EE%=71.351% was found in the experiment with the multiple response prediction parameters. Microcapsules produced with maximized values were examined with an optical microscope and SEM devices. Optical microscope images showed that the microcapsules had a smooth round shape. On the other hand, in SEM images, since the device operates in low vacuum mode, some parts of the image are distorted. However, the presence of microcapsules could also be visualized under the SEM device. Obtained microcapsules were evaluated in terms of release in n-hexane medium at 1 min, 3 min, 5 min, 7 min, 10 min, 30 min, 60 min, 120 min, 240 min, 360 min, 720 min, 1440 min, 2160 min, and 2880 min. The microcapsules started with a rapid release, and the release value was found to be constant as time progressed (0.289% at the 1st min, was 20.140% at the 30th min, 26.128% at the 60th min, 49.319% at the 360th min, 58.689% at the 720th min and 67.282% at the 2160th min). In our study, hemp oil was successfully encapsulated with gelatin and gum Arabic polymers by complex coacervation method, which is a chemical method. It is thought that the findings obtained from our study will contribute to the literature and further studies.

5. CONFLICT OF INTEREST

There is no conflict of interest.

6. ACKNOWLEDGMENTS

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