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Araştırma Makalesi Research Article

OLIVE LEAF POLYPHENOLS LOADED MUCOADHESIVE ORAL FILMS

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Keywords

Mouth Dissolving Film, Oral Film, Carboxymethyl Chitosan, Okra Gum, Olive Leaf Extract.

Abstract

Among drug delivery systems, mouth dissolving films (MDFs) are a form of drug administration with many advantages. Some problems could be experienced, primarily in pediatric and geriatric patients with conventional drug delivery methods as swallowing. It is of great convenience to dispensing the drug with a film dispersed in the mouth in such cases. In this study, for the preparation of films, the solvent casting method was preferred. The effect of biopolymer ratio and loaded olive leaf extract on the film properties were investigated with the central composite experimental design. Characterization studies of the prepared films were done with AFM, SEM, and FTIR analysis. Based on the characterization studies, the four best formulations were chosen, and further investigations were focused on these formulations. Formulation 8 was chosen as the optimum formulation among 17 formulations due to its better film properties as flexibility and homogeneity, lower disintegration time 200s, and a higher antioxidant capacity 3.21 mM TEAC/g MDF. The obtained data revealed that it is possible to deliver olive leaf polyphenols with the prepared mouth dissolving films.

ZEYTİN YAPRAĞI POLİFENOLLERİ YÜKLÜ MUKOADHESİF ORAL FİLMLER

Anahtar Kelimeler

Ağızda Çözünen Film, Oral Film, Karboksimetil Kitosan, Bamya Zamkı, Zeytin Yaprağı Ekstraktı.

Öz

İlaç aktarım sistemleri arasında, ağızda çözünen filmler (AÇF'ler) birçok avantajı olan bir ilaç uygulama şeklidir. Yutma gerektiren geleneksel ilaç verme yöntemleri ile özellikle pediatrik ve geriatrik hastalarda bazı sorunlar yaşanabilir. Bu gibi durumlarda, ilacın ağızda dağılmış bir filmle verilmesi büyük kolaylık sağlar. Bu çalışmada, filmlerin hazırlanması için solvent döküm yöntemi tercih edilmiştir. Biyopolimer oranı ve yüklenmiş zeytin yaprağı ekstraktının film özellikleri üzerindeki etkisi, merkezi kompozit deneysel tasarım ile araştırılmıştır. Hazırlanan filmlerin karakterizasyon çalışmaları AFM, SEM ve FTIR analizleri ile yapılmıştır. Karakterizasyon çalışmalarına dayanarak, en iyi dört formülasyon seçilmiş ve araştırmada bu formülasyonlara odaklanılmıştır. Formülasyon 8, esneklik ve homojenlik gibi daha iyi film özellikleri, daha düşük parçalanma süresi (200 s) ve daha yüksek antioksidan kapasitesi (3.21 mM TEAC/g AÇF) nedeniyle 17 formülasyon arasından optimum formülasyon olarak seçilmiştir. Elde edilen veriler, hazırlanan ağızda çözünen filmlerle zeytin yaprağı polifenollerinin aktarımının mümkün olduğunu ortaya koymuştur.

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1. Introduction

Oral administration is the most preferred drug delivery for pediatrics, geriatrics, bedridden, nauseous, or noncompliant patients (Irfan et al. 2016). However, in some cases, handicaps like choking hazards or lack of patient compliance can lead to solid dosage problems. In order to overcome these problems, mouth dissolving films (MDF)(Verma et al. 2018) were proposed. With MDFs, it is possible to directly enter into the systemic circulation without water to swallow (Dahiya et al. 2009; Patel et al. 2011). Recently, worldwide interest in medicinal and aromatic plants has increased, providing many raw materials for the food, cosmetic and pharmaceutical industries (Rahmanian et al. 2015). Due to synthetic polymers' unseeable effects, natural raw materials are now more preferred than synthetic raw materials in medicines and material science (Vilar et al. 2012).

Natural polymers usage has become widespread while obtaining MDF (Verma et al. 2018). Hydrophilic polymers are used to prepare the MDFs because of the rapid dissolution of these polymers on the tongue or buccal cavity (Ashok Pawar and Kamat 2017). Water solubility is an important parameter because the drug should be dispersed in the mouth mucosa and directly enter the systemic blood circulation (Verma et al. 2018).

Chitosan is a natural polymer with essential properties such as biocompatibility, biodegradability, mucoadhesion, non-toxicity, antimicrobial activity, low immunogenicity, inexpensive, and attainability(Shariatinia 2018). In the medical field, chitosan is widely used due to its properties as quickly forming gels and films(Wang et al. 2007). In order to increase the water solubility, a reaction between chitosan and chloroacetic acid in alkaline conditions was done to obtain carboxymethyl-chitosan, which has better water solubility than chitosan. Carboxymethyl-chitosan (CMC) is a useful polymer for drug delivery due to some benefits like pH-sensitivity, solubility, absorbability, non-toxicity of its degradation products, controlled biodegradability, bioadhesion, simple administration, and continuous drug release (Shariatinia 2018).

In recent years, okra gum has been used in the food and confectionery industry as a thickener, emulsifier, foam stabilizer, and suspending agent in many products. OG's dissolution in water and its rheological properties in an aqueous medium facilitate its use as a potential pharmaceutical adjuvant in several pharmaceutical dosage formulations (Nayak, Ahmad, et al. 2018). Okra gum (OG) is a natural polymer often used in the pharmaceutical industry. It is inert, nonirritant, biodegradable, biocompatible, and eco-friendly (Zaharuddin et al. 2014). OG is insoluble in organic solvents while soluble in water. In the aqueous medium, OG swells and generates adhesive solutions(Nayak, Ara, et al. 2018). OG was used as a binding agent in tablet dosage forms in drug delivery systems. The resulting tablets showed excellent properties in terms of hardness, friability, and drug release profiles (Kalu et al. 2007). OG was also used as a film-coating agent in drug tablets. Tablets coated with okra gum have better physicochemical properties than core tablets, such as uniformity of weight, friability, disintegration time, and dissolution profiles (Ogaji and Nnoli 2014).

Olea europaea L. leaves are the by-product of olive farming and have high phenolic compounds. So far, olive leaf extract (OLE) has been used in the pharmaceutical industry(Navarro and Morales 2017). Olive leaf extract includes different phenolic compounds, such as secoroids, flavonoids, simple phenols, flavones, flavonols, and acid phenols. The predominant polyphenol in it is oleuropein. Various studies stated OLE's antioxidant properties due to phenolic compounds present in the olive leaf extract(Moudache et al. 2016). These phenolic compounds have antioxidant, antimicrobial, and antifungal properties(Cejudo Bastante et al. 2018). The synergistic effects of all polyphenols are essential, as well as the individual polyphenols it contains (Navarro and Morales 2017).

Olive leaf is one of the leading natural sources of therapeutically active compounds. Various studies have shown that the untreated olive leaf extract comprises oleuropeosides, flavones, flavonols, flavan-3ols, and substituted phenols.(Erdoğan et al. 2018) The concentration of polyphenols varies depending on the quality, origin, and variety of olive leaves(Altıok et al. 2008). OLE is one of the most effective natural sources in removing free radicals. The bitter compound oleuropein, the dominant secoiridoid in olive leaf extract, has an antimicrobial, anti-inflammatory, and antioxidant effect. It also has a protective effect against plant pathogens(El and Karakaya 2009; Erdoğan et al. 2018). When combined with a biomaterial, OLE increases functionality, improves biocompatibility, reduces inflammation, and increases cellular proliferation (Erdogan et al. 2015).

In recent years, drug-loaded various oral films have been popular. For many drugs, oral mucosa has better permeability. The mucoadhesive film is used as a new approach. It is so crucial to dissolve rapidly in the mouth and directly reaching the systemic circulation. The future looks promising as new technologies are discovered to produce thin films day by day (Karki et al. 2016; Tomar et al. 2012). Garcia and coworkers gathered the literature in a book chapter named "Orally disintegrating films of biopolymers for drug delivery" recently (Garcia et al. 2020). Even though CMC has been preferred in the literature for delivery systems, it has been used with biopolymers as guar gum, whey protein, starch etc. in order to improve mechanical properties and thermal stability (Iqbal et al.

2020; Jiang et al. 2020; Suriyatem et al. 2018). Nagpal and coworkers used okra gum and chitosan to obtain polymer films. In the study, formulation, and evaluation of okra fruit gum (OFG)-chitosan (CH) impregnated polymer network films were investigated. Their results revealed that $-COO^-$ moieties present in OFG and $-NH3^+$ moieties present in CH interaction improved the chitosan okra gum film-forming properties (Nagpal et al. 2017). Unlike this study in the present study, carboxymethyl-chitosan was preferred due to better solubility and active bioactive compound added to prepared biopolymer films.

In this study, OLE was chosen as an active compound for the delivery system. CMC and OG were used in the biopolymer blend in order to deliver olive leaf polyphenols to the systemic circulation.

2. Material and Method

2.1. Materials

Dried okra was purchased from a local market from Mugla province, and olive leaves were collected from the Muğla province in the Aegean region. Absolute Ethanol and HPLC grade acetonitrile obtained from Isolab Chemicals, Germany. Carboxymethyl chitosan supplied from Santa Cruz Biotechnology, USA. Acetic acid and oleuropein standard (HPLC grade) were purchased from Merck, USA. Phosphate buffer saline, acetone, and ABTS supplied from Sigma-Aldrich, Germany.

2.2. Extraction of Okra Gum

For the okra gum extraction, a study done by Zaharuddin et al. was modified. 44 g dry okra was ground and added to the distilled water with 1:20 solid to liquid ratio and left overnight at room temperature 24^0C. The obtained mixture was filtered with a muslin cloth. Acetone was added to the viscous adhesive part with 1:1 v/v and waited until the separation was observed. Excess acetone was removed after phase separation was observed (Zaharuddin et al. 2014). The residue was dried in a forced convection oven (JSR JSOF-050 Model, Korea), milled with a grinder, and labeled as okra gum.

2.3. Preparation of Olive Leaf Extract (OLE)

Dried olive leaves were ground and mixed with 70 % ethanol-water solution for 24 h at a 1:20 solid to liquid ratio. When the extraction was over, filtration was done to remove insoluble parts. For ethanol removal from the extract, a rotary evaporator was used. The remaining aqueous solution was lyophilized, and OLE was obtained (Erdogan et al. 2015).

2.4. Determination of Total Biopolymer Amount

Two parallel experiments were performed with a total amount of biopolymer of 140 mg and 170 mg. Stock solutions were prepared by dissolving biopolymers in the water at room temperature with stirring overnight. Stock solutions for the preparation of polymer blend solutions with a total biopolymer of 140 mg were 5 mg/ml CMC and 2 mg/ml OG. Stock solutions for the preparation of solutions with a total biopolymer of 170 mg were 6 mg/ml CMC and 2.5 mg/ml OG. Polymer solutions in the ratios specified in Table 1 and 2 were prepared with stirring for 6 hours and then was cast in petri-dish and dried at 50 ^{0}C for 30 h in a forced convection oven (JSR JSOF-050 Model, Korea). The dried films were carefully removed from the petri dish.

Table 1. Preparation Conditions of Polymer Solution for Total Biopolymer 140 mg

Okra gum % (w/w)	Carboxymetyl chitosan % (w/w)	Mixing
75	25	7 ml CMC solution (35 mg CMC) + 52.5 ml OG solution (105 mg OG)
50	50	14 ml CMC solution (70 mg CMC) + 35 ml OG solution (70 mg OG)
25	75	21 ml CMC solution (105 mg CMC) + 17.5 ml OG solution (35 mg OG)
0	100	28 ml CMC solution (140 mg CMC)
100	0	70 ml OG solution (140 mg OG)

Table 2. Preparation Conditions of Polymer Solution for Total Biopolymer 170 mg

Okra gum % (w/w)	Carboxymetyl chitosan % (w/w)	Mixing
75	25	7.1 ml CMC solution (42.5 mg CMC) + 51 ml OG solution (127.5 mg OG)
50	50	14.2 ml CMC solution (85 mg CMC) + 34 ml OG solution (85 mg OG)
25	75	21.3 ml CMC solution (127.5 mg CMC) + 17 ml OG solution (42.5 mg OG)
0	100	28.3 ml CMC solution (170 mg CMC)
100	0	68 ml OG solution (170 mg OG)

2.5. Preparation of Loaded Mouth Dissolving Films

In experimental studies, different values were assigned to independent variables in order to examine the effect of parameters on response function. The first stage of the experimental design is to determine the effective process parameters of the experiment. With the experimental design, the maximum amount of data can be obtained with the minimum possible experiment.

Response surface methodology was used to analyze the effects of parameters on the responses. OG and OLE percent were determined as parameters. The effect of biopolymers concentration on the film properties was investigated with Central Composite Experimental Design (CCD) by using Design Expert® Version 7.0.0 (Stat-Ease Inc., Minneapolis, MN, USA). The responses were thickness, weight, disintegration time, and pH. Total biopolymer amount was kept constant at 140 mg by preparing polymer blends from stock solutions of 5 mg/ml CMC and 2 mg/ml OG. All 17 experiments were done, and responses were recorded. The ratios of the polymer solutions with OLE were given in Table 3.

Table 3. Preparation Conditions of OLE Loaded MDFs

Experiment Run no	Okra gum % (w/w)	Olive leaf extract % (w/w)	Mixing
1 2	20	1	22.4 ml CMC solution (112 mg CMC) + 14 ml OG solution (28 mg OG) + 1.4 g OLE
3	42.5	5	16.1 ml CMC solution (80.5 mg CMC) + 29.75 ml OG solution (59.5 mg OG) + 7.4 mg OLE
4 12	50	1	14 ml CMC solution (70 mg CMC) + 35 ml OG solution (70 mg OG) + 1.4 mg OLE
5 6 7 11 16	35	5	18.2 ml CMC solution (91 mg CMC) + 24.5 ml OG solution (49 mg OG) + 7.4 mg OLE
8 10	20	9	22.4 ml CMC solution (112 mg CMC) + 14 ml OG solution (28 mg OG) + 13.9 mg OLE
9	27.5	5	20.3 ml CMC solution (101.5 mg CMC) + 19.3 ml OG solution (38.5 mg OG) + 7.4 mg OLE
13 17	50	9	14 ml CMC solution (70 mg CMC) + 35 ml OG solution (70 mg OG) + 13.9 mg OLE
14	35	7	18.2 ml CMC solution (91 mg CMC) + 24.5 ml OG solution (49 mg OG) + 10.5 mg OLE
15	35	3	18.2 ml CMC solution (91 mg CMC) + 24.5 ml OG solution (49 mg OG) + 4.3 mg OLE

2.6. Characterization of Loaded Mouth Dissolving Films

2.6.1. Thickness and Weight

Film thicknesses were measured and averaged from 5 points with a digital micrometer (Electronic digital micrometer, 0-25-0.001 mm). The weight of each film was measured and recorded.

2.6.2. Disintegration Time

The film was placed in a petri dish with 25 ml of distilled water. The petri dish was swung every 10 seconds. The hydrophilic film's disintegration time is stated as the time it starts to break when in contact with water.

2.6.3. pH

pH determination is vital to avoid irritation of the oral mucosa (Irfan et al., 2015). A piece of film was placed in a petri dish and soaked with distilled water. The pH meter electrode was touched to the surface of the wet film, and the measured pH value was recorded.

2.6.4. Scanning Electron Microscopy (SEM) Analysis of OLE Loaded MDFs

SEM was used to investigate the surface morphology of the mouth dissolving films. The prepared films were coated with Au/Pd before the analysis. SEM analysis was performed with Jeol-JSM-7600F Field Emission SEM.

2.6.5. Fourier Transform Infrared Spectroscopy (FTIR) Analysis of OLE Loaded MDFs

The molecular structures of mouth dissolving films and prepared OLE were determined with FTIR analysis. Analyses were made in the range of 650 to 4000 cm^{-1} with the attenuated total reflectance (ATR) unit using 100 FTIR Spectrometer (Perkin Elmer Spectrum, USA).

2.6.6. Atomic Force Microscopy (AFM) Analysis of OLE Loaded MDFs

AFM can allow molecular and surface forces to be measured on a near molecular scale. AFM is used to determine macromolecules' binding from solution to the mucosal surface (Pinhas and Peled, 2010). AFM analysis was done with ezAFM (Nanomagnetics Instruments, Turkey) to determine the surface roughness and topography of the films.

2.7. Determination of Oleuropein Amount in the Olive Leaf Extract and Loaded Mouth Dissolving Films

HPLC analysis was performed to determine the amount of oleuropein in the olive leaf extract. The mobile phase was prepared with water with 0.5% acetic acid: acetonitrile (1:1 v/v). 1000 ppm stock standard oleuropein solution was prepared for calibration. Various oleuropein standard solutions with different concentrations (800, 500, 300, and 150 ppm) were prepared by diluting with the mobile phase. Oleuropein calibration was obtained at 280 nm with these standard solutions (Al-Rimawi 2014). OLE stock solution was prepared by dissolving 0.1 g of OLE in 10 ml of ethanol. The sample from this solution was delivered directly to the device, and the results were recorded. Four selected films were kept in 15 ml phosphate buffer for a day. Film solutions were centrifuged and filtered on a 0.45 µm HPLC filter (Sartorius Minisart). Samples were analyzed with HPLC, and results were recorded.

2.8. Determination of Total Antioxidant Capacity

Trolox equivalent antioxidant capacity (TEAC) was evaluated for radical scavenging ability according to the ABTS method. 5 ml of 7 mM ABTS activated with 5 ml of 2.45 mM $K_2S_8O_2$. The solution was allowed to stand in the dark for 16 hours. The ABTS solution was diluted with ethanol to obtain 0.70 absorbance at 734 nm and equilibrated at 30 ^{0}C. 1490 µl ABTS solution, and 10 µl sample was placed in cuvettes and kept in the dark for 30 min. The absorbance of samples at 734 nm was recorded (Re et al. 1999). Percent inhibition of ABTS cation due to the antioxidant activity of samples was calculated by the following formula (1):

$$\text{ABTS Inhibition } \% = \left(1 - \left(\frac{A_f}{A_0}\right)\right) * 100 \qquad (1)$$

where A_f was the final absorbance value measured on the last measurement, and A_0 was the absorbance value measured directly after dispensing ABTS on the sample. Trolox was used as a standard in the calibration curve (Trolox equation: y=5.1642x). Calculated percent ABTS inhibition values of samples were placed by the Trolox equation to determine TEAC's values as mM Trolox for 1 mg of olive leaf extract (Altıok et al. 2008).

3. Result and Discussion

3.1. Characterization of Components of the OLE Loaded MDFs

The extraction yield of okra gum was determined as 11%. In a study done by Mohan et al., characterization of okra mucilage was done, and components were identified. In addition to the polysaccharides, it was stated that okra mucilage was rich in protein (Chandra Mohan et al. 2018). The extraction yield of OLE was determined as 28%. The amount of oleuropein in 1 g of OLE was 71 mg. The oleuropein percentage in olive leaf extract was 7.1%. Altıok and coworkers stated that 1 g OLE contained 134.4 mg oleuropein. (Altıok et al. 2008)The obtained amount of oleuropein in the prepared OLE was in accordance with the reported values in the literature. The difference may occurred due to the different regions and collection time of the olive leaves.

3.2. Characterization of Loaded Mouth Dissolving Films

As a result of the preliminary tests, it was observed that films with a 140 mg total biopolymer amount were more flexible and easy to remove. So, the total amount of biopolymer was selected as 140 mg. The FTIR spectra of films with increasing CMC ratios are shown in Figure 1.

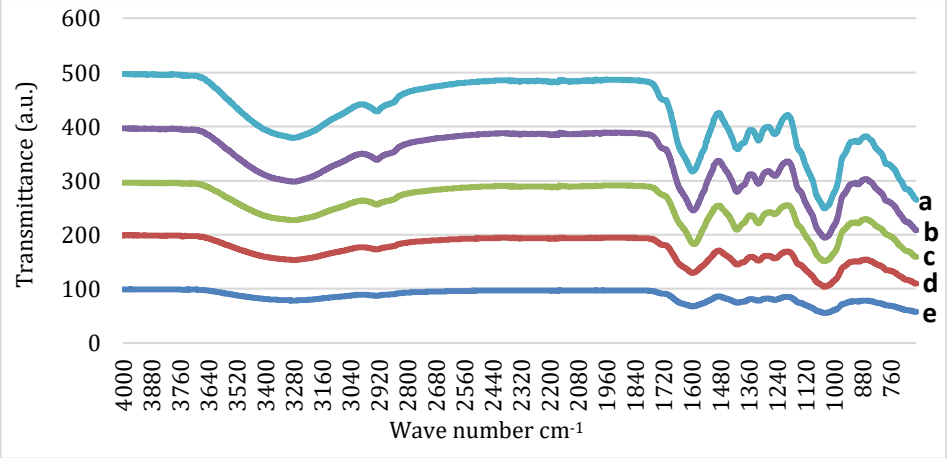

Figure 1. FTIR spectrum of blend films and compounds a) Okra Gum b) Carboxylmethyl chitosan c) OG:CMC (1:3) d) OG:CMC (1:1) e) OG:CMC (3:1)

The broad peak around 3280 cm^{-1} was considered as O-H stretching vibration. While the O-H peak was flat for the 75% OG blend film, the O-H stretching was sharper as CMC's amount increased. This could be due to the N-H stretch from CMC and the bonding between the two polymer molecules. The peak around 2929 cm^{-1} was considered as C-H stretching vibration. In the blend film, which contained 75% OG, the second peak seems indistinct, but when the amount of CMC increases, it becomes apparent at the same point. The peak around 1600 cm^{-1} can be seen as N-H bending. The peak around 1400 cm^{-1} can be assigned to O-H bending. There were several peaks in the region of 750-1300 cm^{-1}. These regions were reported as the fingerprint region for polysaccharides due to the characteristic of the carbohydrate region(Ruiz et al. 2013). As the CMC amount increased intensity of those peaks also increased. Intermolecular bonding between polymers may have been more stable as the OG increases, and this may have occurred as a decrease in the intensity of the functional group's peaks examined. FTIR spectra of loaded films are shown in Figure 2.

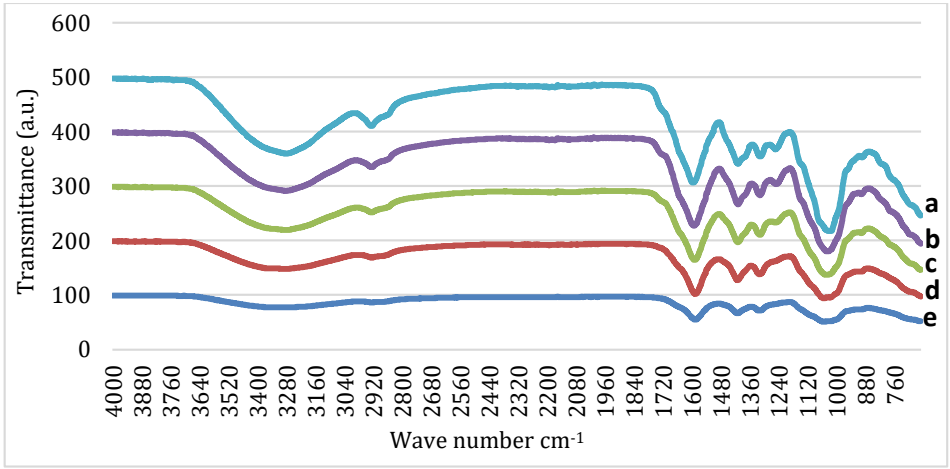

Figure 2. The FTIR spectrum of loaded film and OLE powder a) OLE powder b) Run 15 c) Run 14 d) Run 8 e) Run 1

The broad peak around 3280 cm^{-1} was considered as O-H stretching vibration, and the peak around 2900 cm^{-1} is considered as C-H stretching vibration. When the amount of olive leaf extract increased in the films, it was observed that the peak in the O-H vibration region sharpened. It suggested that OLE was attached to the surface by molecular interaction. The peak around 1600 cm^{-1} can be labeled as N-H bending. The peak around 1400 cm^{-1} can be assigned to O-H bending. There were several peaks in the region of 750-1300 cm^{-1}. They occurred due to the fingerprint region for polysaccharides as a characteristic of the carbohydrate region(Ruiz et al. 2013).

3.3. Characterization of OLE Loaded Mouth Dissolving Films

The pictures of the films prepared according to the central composite design are shown in Figure 3.

Figure 3. Pictures OLE Loaded Mouth Dissolving Films

All films were visually evaluated on their flexibility and how easily they were removed from the petri dish. As seen from Figure 3, some films were broken at one or several points. Drying conditions could cause these fractures. Some film features and evaluations are shown in Table 4.

Table 4. Properties and Evaluations of the Prepared MDFs

Run	Okra Percent (%)	OLE Percent (%)	Visual Inspection	Thickness (mm)	Weight (g)	Disintegration (s)	pH
1	20	1	**Flexible film, easy to remove from petri dish**	0.0408	0.1514	**20**	9.3
2	20	1	**Flexible film, easy to remove from petri dish**	0.0419	0.1536	25	9.4
3	42.5	5	Flexible film, breaks from many points	0.0392	0.1498	198	8.5
4	50	1	**Flexible film, easy to remove from petri dish**	0.0385	0.1477	40	9
5	35	5	**Flexible film, easy to remove from petri dish**	0.0429	0.1555	220	8.9
6	35	5	**Flexible film, easy to remove from petri dish**	0.0425	0.1549	210	8.8
7	35	5	Flexible film, break from **one** point	0.0422	0.1537	180	8.7
8	20	9	**Flexible film, easy to remove from petri dish**	0.0480	0.1590	**200**	8.9
9	27.5	5	Flexible film, breaks from many points	0.0425	0.1552	235	9
10	20	9	**Flexible film, easy to remove from petri dish**	0.0430	0.1557	180	9.5
11	35	5	**Flexible film, easy to remove from petri dish**	0.0478	0.1588	170	9
12	50	1	**Flexible film, easy to remove from petri dish**	0.0350	0.1423	44	8.8
13	50	9	Flexible film, breaks from many points	0.0347	0.1418	290	8.7
14	35	7	Flexible film, breaks from many points	0.0397	0.1501	**258**	9
15	35	3	Flexible film, break from one point	0.0400	0.1505	**50**	9.4
16	35	5	**Flexible film, easy to remove from petri dish**	0.0462	0.1563	166	8.9
17	50	9	Flexible film, breaks from many points	0.0388	0.1488	279	8.7

3.3.1. Thickness and Weight

In order to achieve the same thickness in all films, the total amount of biopolymer was kept constant for the prepared films. Uniformity of thickness is essential for the correct delivery of the dose (Irfan et al. 2016). The thickness of all films was approximately the same and was about 0.04 mm. Likewise, the weight is expected to be the same, and all are about 0.15 g.

3.3.2. Disintegration Time

Disintegration times of films are an essential parameter for patient compliance and easy acceptance. If the film is not dispersed within a certain period, the entire film could be aspirated and led to choking hazards. In the literature, studies were suggested that a film should start to disintegrate between 5 to 30 seconds to be counted among fast-disintegrated films(Arya et al. 2010; Irfan et al. 2016; Speer et al. 2018). The disintegration time of the obtained films changed with the OLE amount. It was observed that films with low OLE had shorter disintegration times. This result can be explained with the cross-linking effect of olive leaf extract(Erdogan et al. 2015).

ANOVA was applied to estimate the significance ($p < 0.05$) of the model. As shown in Table 5, the model chosen for disintegration time was significant, p-value: < 0.0001 and F value: 63.16. Values of "Prob > F" less than 0.0500 indicate model terms were significant.

Table 5. ANOVA for Response Surface Reduced Cubic Model for the Disintegration Time

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.314E+05	7	18765.42	63.16	< 0.0001	
A-Okra percent	684.50	1	684.50	2.30	0.1634	
B-OLE percent	21632	1	21632.00	72.81	< 0.0001	
AB	2812.50	1	2812.50	9.47	0.0132	
A^2	2324.49	1	2324.49	7.82	0.0208	
B^2	5741.83	1	5741.83	19.33	0.0017	
A^2B	5237.76	1	5237.76	17.63	0.0023	
AB^2	2018.94	1	2018.94	6.80	0.0284	
Residual	2673.94	9	297.10			
Lack of Fit	20.14	1	20.14	0.06	0.8116	**not significant**
Pure Error	2653.80	8	331.73			
Cor Total	1.340E+05	16				

Std. Dev.	17.24		**R-Squared**	0.9800
Mean	162.647		**Adj R-Squared**	0.9645
C.V. %	10.60		**Pred R-Squared**	0.9646
PRESS	4748.64		**Adeq Precision**	22.1578

In this case, B, AB, A^2, B^2, A^2B, AB^2 were significant model terms. Values greater than 0.1000 indicate the model terms were not significant. The "Lack of Fit F-value" of 0.06 refers to the Lack of Fit was not significant and relative to the experimental error. These results suggested that the obtained data fit the model, and a non-significant lack of fit was good. The "Predicted R-Squared" of 0.9646 is matched with the "Adjusted R-Squared" of 0.9645. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. This model can be used to navigate the design space.

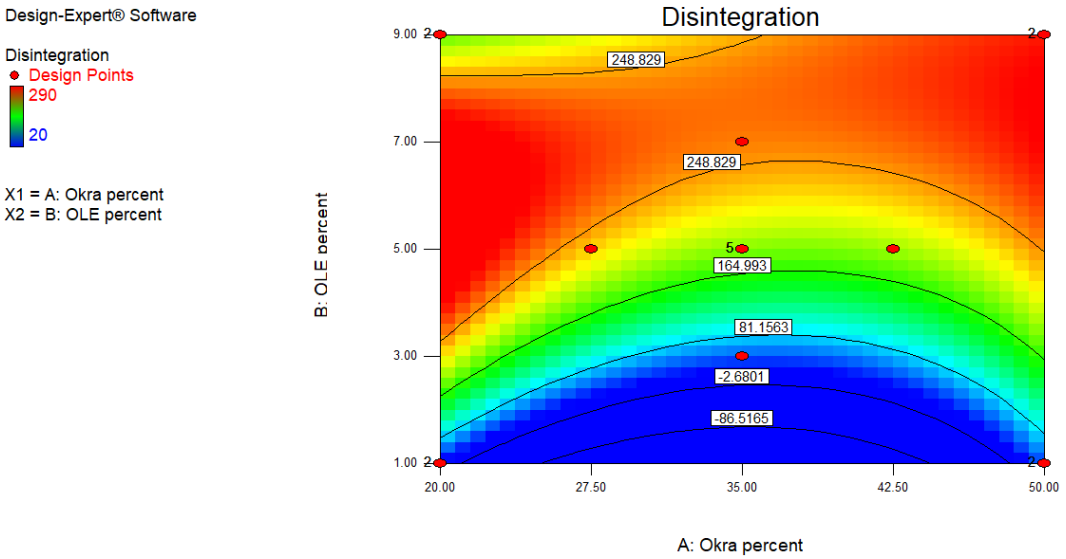

Figure 4. 2D Contour Plot of Disintegration Time

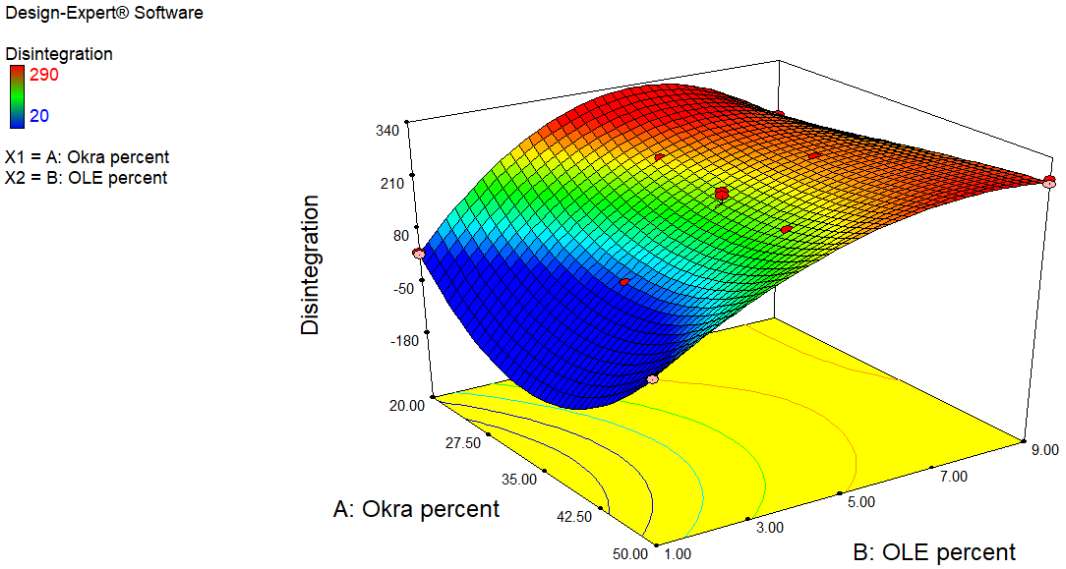

Figure 5. 3D Response Surface Plot of Disintegration Time

2D contour plots and 3D response surfaces were set to visualize the effect of independent variables on response. 3D response surface plots gave the opinion about the independent variables' interaction, whereas the visual representation of the responses' values was given by 2D contour plots(Verma et al. 2018). As can be seen from the 2D contour plot and 3D response surface plot (Figures 4 and 5, respectively), when the OLE percentage increased regardless of the OG percentage, there was an increase in disintegration time at different rates.

3.3.3. pH

The saliva pH has a variable structure from 5.3 to 7.8 (Gittings et al. 2015). pH is expected to be close to neutral since the MDF is too acidic or basic, causing irritations in the mouth. The pH of the obtained films ranges from 8.5 to 9.5.

3.3.4. Scanning Electron Microscopy (SEM) Analysis of Loaded MDFs

Taken SEM images showed a few aggregates, which may have occurred during the film's drying. SEM images revealed that OLE is integrated with the polymers and that prepared formulation can be used as an effective drug delivery system(Bharti et al. 2019; Nair et al. 2018). In Figure 6, SEM images of the selected four films were appeared to be a non-porous whole structure.

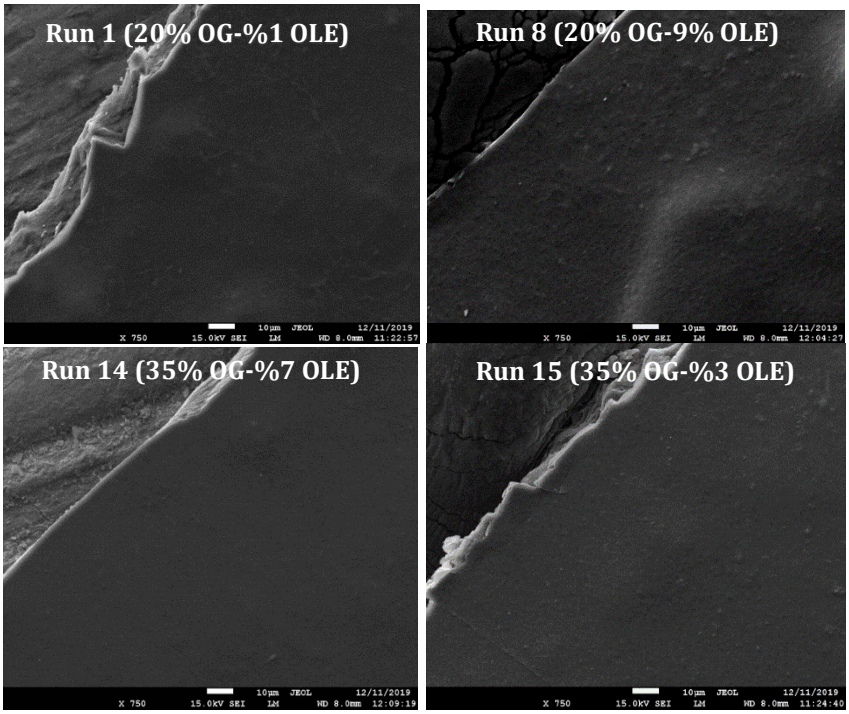

Figure 6. SEM pictures of selected loaded films

When SEM images of polymer films in literature were examined, it was observed that the structures were similar in appearance.

3.3.5. Atomic Force Microscopy (AFM) Analysis of Loaded MDFs

The AFM images of polymeric films were used to visualize morphological structure and determine the prepared films' roughness. In Figure 7, AFM images of the selected films are given.

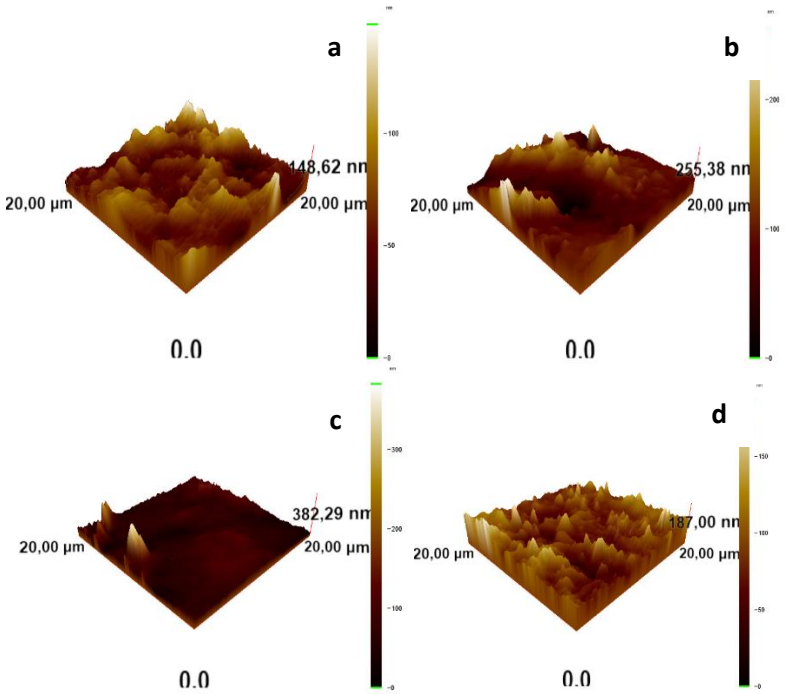

Figure 7. 3D image of prepared MDFs, a) Run 1 (20% OG-1% OLE b) Run 8 (20% OG-9% OLE) c) Run 14 (35% OG-7% OLE) d) Run 15 (35% OG-3% OLE)

The films' roughness values were determined as 14.23, 25.57, 19.10, and 15.79 nm, respectively. As the OLE content of the film increased, the roughness value increased. This result revealed that the OLE amount directly changed the surface properties of the prepared films (Bharti et al. 2019).

3.3.6. Total Antioxidant Capacity of Loaded Mouth Dissolving Films

Antioxidant capacity was determined by the ABTS method. The obtained data are shown in Table 6.

Table 6. Antioxidant capacity of selected MDFs

Experiment Number	Antioxidant Capacity (mM TEAC/g MDF)
Run 1 (20% OG-%1 OLE)	0.96
Run 8 (20% OG-9% OLE)	3.21
Run 14 (35% OG-%7 OLE)	2.58
Run 15 (35% OG-%3 OLE)	1.37

As seen from Table 6, antioxidant activity was increased with increasing concentration of OLE in the films. In the literature, the total antioxidant capacity of OLE using the same method was reported as 11.62±1.22 mM TEAC/g olive leaf extract(Basal et al. 2016). The total antioxidant capacity of OLE in this study was determined as 9.97 mM TEAC/g. The values found were similar to each other. It is expected that the results found in the films will be lower than the results in the extract. These results may be affected by the prepared films' drying conditions and the possibility of OLE interacting with film-forming polymers.

3.3.7. Amount of Oleuropein in Loaded Mouth Dissolving Films

The obtained data are given in Table 7. HPLC results were consistent with the increase of OLE in film content.

Table 7. Amount of oleuropein in loaded MDFs

Experiment	Amount of Oleuropein (theoretical) (mg/g MDF)	Amount of Oleuropein (experimental) (mg/g MDF)	Recovery of oleuropein (%)
Run 1 (20% OG-%1 OLE)	0.66	0.40	60
Run 8 (20% OG-9% OLE)	6.16	3.88	63
Run 14 (35% OG-%7 OLE)	5.00	3.55	71
Run 15 (35% OG-%3 OLE)	2.06	1.40	68

The amount of oleuropein present in the olive leaf extract initially added to the film solutions, and the HPLC results obtained from the experimental study resulted in a recovery of 60 -70%. Due to cross-linking between OLE and biopolymers, the complete recovery of loaded OLE might not be achieved.

4. Conclusion

In this study, the suitability of carboxymethyl chitosan and okra gum as a biopolymer for the carrier system for olive leaf extract was investigated. Both preliminary experiments and experimental design showed that with changing concentrations of the carboxymethyl chitosan and okra gum drastically changed the film properties as homogeneity and flexibility. These results revealed that okra gum worked as a plasticizer in the film mixture. As the experimental design data showed that OLE amount directly related to the prepared films' surface properties and disintegration time. In order to overcome any problems with patients' compliance among 17 formulations, 4 formulations were chosen due to shorter disintegration time. Then further characterizations were carried out. Due to the higher OLE amount in the formulations, higher antioxidant capacity was achieved in formulations 8 (20% OG-9% OLE) and 14 (35% OG-%7 OLE). Due to the higher OG amount in the formulation, a longer disintegration time was recorded for formulation 14 as 258 s. Obtained data revealed that prepared biopolymer blend films were suitable for the olive leaf extract and preserved the olive leaf extract's antioxidant property.

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Conflict of Interest

No conflict of interest was declared by the authors.

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