J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 47(1): 9-19, 2023
Doi: 10.33483/jfpau.1150792

ORIGINAL ARTICLE / ÖZGÜN MAKALE

MUCOADHESIVE ORODISPERSIBLE FILM FORMULATIONS OF RASAGILINE MESYLATE FOR PARKINSON TREATMENT

PARKİNSON TEDAVİSİ İÇİN RASAJİLİN MESİLAT MUKOADEZİF AĞIZDA DAĞILAN FİLM FORMÜLASYONLARI

Meliha GÜNEŞ[1], Gökçe TURAN[1], Armağan DÖNMEZER[1], Fadime AYDIN KÖSE[2], Özgen ÖZER[1], Sinem Yaprak KARAVANA[1]*

[1]Ege University Faculty of Pharmacy, Department of Pharmaceutical Technology, 35040, Izmir, Turkey

[2]Katip Çelebi University Faculty of Pharmacy, Department of Biochemistry, 35620, Izmir, Turkey

ABSTRACT

Objective: *The article is included formulation studies and in vitro evaluation of orally dispersible Rasagiline mesylate (RM) orodispersible films (ODFs) for non-oral treatment of Parkinson's Disease. RM undergoes extensive hepatic biotransformation and is a highly selective and irreversible MAO-B inhibitor. It is metabolized in the liver and has an oral bioavailability of approximately 36% and a half-life of 3 hours. These properties suggest that RM may be an excellent candidate for buccal drug delivery.*

Material and Method: *ODFs of RM were prepared using PEO by the solvent casting method. While evaluating these formulations, parameters such as appearance, weight homogeneity, thickness, surface pH, drug content, swelling ratio, tensile strength, mucoadhesion, in vitro drug release studies and also stability were considered.*

Result and Discussion: *Obtained results show that ODFs are an effective and viable approach for RM delivery and may be a new alternative to existing oral delivery systems for Parkinson's treatment.*

Keywords: *Buccal delivery, orodispersible film, polyethylene oxide, rasagiline mesylate*

* **Corresponding Author / Sorumlu Yazar:** Sinem Yaprak Karavana
e-mail / e-posta: sinemyaprak@hotmail.com, **Phone / Tel.:** +905326113241

Submitted / Gönderilme : 29.07.2022
Accepted / Kabul : 06.10.2022
Published / Yayınlanma : 20.01.2023

ÖZ

Amaç: *Bu makale, Parkinson Hastalığının oral olmayan tedavisi için Rasagilin mesilat (RM) ağızda dağılan filmlerin (ODF'ler) formülasyon çalışmalarını ve in vitro değerlendirmesini içermektedir. RM, özellikle yoğun hepatik biyotransformasyona uğrayan geri döndürülemez ve yüksek seçiciliğe sahip bir MAO-B inhibitörüdür. Karaciğerde metabolize edilir ve oral biyoyararlanımı yaklaşık %36 ve yarılanma ömrü 3 saattir. Bu özellikler, RM'nin bukkal ilaç dağıtımı için mükemmel bir aday olabileceğini göstermektedir.*

Gereç ve Yöntem: *RM'nin ODF'leri, solvent döküm yöntemiyle PEO kullanılarak hazırlandı. Bu formülasyonlar değerlendirilirken görünüş, ağırlık homojenliği, kalınlık, yüzey pH'sı, ilaç içeriği, şişme oranı, gerilme mukavemeti, mukoadezyon, in vitro ilaç salım çalışmaları ve stabilite gibi parametreler göz önünde bulundurulmuştur.*

Sonuç ve Tartışma: *Elde edilen sonuçlar, ODF'lerin RM uygulaması için etkili ve uygulanabilir bir yaklaşım olduğunu ve Parkinson tedavisi için mevcut oral dağıtım sistemlerine yeni bir alternatif olabileceğini göstermektedir.*

Anahtar Kelimeler: *Ağızda dağılan film, bukkal uygulama, polietilen oksit, rasaJilin mesilat*

INTRODUCTION

Parkinson Disease (PD) is a chronic and progressive disease, so the symptoms become worse in the course of time. It's characterized by motor symptoms associated with movement like tremors, stiffness, or rigidity of the muscles. In addition to motor symptoms, non-motor symptoms like sleep problems, depression, anxiety, constipation, and fatigue related to PD can occur in patients [1].

The efficacy of levodopa, which has long been accepted as the 'gold standard' and administered orally for the control of motor symptoms in patients with PD, appears to decrease as the duration of treatment increases. Since the effects of orally used drugs start late, OFF periods are seen in the early morning hours in Parkinson's patients. It has been reported that the OFF period complicates the morning routines of the patients and significantly affects their quality of life. Dysfunctions in the gastrointestinal track occur at all levels of PD and this causes motor fluctuations in the advanced levels of PD, which makes the management of the disease difficult. Transmucosal routes such as intranasal, buccal, sublingual and rectal are used as an alternative to the oral route due to their advantages such as being non-invasive, possibility of self-administration, rapid drug absorption, high bioavailability and successful systemic delivery of the active substance to the circulation. Among the transmucosal routes, buccal administration, excellent accessibility, non-invasiveness of administration of the dosage form, rapid blood supply and permeable mucosa make it a highly attractive route for drug delivery for systemic action [2].

Rasagiline mesylate (RM) is an irreversible MAO-B inhibitor. RM is a freely soluble active substance with a biological half-life of 3 hours and a dose of 0.5-2 mg per day. RM is an irreversible MAO-B inhibitor [3]. RM's absolute bioavailability is 36% due to the metabolism by N-dealkylation and hydroxylation in liver. Because it is an effective active substance with low oral bioavailability, RM is a very suitable candidate for transmucosal administration [4].

Orally dispersible films (ODFs) are drug delivery systems intended to disperse rapidly after administration into the oral cavity. ODFs which are commonly prepared with hydrophilic polymers are dosage forms by means of suitable size, thickness, and easy application which provide easy patient compliance. Solvent casting method is one of the methods frequently used in the production of ODFs. In this method, after the film-forming agents are dissolved in designated solvent, the solution is poured onto a flat surface and dried [5]. When placed on the tongue, formulations immediately moisten with saliva and then rapid disintegration and/or dissolution occurs and the release the active pharmaceutical substance happened [6]. They are preferred over solid dosage forms in terms of flexibility, application without water needed and improved patient comfort. They also provide more accurate drug dosing and longer residence time in buccal mucosa as compared to semi-solid dosage forms [2].

The present research represents the preformulation studies, formulation development and *in vitro* evaluation of mucoadhesive ODFs of RM.

MATERIAL AND METHOD

Materials

RM was gifted from Ali Raif Pharmaceutical Industry, Turkey, polyethylene oxide (PEO) purchased by Sigma-Aldrich, USA, glycerol (GLY) (98%) was purchased by Merck, Germany. Mouse embryonic fibroblast cells (NIH/3T3) and cell lines purchased from ATCC, USA were employed. Dulbecco's modified Eagle medium (DMEM) with L-Glutamine, sterile phosphate-buffered saline (PBS), fetal bovine serum (FBS) and trypsin-EDTA was obtained from Biowest, France. Trypan Blue solution was purchased from Biological Industries (Israel). ThinCert cell culture insert, and other cell culture plastics were supplied from Greiner Bio-One, Germany. 4-[3-(4-iodophenyl)-2-(4-nitro-phenyl)-2H-5-tetrazolio]-1,3-benzene sulfonate (WST-1) cell proliferation reagent was obtained from Roche Diagnostics, Germany. All chemicals were of analytical grade.

Preparation of Mucoadhesive ODFs

In our study, solvent casting method was preferred to prepare mucoadhesive ODFs. 1.56 mg RM added into 35 ml distilled water (DS) per ODF (6 cm^2). Different concentrations of PEO were slowly added to these solutions and stirred using a magnetic stirrer for 24 h. Then glycerin (GLY) added and then mixtures were ultrasonicated to acquire bubble-free gels and kept a side at least 6 hours. Then solutions were poured on petri dishes and kept in controlled room temperature for 24 h. After drying, ODFs were cut to 6 cm^2 (2×3 cm^2).

Table 1. Composition of ODFs*.

	F1	F2	F3	F4
PEO	0.5 g	0.6 g	0.7 g	0.8 g
GLY	0.025 g	0.030 g	0.035 g	0.040 g

*1.56 mg RM added into DW per ODF (6 cm^2) for drug loaded ODFs.

Physical Properties

By examining the general appearances of the ODFs; It was checked whether they can be separated from the petri dish and have sufficient homogeneity, flexibility, softness, and stickiness.

Weight Uniformity and Thickness

The formulations were weighed separately on a precision balance (Sartorious Basic, Göttingen, Deutschland). The average weights and standard deviations were calculated. A digital micrometer (Mitutoyo, Japan) was used to measure the thickness of the ODFs. The average thickness and standard deviations were calculated (n=6).

Drug Content and Content Uniformity

RM amount of ODF formulations was analyzed with a fully validated HPLC method according to the ICH guidelines. Drug content and content uniformity studies are performed with Agilent series 1100-1200 HPLC apparatus with an UV detector at 265 nm, using 5 µm, 4.6 × 250 mm C18 column. A mixture of ammonium acetate buffer/acetonitrile (60:40, v/v) was chosen as the mobile phase and flow rate was determined as 1.0 ml/min at 25°C. Injection volume of 20 µl and run time of 10 min was optimized for the final method [7].

The drug content and content uniformity tests were performed by dissolving an ODF in 40 ml of simulated saliva fluid (SSF) and ethanol mixture (50:50) with stirring for 30 minutes (n=6).

Swelling Ratio Determination

The RM-loaded ODFs were weighed (W1) and adhered to pre-weighed coverslips. The prepared coverslips were then thrown into 20 ml of SSF at 37°C. At certain times the coverslips were removed, and excess of solvent was soaked by a filter paper then weighed (W2). Until a constant weight was

obtained, the increase in the weight of the ODFs was evaluated (n=6) by using the following equation (*Equation 1*) [8].

$$Swelling\ ratio\ \% = (W2-W1)/W1*100 \qquad \text{Equation 1}$$

In Vitro Disintegration

ODFs were placed in a 20 ml SSF beaker at 37°C to determine the *in vitro* degradation time (n=6) and their disintegration time was evaluated only visually (n=6) [9].

Surface pH

Formulations were allowed to swell for 10 minutes in the beaker containing 5 ml distilled water. The probe of the pH meter was brought into contact with the ODF surface and allowed to equilibrate for 1 minute to determine the pH [10].

Determination of Tensile Strength

The study was performed at least six times with a profile analyzer (TA-TX Plus, Stable Micro System, UK) using a 500 g load cell according to the parameters given in Rençber vd. 2019. The following Equation 2 was used to calculate the results:

$$Tensile\ strength\ (N.mm^{-2}) = Force\ at\ break\ /\ Area\ of\ the\ sample\ (mm^2) \qquad \text{Equation 2}$$

Evaluation of the Mucoadhesive Properties

Mucoadhesion properties were determined at least six times with texture profile analyzer at 37°C according to the parameters given in Karavana vd., 2018 and Tomar vd., 2012. Maximum separation force and mucoadhesion values were obtained from the force-distance plot.

In Vitro Dissolution Study

In vitro dissolution studies of ODFs were carried out with 35 ml of SSF at 37±0.5°C using USP type II. Medium was stirred at 100 rpm and samples were taken at 1-minute intervals by adding the same amount of fresh medium as the sample taken to keep the dissolved medium constant (n=6) [11].

Cell Culture and *In Vitro* Cytotoxicity Assay

WST-1proliferation assay evaluated the cytotoxicity potential of the formulations against HEK-293T and NIH-3T3 [12]. WST-1 reagent reduces to the water-soluble formazan dye by the end of the cellular mechanism in live cells. Therefore, the intensity of the color developed in the experimental environment is proportional to the number of living cells. HEK-293T and NIH-3T3 cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in DMEM containing 2 mM glutamine and 10% FBS [12,13].

Cells were suspended in fresh medium for cytotoxicity experiments and seeded at the bottom of 12-well ThinCert™ plates at a density of 5x105 cells. Plates were placed in a CO_2 incubator and incubated for 18 hours. Before the study, the medium was replaced with 1 ml of fresh medium, and ThinCert™ inserts (pore size: 0.4 μm) were placed in each well.

ODF formulations were added to inserts followed by sterilization with UV light for 8 hours, with an extra 0.5 ml of DMEM. Cells were treated with formulations for 12, 24, 48 and 72 hours. Finally, the inserts were removed, and the medium was replaced with WST-1:medium (1:10) and incubated for an additional 4 hours. The absorbance value of the wells was measured at 450 nm using the CLARIOstar Plus Microplate Reader (BMG LabTech, Germany). Results were represented as the % percentage of formazan absorbance (Mean ± Standard Deviation, Mean ± SD). Statistical analysis of the results was calculated by one-way variance analysis using the GraphPad Prism 5.0 software. The significance level was accepted as $p < 0.05$.

Stability Studies

The ODFs were wrapped in aluminum foil, packed in glass container, and kept in stability chambers, at 25±0.5°C and 60±5% relative humidity and 40±0.5°C and 75±5% relative humidity for 3 months respectively. After 1, 2 and 3 months, ODFs were tested for changes in appearance, drug content, pH and disintegration time.

RESULT AND DISCUSSION

RM is one of the most prescribed drugs as monotherapy or in addition to levodopa in people who suffer from PD. This study includes successfully preparing and characterising RM loaded ODFs prepared by solvent casting. The developed ODFs were evaluated for various physicochemical properties such as weight uniformity, thickness, drug content and content uniformity, surface pH, tensile strength, swelling ratio, disintegration time, mucoadhesive properties, *in vitro* drug release, *in vitro* cytotoxicity assay and also stability study.

The PEO was used as film formers and GLY as a plasticizer for ODFs formulations. PEO provided the formulations with optimum physicochemical properties such as high mucoadhesiveness and easy applicability. GLY was added to ODF formulations to increase flexibility and reduce brittleness. For formulation development studies, ODFs without active substance were prepared with different ratios of PEO and GLY. F1 and F2 coded ODFs were separated easily from petri dishes and are homogeneous, flexible, smooth, soft, and adhesive. Studies on the F3 and F4 formulations were not continued because the viscosities of their solutions were too high, and air bubbles could not be removed despite many attempts.

Weight Uniformity and Thickness

Weight uniformity and thickness are parameters that must be considered to ensure precision and homogeneity of the dose administered to patients using ODF. The results of these studies were given in Table 2. The thickness of all formulations was found to be in the range of 0.37 mm to 0.67 mm, meaning that a thin ODF can be prepared that can be beneficial as it causes minimal discomfort to patients. The thickness of all formulations was found to be in the range of 0.37 mm to 0.67 mm, meaning that a thin ODT can be prepared that can be beneficial as it causes minimal discomfort to patients.

The weight of ODFs were found in the range of 0.031± 0.001 to 0.039 ± 0.009 g. The formulation F1 showed minimum weight due to ratio of polymer. The polymer ratio and the presence of RM in the ODFs slightly changed the weight. But the standard deviation values detected in all samples were very low, indicating that the weights of the prepared formulations were uniform. The homogeneity of ODFs is a key parameter to ensure predictable drug release from formulations with uniform distribution of drug [14]. The thickness was determined using a digital micrometer and according to the determined thickness results, as the polymeric amount increased; It was observed that the thickness of the ODFs increased. The thickness of all formulations was found to be in the range of 0.37 mm to 0.67 mm, meaning that a thin ODF can be prepared that can be beneficial as it causes minimal discomfort to patients.

Table 2. Physicochemical properties of ODFs.

Code	Thickness (mm)	Weight (g)	Swelling Ratio	Disintegration time (sec)	Surface pH
F1	0.037±0.01	0.031±0.001	37.7 ± 0.04	29.91±0.52	6.88±0.02
F2	0.067±0.01	0.037±0.004	41.2±0.04	64.73±0.52	7.12±0.05
F1E	0.055± 0.02	0.033±0.004	29.9± 0.06	12.39±0.64	7.33± 0.20
F2E	0.050±0.01	0.039±0.009	35.5± 0.05	20.69±0.64	7.45±0.07

Swelling Ratio Determination

The hydration of ODFs depends on the type and physicochemical nature of the polymers. Swelling, which begins with contact with water, allows the structure of bioadhesive polymers that are initially stretched, twisted, or entangled to relax, resulting in rapid dissolution of polymer chains and the formation of a macromolecular structure that increases the porosity of the ODF and initiates drug release. However, increased swelling of ODFs can cause discomfort for patients [15-17]. The swelling behaviour of formulations shows their bioadhesive property and drug release of the formulation was determined at various time intervals. ODF formulations were found to exhibit good swelling behavior. It was observed that the formulation F2 showed the highest swelling index due to greater swelling of PEO. It indicates that when ODFs placed in an aqueous medium, liquid penetrates into ODFs and a gel is formed. Swelling increases as the time proceeds because the polymer gradually absorbs water due to hydrophilicity of polymer [18].

In Vitro Disintegration

In vitro disintegration time was measured and was approximately 12.39±0.64 to 64.73±0.52 min, respectively. Results of *in vitro* disintegration time of ODFs were shown in Table 2. It was observed that the increase in polymer concentration and viscosity increased the disintegration and dissolution time, and this was found to be consistent with the literature. In addition, *in vitro* disintegration time increased with a higher amount of PEO in all formulations.

Surface pH

If the pH difference between the applied dosage form and the buccal mucosa is not large, the possibility of causing any problems in the application area is reduced. The pH of the ODFs was examined for any sensitization or allergic reaction with buccal use. As the surface pH for all formulations is close to the buccal pH, it is considered that it will not cause irritation to the mucosa. Surface pH of all formulations was seen to be compatible with salivary pH. For this reason, it was determined that the formulations did not have a risk of irritation on the oral mucosa. The pH values of ODFs were found to be almost neutral (pH 6.88-7.45) and near to the buccal pH which was 6.8.

Drug Content and Content Uniformity

Content uniformity is an essential pharmaceutical quality control criteria evaluated to control drug homogeneity in pharmaceutical products. The data displayed in Table 3 signifies higher drug content >98% in ODFs. Consistent values between ODF formulations show that the change in polymer ratio does not affect the RM content and homogeneous formulations can be prepared. The results showed acceptable drug content changes, so the ODFs have uniform drug distribution.

Table 3. Drug content of RM loaded ODFs.

Code	Drug content %
F1E	98.68±2.25
F2E	99.87±3.52

Determination of Tensile Strength

The mechanical strength of ODF formulations is a very important factor not only in production or formulation development stages, but also in terms of ease of use by the patient. Different factors affect the mechanical properties of formulations, such as the type and amount of film-forming agent, plasticizer type and amount, type and amount of residual solvents, weight and thickness of ODF, manufacturing process, storage conditions, and type and amount of active substance. The results of the tensile strength test are shown in Table 4. The mechanical characteristics of polymers are affected by the preparation process of the formulation, and the temperature and humidity of the environment, so it is difficult to compare the values in one study with other studies. No specific values are also determined for tensile

strength for mucoadhesive buccal ODF. The results obtained in this study for ODFs with different PEO ratios were in accordance with the results of other published studies [17].

Table 4. Tensile strength of ODFs.

Formulation Code	Tensile strength (N/mm^2)
F1	0.1±0.05
F2	0.1±0.05
F1E	0.04±0.03
F2E	0.09±0.03

Evaluation of the Mucoadhesive Properties

The mucoadhesive property of ODFs is an important parameter in prolonging the residence time in the buccal region and preventing the removal of the formulation by mucosal secretion. Therefore, it is beneficial to use mucoadhesive formulations to strengthen the interaction between ODFs and mucosa in the development process [19]. The data in Table 5 demonstrates that ODFs possessed adequate mucoadhesive force (>152 mN). Indeed, the high mucoadhesive strength of ODFs favours their long residence time in the buccal mucosa. Studies with a neutral polymer, PEO, show that polymer concentration affects mucoadhesion. This is thought to be due to the viscosity of the polymer solution.

Table 5. Mucoadhesive properties of ODFs.

Formulation Code	Mucoadhesion Force (mN)	Work of Adhesion (mN.mm)
F1	152.37±2.03	52.54±1.03
F2	272.60±2.80	11.58±1.17
F1E	236.86±1.08	81.67±1.07
F2E	302.63±1.02	126.09±2.04

In Vitro Dissolution Study

In vitro drug release studies are important to learn about the release of active substances from ODF and subsequent passage through the buccal mucosa. The correlation between in vitro release data and *in vivo* absorption has also been demonstrated by different studies [14]. In addition, it is known that drug delivery from a formulation is primarily determined by the properties of drugs and polymers. The release of the drug from the ODF depends on the drug/polymer properties, the solubility of the drug, the diffusion of the drug from the ODF, the swelling properties of the polymer matrix and the disintegration time [17]. Although the solubility of RM is not expected to have a negative effect on dissolution and absorption based on the information obtained as a result of the literature searches, a dissolution study was performed at pH 6.8 (physiological pH of the oral cavity). The swelling and dissolution characteristics of some natural polymers are affected by salivary fluid's ion concentration, osmolality, surface tension, viscosity, etc. [18,19]. These features were not taken into account in this study. *In vitro* release of RM from ODFs are shown in Figure 1 and Figure 2. A rapid drug release was seen in both formulations, although there were differences between the two formulations. For F1 formulation, more than 50% of RM was released in the first 2 min and more than 100% in the first 4 min. The release was fast from F1 because of the polymer ratio. For F2 formulation, more than 50% of RM was released in the first 4 min and more than 100% in the first 8 min.

Cell Culture and *In Vitro* Cytotoxicity Assay

Cytotoxicity experiments are a significant step in the pharmaceutical development process. For this purpose, empty and RM-loaded ODF formulations were tested on two healthy well-defined cell lines: human kidney embryonic epithelial cell line (HEK-293T) and mouse embryonic fibroblast cell

line (NIH-3T3). These cell lines are commonly used for preliminary cytotoxic test novel formulations [20].

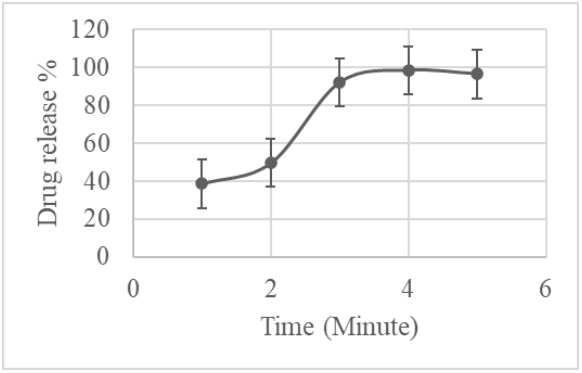


Figure 1. *In vitro* drug release from F1E.

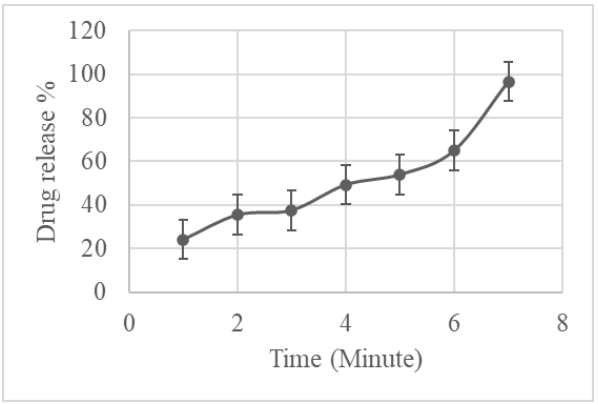


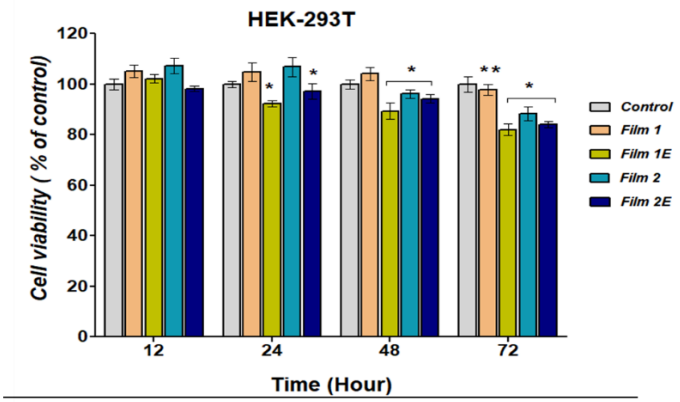
Figure 2. *In vitro* drug release from F2E.

HEK-293T and NIH-3T3 cells were incubated with empty and RM-loaded ODFs for various times (12, 24, 48 and 72 hours) in ThinCert™ plates. Then, the cytotoxic potential of formulations was determined by WST-1 cell proliferation reagent (Figure 3, 4).

In Figure 3 the table shows the % cell viability rate of HEK-293T cell, and the graphs show the control and ODF formulation-treated cell viability ratio. As a result of 12 hours of incubation with ODF formulations in the HEK-293T cell line, cell viability in all groups was similar to control group cells ($p > 0.05$). After 24 hours of incubation, the cell viability of the empty ODF-1 and -2 treated groups was identical to the control. It was observed that ODF-1E and -2E formulations caused a slight decrease in cell viability (92.30%±1.35 and 94.08%±3.11, respectively). As a result of the 48- and 72-hour incubation carried out to determine the long-term effects of the formulations on the cells, it was determined that the formulations except the empty ODF-1 group caused a decrease in HEK-293T cell viability compared to the control ($p < 0.05$) (Figure 4). In addition, the results obtained at the end of the 72-hour incubation period showed that the viability of the cells incubated with the ODF-1 formulation was significantly higher than the other tested formulation ($p < 0.05$). On the other hand, no cytotoxic effect of the empty ODF-1 formulation was found neither towards HEK-293T cells nor towards NIH-3T3 cells at any time tested ($p > 0.05$).

Figure 3 shows the cell viability rate of NIH-3T3 cells, and the graphs show the comparison of cell viability between control and ODF formulation-treated cells. After 12- and 24-hour incubations, cell viability was determined similar to control in all formulations tested. Controversially, it was observed that all tested formulations caused a slight but significant decrease in NIH-3T3 cells. In

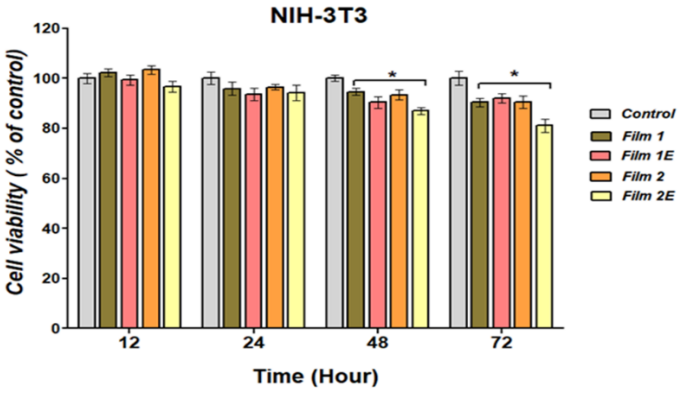
contrast, all formulations tested were observed to cause a slight but significant reduction in NIH-3T3 cell viability after 48 and 72 hours of testing ($p>0.05$). Furthermore, results also showed that cell viability was still almost 90% compared to control cells (Figure3).



HEK-293T Cell Viabilty % (Mean ± Standard Deviation)

HEK-293T	Control	Film-1	Film-1E	Film-2	Film-2E
12 H	100 ± 2,04	105,11 ± 2,50	102,21 ± 1,71	107,32 ± 3,03	98,26 ± 1,14
24 H	100 ± 1,32	104,79 ± 3,77	92,30 ± 1,35	106,86 ± 3,83	97,24 ± 3,06
48 H	100 ± 1,80	104,11 ± 2,59	89,36 ± 3,23	96,21 ± 1,74	94,26 ± 1,69
72 H	100 ± 3,02	97,78 ± 2,11	82,08 ± 2,43	88,37 ± 2,86	84,01 ± 1,18

Figure 3. Evaluation of the cytotoxic potential of ODF formulations on HEK-293T cell.



NIH-3T3 Cell Viabilty % (Mean ± Standard Deviation)

	Control	Film-1	Film-1E	Film-2	Film-2E
12 H	100 ± 2,01	102,33 ± 1,53	103,36 ± 1,72	99,35 ± 2,09	96,63± 2,29
24 H	100 ± 2,41	95,66 ± 2,73	96,42 ± 1,02	93,44 ± 2,49	94,08 ± 3,11
48 H	100 ± 1,33	94,60 ± 1,48	93,33 ± 1,96	90,34 ± 2,35	86,91 ± 1,45
72 H	100 ± 2,87	90,33 ± 1,60	90,36 ± 2,49	91,96 ± 1,87	81,03 ± 2,74

Figure 4. Evaluation of the cytotoxic potential of ODF formulations on NIH-3T3 cell.

Stability Studies

The product must maintain its optimum properties over time, thus ensuring the physical and chemical stability of the formulation. These properties depend on the formulation structure, production process and ambient conditions. Stability studies of ODFs were carried out as per ICH guidelines, and the formulation was stable after 90 days at 25±0.5ºC/ 60±5% relative humidity and 40±0.5ºC/75±5% relative humidity. According to the stability results, there was no meaningful change on followed stability parameters of ODFs after 3 months.

In the current study, we aimed to develop RM-loaded ODFs for PD. Development of the ODFs of RM could be a preferable alternative to the conventional oral route improving the quality of life and patient acceptability. ODF formulation of RM was prepared using PEO by solvent casting method using GLY as a plasticizer. All the formulations were evaluated for various parameters such as appearance, tensile strength, weight uniformity, thickness, surface pH, drug content swelling index, *in vitro* drug release and stability. F1 and F2 were smooth and flexible with an opaque appearance. The weights and thicknesses of the ODFs were found to be uniform. In addition, when homogeneity is evaluated in terms of drug content, the low SD value shows that the prepared ODFs are homogeneous.

The pH values were found to be around 6.8 that comply with the range of buccal mucosa. Formulations exhibited good swelling behavior and higher tensile strength due to their elastic behavior. All the formulations were subjected to *in vitro* drug release study which were carried out using USP apparatus II. The above study concluded that possibilities of the making of mucoadhesive ODFs for RM which will be more effective in a short time and having satisfactory properties which may provide increased therapeutic efficacy and patient compliance. The findings obtained from the results of the cytotoxicity experiment using two different healthy cell lines reveal that both F1 and F2 formulations are suitable for non-toxic drug-carrier formulations. Moreover, our results strongly indicate that the active ingredient RM prepared in F1 and F2 ODFs may be significantly masking the potential side effects of RM on healthy tissue during treatment. After all, in the light of all data in this study, we think that RM loaded ODFs can be considered as a potential and viable approach for successful treatment of PD soon.

ACKNOWLEGMENTS

This study was supported by the Scientific and Technological Research Council of Turkey (grant TUBITAK-219S545). The authors would also like to thank the Ege University Pharmaceutical Sciences Research Center (FABAL) for enabling us to use their laboratory instruments.

AUTHOR CONTRIBUTIONS

Concept: M.G., F.A.K., Ö.Ö., S.Y.K.; Design: M.G., F.A.K., Ö.Ö., S.Y.K.; Control: M.G., F.A.K., Ö.Ö., S.Y.K.; Sources: M.G., G.T., A.D., F.A.K., Ö.Ö, S.Y.K; Materials: M.G., G.T., A.D., F.A.K., S.Y.K.; Data Collection and/or Processing: M.G., G.T., A.D., F.A.K., Ö.Ö., S.Y.K.; Analysis and/or Interpretation: M.G., F.A.K., Ö.Ö., S.Y.K.; Literature Review: M.G., F.A.K., S.Y.K.; Manuscript Writing: M.G., F.A.K., Ö.Ö., S.Y.K.; Critical Review: M.G., G.T., A.D., F.A.K., Ö.Ö, S.Y.K; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Pfeiffer, R.F. (2016). Non-motor symptoms in Parkinson's disease. Parkinsonism & Related Disorders, 22, S119-S122. [CrossRef]
2. Himanshu, Bhaskar R., Sharma N., Mehta M., Singh A., Yashwant, Yadav N.S., Khatik G.L., Verma S. (2017). Development and evaluation of buccoadhesive film of ropinirole hydrochloride for the treatment of Parkinson's disease. International Journal of Drug Delivery Technology, 7, 106-112. [CrossRef]
3. Rabey J.M., Sagi I., Huberman M., Melamed E., Korczyn A., Giladi N., Inzelberg R., Djaldetti R., Klein C., Berecz G. (2000). Rasagiline mesylate, a new MAO-B inhibitor for the treatment of Parkinson's disease: A double-blind study as adjunctive therapy to levodopa. Clinical Neuropharmacology, 23, 324-330. [CrossRef]
4. Bukka, R., Prakasam, K., Patel, C.D. (2010). Preparation and evaluation of intraoral drug delivery system for rasagiline mesylate. International Journal of Pharmaceutical Sciences and Drug Research, 2, 294-301.

5. Hoffmann, E.M., Breitenbach, A., Breitkreutz, J. (2011). Advances in orodispersible films for drug delivery. Expert Opinion on Drug Delivery, 8(3), 299-316. [CrossRef]
6. Irfan, M., Rabel, S., Bukhtar, Q., Qadir, M.I., Jabeen, F., Khan, A. (2016). Orally disintegrating films: A modern expansion in drug delivery system. Saudi Pharmaceutical Journal, 24(5), 537-546. [CrossRef]
7. Ravi, P.R., Aditya, N., Cherian, L., Patil, S. (2013). LC method for determination of rasagiline mesylate in different plasma matrices and its application to oral pharmacokinetic study in rabbits. Journal of Chromatographic Science, 51(1), 1-7. [CrossRef]
8. Tomar, A., Sharma K., Chauhan N.S., Mittal, A., Bajaj U. (2012). Formulation and evaluation of fast dissolving oral film of dicyclomine as potential route of buccal delivery. International Journal of Drug Development and Research, 4(2), 408-417.
9. Hosny K.M., El-Say K.M., Ahmed O.A. (2016). Optimized sildenafil citrate fast orodissolvable film: a promising formula for overcoming the barriers hindering erectile dysfunction treatment. Drug Delivery, 23(1), 355-361. [CrossRef]
10. Bahri-Najafi, R., Tavakoli, N., Senemar, M., Peikanpour, M. (2014). Preparation and pharmaceutical evaluation of glibenclamide slow release mucoadhesive buccal film. Research in Pharmaceutical Sciences, 9(3), 213-223.
11. Mashru R.C., Sutariya V.B., Sankalia M.G., Parikh P.P. (2005). Development and evaluation of fast-dissolving film of salbutamol sulphate. Drug Development and Industrial Pharmacy, 31(1), 25-34. [CrossRef]
12. Çoban G., Aydın-Kose, F. (2019). Synthesis, biological evaluations and molecular modelling studies of novel indolin-2-ones designing as FGFR inhibitors. Saudi Pharmaceutical Journal, 27(7), 952-967. [CrossRef]
13. Cheaburu-Yilmaz C.N., Yilmaz O., Aydin-Kose F., Bibire N. (2019). Chitosan-graft-poly (N-isopropylacrylamide)/PVA cryogels as carriers for mucosal delivery of voriconazole. Polymers, 11(9), 1432. [CrossRef]
14. Nair A.B., Shah J., Jacob S., Al-Dhubiab B.E., Patel V., Sreeharsha N., Shinu P. (2021). Development of mucoadhesive buccal film for rizatriptan: *In vitro* and *in vivo* evaluation. Pharmaceutics, 13(5), 728. [CrossRef]
15. Nair A.B., Al-Dhubiab B.E., Shah J., Jacob S., Saraiya V., Attimarad M., SreeHarsha N., Akrawi S.H., Shehata T.M. (2020). Mucoadhesive buccal film of almotriptan improved therapeutic delivery in rabbit model. Saudi Pharmaceutical Journal, 28(2), 201-209. [CrossRef]
16. Szabó P., Daróczi T.B., Tóth G., Zelkó R. (2016). *In vitro* and in silico investigation of electrospun terbinafine hydrochloride-loaded buccal nanofibrous sheets. Journal of Pharmaceutical and Biomedical Analysis, 131, 156-159. [CrossRef]
17. Jovanović M., Tomić N., Cvijić S., Stojanović D., Ibric S., Uskoković P. (2021). Mucoadhesive gelatin buccal films with propranolol hydrochloride: Evaluation of mechanical, mucoadhesive, and biopharmaceutical properties. Pharmaceutics, 13(2), 273. [CrossRef]
18. Singh T.P., Singh R.K., Shah J.N., Mehta T.A. (2014). Mucoadhesive bilayer buccal patches of Verapamil hydrochloride: Formulation development and characterization. International Journal of Pharmacy and Pharmaceutical Sciences, 6(4), 234-241.
19. Rençber S., Karavana S.Y., Yilmaz F.F., Eraç B., Nenni M., Gurer-Orhan H., Limoncu M.H., Güneri P., Ertan G. (2019). Formulation and evaluation of fluconazole loaded oral strips for local treatment of oral candidiasis. Journal of Drug Delivery Science and Technology, 49, 615-621. [CrossRef]
20. Arslan-Azizoglu G., Tuncay-Tanriverdi S., Aydin-Kose F., Ballar-Kirmizibayrak P., Ozer O. (2017). Dual-Prevention for UV-Induced Skin Damage: Incorporation of Melatonin-Loaded Elastic Niosomes into Octyl Methoxycinnamate Pickering Emulsions. AAPS PharmSciTech, 18, 2987-2998. [CrossRef]