FABAD J. Pharm. Sci., 49, 1, 65-80, 2024
Doi: 10.55262/fabadeczacilik.1327446
RESEARCH ARTICLE

Development and Evaluation of Pullulan-Based Mouth Dissolving Film of Furosemide

Aafreen SAHA[*], Umesh Kumar ATNERIYA[**°], Umashankar JOSHI[***], Dharmendra SOLANKI[****]

Development and Evaluation of Pullulan-Based Mouth Dissolving Film of Furosemide

SUMMARY

Mouth-dissolving oral film is an innovative oromucosal route for the systemic delivery of therapeutically/medicinally active drug substances. As we know, furosemide belongs to class IV in BCS classification of drugs, having low solubility and permeability due to strong inter and intramolecular bonds. Due to low aqueous solubility, it exhibits low oral bioavailability. Here, we prepare mouth dissolving film of furosemide to increase its solubility, ultimately enhancing its bioavailability. In this way, we will achieve more rapid drug absorption and the desired pharmacological effect with a quick onset of action by avoiding degradation in gastrointestinal tract (GIT) and first-pass metabolism. This system will provide maximum therapeutic efficacy and stability by reducing the frequency of dosage and improving patient compliance. The present research aimed to prepare pullulan-based mouth dissolving oral films of furosemide used to treat and control hypertension. Pullulan was selected as a film former that exhibits better moisture retention, oxygen barrier, and uniform film-forming properties. Due to the hydrophilic nature of pullulan, it provides quick dissolution. By considering the above points, the film was prepared through the solvent casting method by pullulan as a film-forming polymer, glycerol as a plasticizer, and lepidium sativum mucilage as a natural disintegrating agent known as asaliyo. Disintegration time, drug release, moisture loss, and folding endurance of film were evaluated. Promising batch F6 exhibited better drug dissolution, 98.75±0.09% within 8±0.4 minutes than the other films. The disintegration time for batch F6 was 23±5 sec. It is evident from the above results that it is an innovative dosage form to improve drug delivery, onset of action, and patient compliance.

Key Words: *Mouth Dissolving Film, Furosemide, Pullulan, Solvent Casting Method, Bioavailability.*

Pullulan Bazlı Ağızda Çözünen Furosemid Filminin Geliştirilmesi ve Değerlendirilmesi

ÖZ

Ağızda çözünen oral film, terapötik/tıbbi açıdan aktif ilaç maddelerinin sistemik olarak verilmesi için yenilikçi bir oromukozal yoldur. Bildiğimiz gibi furosemid, BCS ilaç sınıflandırmasında sınıf IV'e aittir ve güçlü moleküller arası ve molekül içi bağlar nedeniyle düşük çözünürlük ve geçirgenliğe sahiptir. Suda çözünürlüğünün düşük olması nedeniyle oral biyoyararlanımı düşüktür. Bu çalışmada, çözünürlüğünü arttırmak ve sonuçta biyoyararlanımını arttırmak için ağızda çözünen furosemid filmi hazırlıyoruz. Bu sayede gastrointestinal sistemde (GİS) bozulmanın ve ilk geçiş metabolizmasının önüne geçerek daha hızlı ilaç emilimi ve istenen farmakolojik etkiyi elde etmiş olacağız. Bu sistem, dozaj sıklığını azaltarak ve hasta uyumunu artırarak maksimum terapötik etkinlik ve stabilite sağlayacaktır. Mevcut araştırma, hipertansiyonu tedavi etmek ve kontrol etmek için kullanılan furosemidin pullulan bazlı ağızda çözünen oral filmlerini hazırlamayı amaçlamaktadır. Pullulan, daha iyi nem tutma, oksijen bariyeri ve tekdüze film oluşturma özellikleri sergileyen bir film oluşturucu olmasından dolayı seçilmiştir. Pullulan hidrofilik yapısı nedeniyle hızlı çözünme sağlar. Yukarıdaki hususlar dikkate alınarak, film oluşturucu polimer olarak pullulan, plastikleştirici olarak gliserol ve asaliyo olarak bilinen doğal parçalayıcı madde olarak lepidium sativum müsilajı kullanılarak solvent döküm yöntemiyle film hazırlanmıştır. Filmin dağılma süresi, ilaç salımı, nem kaybı ve katlanma dayanıklılığı değerlendirilmiştir. Gelecek vaat eden F6 serisi, diğer filmlere göre 8±0,4 dakika içinde % 98,75±0,09 ile daha iyi ilaç çözünmesi göstermiştir. F6 serisinin parçalanma süresi 23±5 saniye olarak bulunmuştur. Yukarıdaki sonuçlardan, bunun iyileştirilmiş ilaç uygulamasını, etki başlangıcını ve hasta uyumunu iyileştirmeye yönelik yenilikçi bir dozaj formu olduğu açıkça görülmektedir.

Anahtar Kelimeler: *Ağızda Çözünen Film, Furosemid, Pullulan, Çözücü Dökme Yöntemi, Biyoyararlanım.*

Received: 14.07.2023
Revised: 21.11.2023
Accepted: 22.11.2023

[*] ORCID: 0009-0004-7250-8492, BM College of Pharmaceutical Educatipon and Research, Indore (Madhya Pradesh) India
[**] ORCID: 0000-0003-2402-6048, School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (Madhya Pradesh) India
[***] ORCID: 0009-0006-5426-867X, Shri Bherulal Pharmacy Institute, Indore (Madhya Pradesh) India
[****] ORCID: 0000-0001-7093-5985, BM College of Pharmaceutical Educatipon and Research, Indore (Madhya Pradesh) India

[°] Corresponding Author;Umesh Kumar ATNERIYA
E-mail: atneriya@gmail.com

INTRODUCTION

Mouth dissolving oral film or strip can be defined as "A dosage form that employs aqua dissolving polymer which allows the dosage form to hydrate by saliva, adhere to the mucosa quickly, and disintegrates within few seconds, dissolves and releases medication for oromucosal absorption when placed on the tongue or oral cavity"(Fitzpatrick et. al., 2019). The oral mucosal absorption directly enters the systemic circulation due to being highly vascularized and hence highly permeable by passing gastrointestinal tract (GIT) and first-pass metabolism in the liver. This may be due to a large surface area, facilitating better absorption and patient compliance(Shakya et. al., 2018). The conventional dosage form is substituted by modern developed drug delivery systems as orally fast disintegrating and dissolving films for pediatric, geriatric, dysphagic, schizophrenic, and bed pron-patients (Homayun et. al., 2019). These films get hydrated very quickly by soaking saliva when placed on the tongue (Rabe et al., 2019). Generally, hydrophilic polymers with plasticizers are used to prepare films that allow them to quickly release incorporated Active Pharmaceutical Ingredient (API) in just seconds (Orsuwan & Sothornvit, 2018). The Food and Drug Administration (FDA) has approved, furosemide as a loop diuretic that acts on the kidney to ultimately increase the water loss from the body(Khan et. al., 2018). It is most commonly used in edema and various clinical conditions with volume overload, congestive heart failure, high blood pressure, liver and renal failure, including nephrotic syndrome (Gulsun et. al., 2018). Furosemide mouth dissolving film is beneficial as there is ease of administration in patients who have difficulty swallowing, like the elderly, pediatric, bedridden patients, stroke victims, and psychiatric patients (Pawar et. al., 2019). Also, the absorption of drugs is prompt from the pre-gastric area like mouth, pharynx, and esophagus, which show the rapid onset of action. Generally, super disintegrating agents are added to decrease the disintegration time, which in turn enhances the drug dissolution rate (Dhiman et. al., 2022). Pullulan, a neutral polysaccharide produced using a black yeast-like fungus called aureobasidium pullulans from sucrose, and starch as carbon substrates. It is a colorless, odorless, tasteless, white powder that has increased solubility, flexibility, strength, and stability over a wide temperature range (Agrawal et. al., 2022). Due to excellent film formation, mouth feel, edible, water soluble and better mechanical properties, pullulan is extensively used in rapid film making. Despite -these features, pullulan provides better moisture retention and oxygen barrier properties (Liu et al., 2019). Some recent developments show pullulan-based rapid films incorporated APIs like amlodipine besylate (Pezik et. al., 2021), bilastine (Kanugo & Gandhi, 2022), aprepitant (Bala & Sharma, 2018), ropinirole (Meher & Dighe, 2019), for their formulation and evaluation. The type of plasticizer and its concentration play a significant role in producing the film of desired flexibility and reducing the brittleness of the formed film (Edwards et al., 2022). Here, we used glycerol as a plasticizer because it has unique features as an antimicrobial agent, cosolvent, emollient, humectant, plasticizer, sweetening agent, and many more (Singh et. al., 2021). Lepidium sativum is used as a natural super disintegrating agent for film (Al-Snafi, 2019). Mucilage of natural origin is advantageous over semi-synthetic and synthetic substances due to its cost-effectiveness, non-toxic and non-irritable properties, easy availability, eco-friendly, biodegradable and biocompatible nature (Soukoulis et. al., 2018). Fenugreek seed mucilage, and ocimum basilicum gum are used earlier for the fast disintegrating tablet of amlodipine besylate (Keisandokht et. al., 2022). In the present study, pullulan is mixed with glycerol and lepidium sativum in various concentrations to study the film properties like brittleness, folding endurance, disintegration, and dissolution time (Maske et. al., 2022).

MATERIAL AND METHODOLOGY

Material

Furosemide was gifted from IPCA Laboratories, Pithampur, Indore (M.P., India). Pullulan was obtained as a gift sample from Gangwal Chemicals Pvt Ltd, Mumbai. Glycerol was gifted from Glister Pharmaceutical, Rau, Indore (M.P., India). Lepidium sativum was procured from SK Traders, Indore (M.P., India). Citric acid and Sucralose were obtained as gift samples from Loba Chemie, Meghnagar (M.P., India)

Method

UV Spectra of Furosemide

10 mg furosemide was accurately weighed (Shimadzu Corporation - EL2204, Japan) and transferred to a 100 mL volumetric flask. It was dissolved and diluted to 100 mL with pH 6.8 phosphate buffer (as per IP, 2014) to obtain a final 100 µg/mL concentration. Dilutions were made to obtain a concentration of 10 µg/mL and scanned by using a UV visible spectrophotometer (Shimadzu-1601, Japan) for λ_{max} in a range of 200–400 nm in the spectrum for three consecutive times (Kumar & Kumar, 2016). The UV spectra of furosemide were represented in Figure 1.

Solubility Study of Furosemide

For the determination of solubility, an excess amount of furosemide was dissolved in each of 20 mL of different below solvents like water, 0.1 N HCl, dimethyl formamide (DMF), sodium citrate, sodium acetate, phosphate buffer pH 6.8, sodium benzoate, 15% glycerine, dimethyl sulfoxide (DMSO), acetone, methanol separately in the flask. Kept it closed with a rubber cap and placed on a mechanical shaker (REMI Elektrotechnik Motor, Vasai, India) at 37±5 °C for 12 hours, and the solution was allowed to equilibrate for 24 hours undisturbed. Then, the solution is filtered through filter paper. The supernatant was analyzed using a double beam UV spectrophotometer (Shimadzu-1601, Japan) (Zhang et. al., 2022).

Compatibility Study of Furosemide and Excipients by FTIR

The individual pellet of furosemide, pullulan, and the physical mixture was formed to determine the compatibility study. In order to do this, 10 mg of each sample was mixed with dried potassium bromide (KBr) of equal weight. The mixture was properly ground using a pestle and mortar. Pellets were formed by compressing the mixture using a hydraulic press. Transparent pellets formed in this way are scanned by FTIR spectrometer (Nicolet-380, Thermo Scientific, Madison, USA). The spectra are examined over a frequency range of 4000-200cm^{-1} (Vimalson et al., 2019).

Isolation of Mucilage from *Lepidium sativum* Seeds

Mucilage is present in the outer layer of the seeds.100 g of seeds were soaked in 1000 mL of distilled water and 5 mL of chloroform for 24 hr. The obtained viscous solution was filtered through a muslin cloth. To the mucilaginous solution, add 1 L of 95% ethanol in the viscous mucilaginous solution to precipitate the mucilage. The precipitated mucilage was collected and dried in a hot air oven (Rasayana scientific-CFC 1001, Surat) at a temperature not exceeding 40 to 45±5°C until completely dried. The powder was sieved through 80 mesh, weight, and stored in a desiccator for further use (Mahapatra et al., 2021).

Preparation of Mouth dissolving Film of Furosemide

It is an easy and accurate method of formulation of mouth-dissolving film. Water soluble polymer was dissolved in water separately. Furosemide and other excipients were dissolved in methanol. This solution was added to the polymeric solution. Then, the solution was mixed using a mechanical stirrer (REMI Electrotechnik Motor, Vasai, India) for 45±2 minutes with a rotating speed of 60-80 rpm. The entrapped air was removed by vacuum. The resulting solution

was uniformly spread in a petri plate and dried in a hot air oven (Rasayana scientific-CFC 1001, Surat) at 40±3°C for 24 hours. After proper drying, films were cut into the desired dimensions i.e., 2×2 cm^2, which 40 mg of furosemide was present (Pattewar et. al., 2019). The formula of mouth dissolving film is shown in Table 1.

Table 1. Formulation batches

S. NO.	Ingredients	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8
1.	Furosemide	40	40	40	40	40	40	40	40
2.	Pullulan	40	42	45	50	52	55	57	60
3.	Glycerol	05	08	11	14	15	17	23	28
4.	Lepidium Sativum	10	10	10	11	13	14	25	25
5.	Citric acid	15	15	15	15	15	15	15	15
6.	Tween 80	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
7.	Sucralose	3	3	3	3	3	3	3	3
8.	Menthol	2	2	2	2	2	2	2	2
9.	Methanol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
10.	Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Sensory evaluation

Appearance is also a key component of sensory evaluation. The vision system is the detector of appearance. We can sense color, intensity, transparency, shininess, and other physical characteristics, including the size and shape of the film. The aroma of mouth dissolving film is perceived through the olfactory system. By somesthesis system, we can evaluate its texture (uniformity, stickiness, rough/smooth). Kinesthesis is also an essential type of sensory evaluation that evaluates the film's mechanical movement like, flexibility, tension, and relaxation (Kemp et. al., 2018).

Film thickness

First of all, make the micrometer screw gauge (Al-Anwar traders, Mumbai, India) reading at zero. Place the film between the spindle and anvil by turning the thimble. Hold the film tightly by turning the ratchet knob until the rachet slips (a "tick" sound is heard). Now, we will note the reading of film thickness. Each film was measured at five positions (central and the four corners) and in terms of its length and width. The mean thickness was calculated as measurement results were divided by the number of samples (Wang et al., 2018).

Folding endurance

Folding endurance indicates the brittleness of the film. Schopper type tester (Fibretec Instrument Roorkee-247667, Uttarakhand, India) is another type of apparatus having opposed and moveable jaws (Perdoch et al., 2022). Firstly, we tighten both jaws, insert the film between the place held by both jaws and tighten both sides. Now, release the jaws and apply various tension to the film. As we press the start button, the jaws move and fold numbers are recorded on the screen. During the process, when the film is broken, it is noted as a folding endurance reading. This test was performed on six films of each formulation and mean±S.D calculated value (Malviya & Pande, 2021).

Moisture content

It is determined by cutting the film strip of 2×2 cm^2 dimension and measuring its initial weight by analytical balance (Shimadzu Corporation - EL2204, Japan). Afterward, these strips were placed in a desiccator containing activated silica, and the desiccator was placed inside the incubator to ensure a constant temperature of 37±5 °C for 24 hours. Now, calculate the film's final weight individually until it show a constant weight. Moisture content is estimated by analyzing the weight gain of the film or the difference between the initial and final weight (Fang et. al., 2021).

Surface pH

The test was performed by placing the film of 2x2cm^2 in a petri dish. The film is moistened with 1 mL of phosphate buffer pH 6.8 and kept for 30 sec. The pH was noted by bringing the electrode of the pH meter (Chemiline-CL110, Aqua mart, Kolkata, India) in contact with the film's surface and allowing equilibration for 1 min. The average of three determinations for each formulation was taken (Kumorek et al., 2020).

Weight variation

It confirms uniformity and supports the quality of the dosage unit. At least six films of each formulation batch are taken of uniform dimension and individually weighed on an analytical balance (Shimadzu Corporation - EL2204, Japan). Note the balance recorded on the screen. Now calculate the average weight of films. Films should have nearly constant weight. As the amount of pullulan increases, the weight of the film also increases. It is helpful to ensure that a film contains the proper amount of excipients and API (Vishvakarma, 2018).

Drug Content Uniformity

Drug content was determined by dissolving the film containing 10 mg of furosemide in 100 mL phosphate buffer pH 6.8 to get 100 µg/mL solutions. An aliquot of 1 mL sample was withdrawn and diluted to 10 mL with phosphate buffer pH 6.8. Then, the solution was filtered through the whattman filter paper and analyzed by UV-spectrophotometer (Shimadzu-1601, Japan) at 272 nm against a blank prepared using dummy film treated similarly. Content uniformity studies were carried out in triplicate for each batch of the film. The limit of content uniformity is 90-110%.

***In vitro* disintegration studies**

In the petri dish method, the film (2x2 cm^2) was placed on a stainless steel wire mesh, which was placed in a petri dish containing 10 mL phosphate buffer pH 6.8with 37±5 °C temperature. The time required for the film to break and disintegrate was noted as in vitro disintegration time. Since the film is expected to disintegrate in the mouth in the presence of saliva, only 10 mL of medium was used. All the measurements are done in triplicate, and the average value is reported (Pawar et. al., 2019).

***In vitro* Dissolution Study**

900 mL phosphate buffer pH 6.8 poured into a stainless steel vessel. Put a single film into the dry basket and immerse the basket into the USP I apparatus (Electrolab- ERD 07, Mumbai). Set the flask at 50 rpm with 37±5 °C temperature. The sink condition is maintained by withdrawing the 5mL sample of the dissolved drug through the pipette at intervals like 1, 2, 4, 6, and 8 minutes and replacing it with fresh dissolution media. The dissolved drug was analyzed by ultraviolet spectrophotometer (Shimadzu-1601, Japan) at λ max 272 (Raza et. al., 2019).

***In vitro* Drug Release Kinetics**

The mechanism of drug release kinetics of furosemide films was analyzed through an in vitro study by exploring the following kinetic models (zero order, first order, and Higuchi equations), and release was determined (Sheikh et al., 2020).

Zero order kinetic(constant rate process)

Release of the drug does not depend on reactant concentration(does not vary with increase or decrease

with the concentration of reactant), The zero power of the reactant concentration.

$$C = K_0 t \ldots\ldots(1)$$

Here, C is the concentration of the drug undergoes reaction at time t.

K_0 is zero order rate constant.

The Concentration vs time plot was plotted with a straight line with a slope equal to K_0. The graph proves the above theory that drug release from the film does not depend on concentration.

First order kinetic

Release of the drug depends on reactant concentration (vary with increase or decrease with the concentration of reactant).

$$\text{Log } C = \text{Log } C_o - kt / 2.303 \ldots\ldots(2)$$

Where C_0 is the initial concentration of the drug, k is the first-order constant, and t is the time.

It proves that the reaction rate is directly proportional to the reactant concentration.

Higuchi's Model

Higuchi's model as cumulative percentage of drug released vs square root of time.

$$Mt/M\infty = Kht_{1/2} \ldots\ldots(3)$$

Where Mt/M∞ is the fraction of drug released at each time t (minutes), Mt is the amount of the drug released in time t (minutes).

M∞ is the amount of drug released after time ∞.

Kh represents the Higuchi release kinetic constant.

The system ensures the release of drugs based on Fickian diffusion as a square root of time-dependent process from mouth dissolving film.

Stability Study of Mouth Dissolving Film of Furosemide

The stability study aims to observe the formulation quality, which fluctuates under certain environmental factors like temperature, relative humidity (RH), and light of which storage conditions are to be established. The International Conference of Harmonization (ICH) Guidelines specified the length of study and storage conditions. Stability studies were carried out by placing the film in storage at $25^0C\pm2^0C/60\pm5\%RH$ and $40^0C\pm2^0C/75\pm5\%$ RH for six months. The effects of temperature and time on the F6 formulation were evaluated. Parameters like folding endurance, surface pH, weight variation, % drug content, disintegration time, and release rate were analyzed (Sahu et al., 2018).

RESULT AND DISCUSSION

UV spectra of Furosemide

10 mg furosemide was accurately weighed (Shimadzu Corporation - EL2204, Japan) and dissolved in 100 mL phosphate buffer pH 6.8 in a volumetric flask and obtained the concentration of 100 µg/mL. Further dilutions were made to obtain a concentration of 10 µg/mL and scanned using a UV visible spectrophotometer (Shimadzu-1601, Japan), and λmax was found to be 272nm.

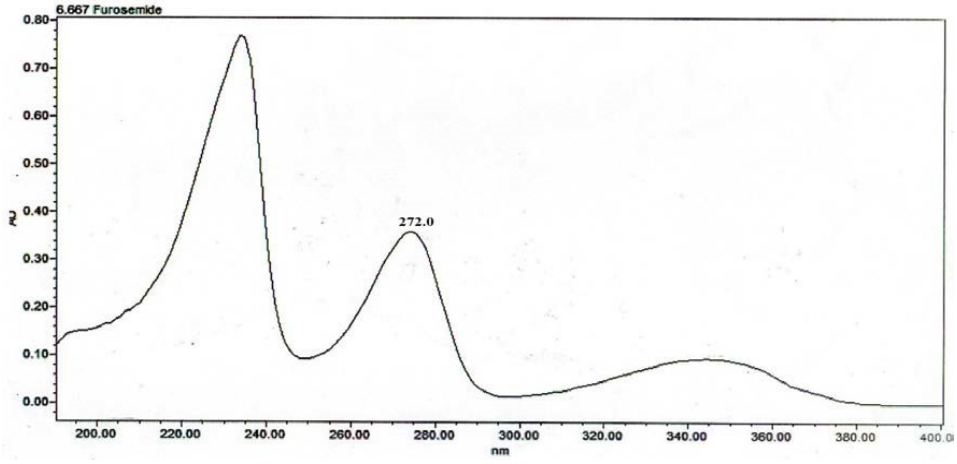

Figure 1. UV Spectra of Furosemide

Solubility Study of Furosemide

Solubility of furosemide in different solvents was done by dissolving it in various below solvents separately. The conclusion shows that furosemide is practically insoluble in aqua and freely soluble in acetone, and methanol, while 15% glycerine possesses good solubility. The solubility data were expressed in Table 2.

Table 2. Solubility of Furosemide in Different Solvent

S.NO	Solvent	Solubility of Furosemide (mg/ml)
1.	Water	0.01825±0.008
2.	0.1 N HCL	0.0570±0.003
3.	Dimethyl formamide(DMF)	0.0792±0.001
4.	Sodium citrate	0.129±0.009
5.	Sodium acetate	0.239±0.005
6.	Phosphate buffer (pH 6.8)	1.6500 ±0.001
7.	Sodium benzoate	2.157±0.003
8.	15% glycerin	14.6600±0.007
9.	Dimethyl sulfoxide(DMSO)	30±0.002
10.	Acetone	50±0.007
11.	Methanol	50 ±0.009

Solubility result of furosemide in various solvents shown as mean ±S.D.(n=3)

Compatibility study of Furosemide and Excipients by FT-IR

The drug excipient compatibility study was performed by FT-IR spectrometer (Nicolet-380, Thermo Scientific, Madison, USA) to ensure compatibility . No drug-polymer interaction was observed between the drug and excipients in the entire FT-IR spectrum. The observed peaks indicated the stable nature of furosemide with pullulan and other excipients. The FT-IR spectrums of furosemide, pullulan, and physical mixture of formulation were represented in Figure 2 (a), (b), and (c).

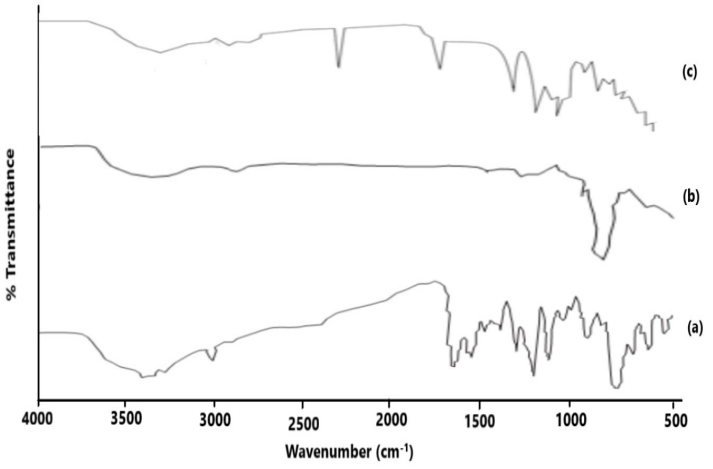

Figure 2. FT-IR Spectrum of Furosemide (a), Pullulan (b) Physical Mixture of Prepared F6 Formulation (c)

FT-IR spectra of pure furosemide are shown in Figure 2 (a). The prominent IR absorption peak of furosemide was attributed to 3647.51 cm-^{1}, indicating O-H hydrogen stretching. 3286.81cm-^{1}represent N-H stretching, peaks near 3147.05 cm^{-1} showed C-H stretching. It also indicated N-H bending at 1672.34 cm^{-1}. The carbonyl group indicated as stretching at 1568.18 cm-^{1}, and the sulfoxide group showed asymmetric stretching at 1263.44 cm^{-1}. FT-IR spectra of pure pullulan are shown in Figure 2 (b). The prominent IR absorption peaks of pullulan at 3423 cm^{-1} indicated O-H hydrogen stretching. Peaks near 2928 cm^{-1} attributed to C-H asymmetric stretching. Peaks 1636 cm^{-1}, 1460 cm^{-1} indicated as C=O stretching. The glycosidic bond represented as stretching and rocking at 928 cm^{-1}, 849 cm^{-1}, 757 cm^{-1}. The FT-IR spectra of the physical mixture of the prepared formulation were found to be recorded and represented in Figure 2 (c). The band at 3423 cm^{-1} was attributed to O-H stretching vibration. The absorption band 2928 cm^{-1} was also represented for C-H vibrations of stretching and bending. Wave numbers between 1700-1200 were attributed to C-O and C-N stretching vibration due to the presence of protein. Region of glycosidic bond vibration was found at 1058 cm^{-1} while 849 cm^{-1} represents carbohydrate group presence.

Sensory Evaluation

Results from trial studies showed that film is transparent, clear, soft, without air bubbles, uniform in thickness, colorless, and easily removed from a petri dish. It exhibits a shinning and glossy appearance with no stickiness, sufficient hardness, and proper plasticity due to the adequate amount of pullulan, glycerol, and lepidium sativum used. Pullulan at 45-55%, glycerol at 14–17%, and 11-14% lepidium sativum form a nontacky, uniform, transparent, and clear film. With less amount of pullulan 5–45%, the film's surface did not form. Less than 14% glycerol and less than 10% lepidium sativum negatively impacted the film's hardness. It possesses no stickiness in film's but has no

clarity. In contrast, the films prepared with more than 55% pullulan, more than 17% glycerol, and more than 14% lepidium sativum form tacky, turbid, and rigid or brittle film (Choi et. al., 2018). The sensory result was noted in Table 3.

Table 3. Sensory evaluation result

S.NO.	Batch	Surface appearance	Stickiness	Film clarity
1.	F1	Film did not form	Non-sticky	--
2.	F2	Film did not form	Non-sticky	--
3.	F3	Film did not form	Non-sticky	--
4.	F4	Film formed is loose	Non-sticky	Non-clear
5.	F5	Semitransparent film	Non-sticky	Clear
6.	F6	Uniform and transparent film	Non-sticky	Glossy
7.	F7	Brittle	Sticky	Muddy
8.	F8	Brittle	Sticky	Muddy

Film Thickness

Calibrated micrometer screw gauge (Al-Anwar traders, Mumbai, India) was used for the determination of film thickness. With little pullulan concentration from F1 to F3, no film was formed. Hence, no thickness was observed. In F4 and F5, the film developed was loose and semi-transparent, respectively with no sufficient thickness. In comparison, The F6 formulation possessed proper thickness with the appropriate pullulan concentration of 55%. But with more than 55% pullulan in the F7 and F8 batches, the film formed was too thicky. That's why F6 was considered best formulation in the context of thickness. The result of film thickness was expressed in Table 4.

Folding Endurance

Schopper type tester (Fibretec Instrument Roorkee-247667, Uttarakhand, India) was utilized to estimate the film's brittleness. Folding endurance was analyzed that batch F1 to F3 film had no flexibility due to very low glycerol, *Lepidium sativum* and pullulan concentration. Then, slightly increased the concentration of the above ingredients in the F4, F5, and F6 batches and improved the films as the F6 formulation possessed exact flexibility and no film breakage was observed. In contrast, more than 17% glycerol and 14% lepidium sativum with higher pullulan concentrations give a brittle film. Hence, the F6 was our best formulation in terms of folding endurance. The Folding endurance result was noted in Table 4.

Moisture Content

Accurate moisture content capacity was observed in formulation F6 because pullulan exhibits suitable moisture retention properties in between 40-55% of the pullulan amount. There is no effect of glycerol and *Lepidium sativum* on the formulations. The result data of moisture content of furosemide film was noted in Table 4.

Surface pH

A digital pH meter with electrode (Chemiline-CL110, Aqua mart, Kolkata, India) was utilized to estimate pH, ultimately to check whether the film exhibits irritation to oral mucosa. All the films had almost near neutral pH but F6 had pH near to saliva pH. There is no effect of the above ingredients on pH observation. The result of the pH of furosemide mouth dissolving film was noted in Table 4.

Weight Variation

Analytical balance (Shimadzu Corporation -

EL2204, Japan) ensured that a film contained the proper amount of excipients and furosemide. The weight variation of the films is directly proportional to the amount of pullulan, glycerol, and lepidium sativum used. As we enhanced the concentration of all ingredients, the weight of the film was also enhanced. But batch F6 had the least weight variation due to sufficient amount of pullulan, glycerol, and *Lepidium sativum* used. The weight variation of furosemide film was noted in Table 4.

Drug Content

Drug content was detected by withdrawing the 1 mL sample from 100 µg/mL solutions diluted to 10 mL with phosphate buffer pH 6.8, and analyzed by UV-spectrophotometer (Shimadzu-1601, Japan) at 272 nm wavelength. The drug content uniformity of furosemide film was showed in Table 4.

Disintegration Time

The test was performed by the petri dish method to ensure the onset of action of furosemide film. Pullulan, glycerol, and *Lepidium sativum* are all widely attributed to disintegrating properties of films. With very low concentrations, they had a negative impact on disintegration. But the film of the F6 batch was produced with sufficient disintegration time with an adequate concentration of contents used, such as 55% pullulan, 17% glycerol, and 14% *Leidium sativum*, which exhibit proper film dissolution. The disintegration time of the furosemide film was noted in Table 4.

Table 4. Evaluation result of Film Thickness

S. NO.	Batch	Film Thickness (mm)	Folding endurance	Moisture Content (%)	Surface pH	Weight variation (mg)	Drug contents (%)	Disintegration Time (Sec)
1.	F1	0.005±0.01	97±1	12.30±0.07	6.4±04	55.21±0.31	92±0.48	32±5
2.	F2	0.07±0.01	98±5	13.01±0.12	6.2±0.7	57.71±0.22	88±0.36	37±2
3.	F3	0.08±0.02	110±6	14.13±0.40	6.4±0.3	62.31±0.44	86±0.53	43±4
4.	F4	0.09±0.01	94±3	13.24±0.12	6.3±0.22	57.41±0.38	87±0.23	35±4
5.	F5	0.09±0.02	101±4	15.15±0.27	6.7±0.5	63.71±0.17	95±0.51	28±3
6.	F6	0.11±0.02	114±2	10.11±0.33	6.9±0.1	60.21±0.77	97±0.57	23±5
7.	F7	0.12±0.01	99±1	10.44±0.16	6.7±0.15	66.21±0.29	89±0.35	47±4
8.	F8	0.15±0.01	95±2	14.23±0.58	6.5±0.3	57.21±0.56	89±0.46	50±3

The thickness result is shown as mean ±S.D.(n=3), which determines that thickness of the film increases with increasing amount of pullulan. F6 has proper film thickness with appropriate concentration. The folding endurance result is shown as mean ±S.D.(n=3), which expresses the exact brittleness of the film. The folding endurance of the film increases as the amount of plasticizer and polymer increases. F6 shows better mechanical properties like flexibility and strength. Moisture retention result is shown as mean ±S.D.(n=3). F6 has proper moisture retention with a proper concentration of pullulan. Surface pH results are expressed as mean ±S.D.(n=3) and there is no effect of pullulan concentration on film pH, but pH was near to neutral. Hence, no irritation to mucosa occurred. The weight variation result is presented as mean ± S.D.(n=3,) which shows that the amount of pullulan increases, and weight of the film also increases. Drug content results are expressed as mean ± S.D.(n=3), which estimated that F6 possessed the strength of furosemide remains within its specified limit. The disintegration time of each film was measured in triplicate as mean ± S.D. (n=3). The amount of pullulan above 55% showed an increase in disintegration time, but an increase in plasticizer concentration decreased disintegration time.

***In vitro* Dissolution Study**

The test was analyzed through USP apparatus I, Basket apparatus (Electrolab- ERD 07, Mumbai) to explore the rate of drug dissolution (Zayed et. al., 2020). The dissolution profiles of the furosemide mouth dissolving film were presented in Figure 5, and result was recorded in Table 5.

Table 5. Dissolution Profile of Mouth Dissolving Films

Time (Min)	F1	F2	F3	F4	F5	F6	F7	F8
0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
1	34.54±0.13	20.35±0.43	25.07±0.46	32.7±0.22	29.24±0.29	27.52±0.37	34.18±0.56	30.94±0.49
2	63.22±0.31	46.47±0.28	46.65±0.47	67.52±0.32	64.49±0.35	48.29±0.54	62.02±0.67	60.32±0.36
4	82.1±0.47	57.98±0.12	78.63±0.36	88.21±0.28	83.74±0.23	83.88±0.76	59.08±0.88	87.63±0.35
6	85.96±0.22	62.98±0.45	85.57±0.34	92.45±0.29	89.41±0.26	92.83±0.39	94.02±0.07	92.15±0.73
8	91.26±0.40	91.44±0.09	92.45±0.29	94.59±0.33	94.79±0.17	98.75±0.09	96.55±0.39	95.47±0.08

The dissolution rate of different sets of furosemide mouth dissolving film was performed by a Basket Dissolution apparatus (USP Type I) at temperature of 37 °C and 50 rpm. The results are shown as Mean±SD (n=3).

With increasing lepidium sativum concentration, disintegration time increases, which ultimately enhances the drug release. The release rate of furosemide was enhanced with pullulan (45 to 55%), which spread the hydrophilic chain around the matrix system. F6 formulation showed a rapid release of 98.75±0.09 within 8±0.4 min. However, the release profile was decreased at a higher percentage of polymer and disintegrating agent by forming a layer around the drug, which allows the drug release at a slow rate.

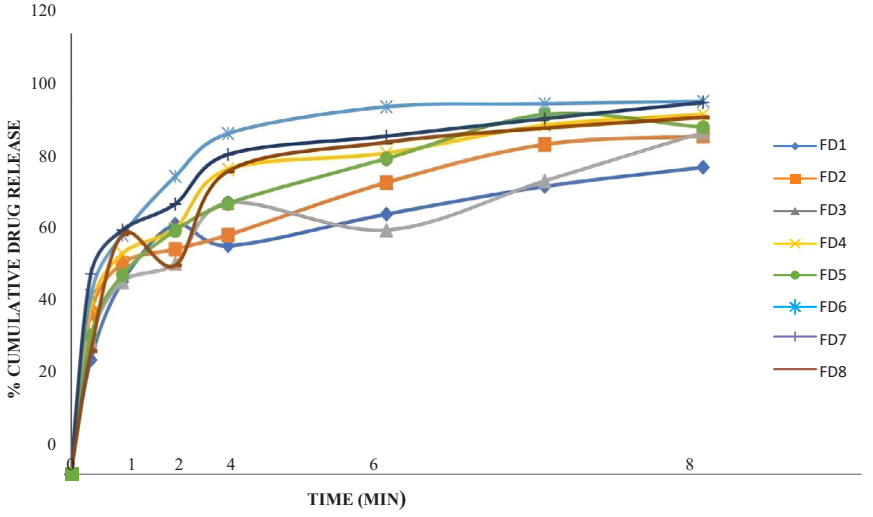

Figure 3. % Cumulative Drug Release v/s Time Plot in Phosphate Buffer pH 6.8

***In vitro* drug release kinetics**

In vitro release data of kinetic models like zero-order, first-order, and Higuchi models were analyzed to describe the release kinetics. The regression values of all formulations were noted in Table 6.

Table 6. *In vitro* drug release kinetic data

S. NO.	Batch	Zero order	First order	Higuchi model
1	F1	0.930	0.956	0.964
2	F2	0.884	0.960	0.943
3	F3	0.923	0.955	0.968
4	F4	0.886	0.973	0.960
5	F5	0.833	0.960	0.953
6	F6	0.862	0.976	0.930
7	F7	0.921	0.914	0.905
8	F8	0.928	0.974	0.975

The best model for the furosemide mouth dissolving film release pattern was determined as per the regression data,for the dissolution study, basket dissolution apparatus (USP TypeI) was employed. Values are presented as mean±SD (n = 3).

To analyze the release kinetic model, the in vitro release study was performed according to zero order, first order, and diffusion-controlled mechanism through a simplified higuchi model. A certain mechanism was based on the coefficient to determine the parameters studied. If the highest coefficient of the determination is observed, it is favoured for selecting the order of release. Release of all the formulations followed first-order kinetics exhibited a diffusion-controlled mechanism as indicated by the highest coefficient of determination (R^2). It was found that the optimized formulation F6 follows first order-kinetic system as it has the highest value of R^2.

Stability study of Mouth Dissolving Film of Furosemide

The developed pullulan-based film of furosemide was evaluated for stability studies. The evaluated parameter showed that no notable changes were found in the prepared film. As per the data gathered during six months of storage, it was recapitulated that the fabricated film formulation was stable, and the stability study result was recorded in Table 7.

Table 7. Stability Study of Mouth Dissolving Film of Furosemide.

Evaluation parameters	Observation in months						
	Initial	25°C±2°C/60±5%RH			40°C±2°C/75±5% RH		
	0	2	4	6	2	4	6
Weight variation (mg)*	60.21±0.77	60.09±0.61	59.98±0.22	59.28±0.87	60.18±0.67	59.88±0.56	59.21±0.25
Surface pH **	6.9±0.19	6.9±0.55	6.8±0.89	6.8±1.0	6.9±0.51	6.8±0.24	6.8±0.09
Folding endurance **	114±2	113±1	112± 3	112±2	114±3	113±1	113±2
Drug content (%)**	97.57±0.57	97.62±0.21	97.51±0.11	97.25±0.51	97.68±0.78	97.33±0.29	97.12±0.18
Disintegration time (Sec)**	23±5 sec	23±7sec	24±8 sec	24±9sec	23±6 sec	23±9 sec	24±5 sec
Cumulative drug release (%)***	98.75±0.12	98.54±0.51	98.22±0.18	97.98±0.88	98.78±0.41	98.73±0.57	98.26±0.59

* Each value represent the mean±standard deviation (n=20)

**Each value represent the mean ± standard deviation (n=6)

*** Each value represent the mean ± standard deviation (n=3)

CONCLUSION

The present research amied to formulate and develop mouth-dissolving oral film and evaluate its different formulations of furosemide to achieve faster drug release to control hypertension. Mouth-dissolving films of diuretic drugs were found to be a better option to control hypertension by way of fast onset of action. Preliminary trials indicated better results for sensory, mechanical properties, and disintegration time. The in vitro release of the drug was found to be 98.75±0.09% within 8±0.4 minutes. The drug content of the optimized formulation was found to be 97±0.57, no significant difference in drug content was found, and hence, the drug was uniformly distributed in the film. Folding endurance indicates the film brittleness. As concentration of pullulan & glycerol increased, folding endurance also increased, affecting the overall flexibility of the furosemide loaded mouth-dissolving film. The higher value of folding endurance for film proves that the films were strong enough to withstand handling that is 114±2 folds. The *in vitro* disintegration study indicates that the drug's onset of action, 23±5 sec., was found because the increase in plasticizer concentration decreased disintegration time in our formulation F6. More than 14% of *Lepidium sativum* enhances disintegration time, while between 10-14% lepidium sativum exhibit proper disintegration profile, and less than 10% possess a negative impact on disintegration time. The mean thickness values for all the batches were 0.07–0.12 mm, indicating uniform cast of respective batches. The surface pH was in the range of 6.5–6.9, close to saliva pH. F6 shows faster release within 8±0.4 minutes. Glycerol also acts as co-solvent hence, it enhances dissolution. Therefore, the F6 formulation is considered as a best and can be used as a fast dissolving oral film as novel drug delivery method. It is a pullulan-based film that exhibits desired moisture retention with faster disintegration time. Due to speedy drug dissolution, it provides quick effects and control hypertension. This film is beneficial for patient convenience and compliance in disease state.

ACKNOWLEDGMENT

The authors would like to express their gratitude to the late Dr. Vimukta Sharma for granting permission to carrying out research work at Department of Pharmaceutics, BM College of Pharmaceutical Education and Research, Indore (MP) India 452020 during the development of this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Aafreen Shah: Draft manuscript preparation. Umesh Kumar Atneriya: Study conception and design, Analysis and interpretation of results. Umashankar Joshi: Data collection. Dr. Dharmendra Solanki & Umesh K Atneriya: Reviewed the results and approved the final version of the manuscript.

REFERENCES

Agrawal, S., Budhwani, D., Gurjar, P., Telange, D., & Lambole, V. (2022). Pullulan based derivatives: Synthesis, enhanced physicochemical properties, and applications. *Drug Delivery, 29*(1), 3328-3339.

Al-Snafi, A. (2019). Chemical constituents and pharmacological effects of lepidium sativum. *Int J Curr Pharm Res, 11*(6), 1-10.

Bala, R., & Sharma, S. (2018). Formulation optimization and evaluation of fast dissolving film of aprepitant by using design of experiment. *Bulletin of Faculty of Pharmacy, Cairo University, 56*(2), 159-168.

Choi, I., Lee, S. E., Chang, Y., Lacroix, M., & Han, J. (2018). Effect of oxidized phenolic compounds on cross-linking and properties of biodegradable active packaging film composed of turmeric and gelatin. *Lwt, 93*, 427-433.

Dhiman, J., Dev, D., & Prasad, D. (2022). Superdisintegrants: Brief Review. *Journal of Drug delivery and Therapeutics, 12*(1), 170-175.

Edwards, L., McCray, N. L., VanNoy, B. N., Yau, A., Geller, R. J., Adamkiewicz, G., & Zota, A. R. (2022). Phthalate and novel plasticizer concentrations in food items from US fast food chains: a preliminary analysis. *Journal of exposure science & environmental epidemiology, 32*(3), 366-373.

Fang, L., Zeng, J., Zhang, X., & Wang, D. (2021). Effect of veneer initial moisture content on the performance of polyethylene film reinforced decorative veneer. *Forests, 12*(1), 102.

Fitzpatrick, S. G., Cohen, D. M., & Clark, A. N. (2019). Ulcerated lesions of the oral mucosa: clinical and histologic review. *Head and neck pathology, 13*, 91-102.

Foo, W. C., Khong, Y. M., Gokhale, R., & Chan, S. Y. (2018). A novel unit-dose approach for the pharmaceutical compounding of an orodispersible film. *International journal of pharmaceutics, 539*(1-2), 165-174.

Gulsun, T., Borna, S. E., Vural, I., & Sahin, S. (2018). Preparation and characterization of furosemide nanosuspensions. *Journal of Drug Delivery Science and Technology, 45*, 93-100.

Homayun, B., Lin, X., & Choi, H.-J. (2019). Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. *Pharmaceutics, 11*(3), 129.

Kanugo, A., & gandhi, Y. (2022). Formulation optimization and evaluation of oral thin film of bilastine for the treatment of allergic conditions. *Journal of Research in Pharmacy, 26*(5).

Keisandokht, S., Orsat, V., Karboune, S., & Gatamaneni Loganathan, B. (2022). Microwave-assisted extraction of Ocimum basilicum L. seed, Trigonella foenum-graecum seed, and Plantago ovata Forsk seed husk hydrocolloids compared with conventional heating extraction at optimum extraction conditions. *Arabian Journal for Science and Engineering, 47*(5), 5859-5874.

Kemp, S. E., Hort, J., & Hollowood, T. (2018). Descriptive analysis in sensory evaluation. 1st ed. *Wiley-Blackwell,* 744.

Khan, T. M., Patel, R., & Siddiqui, A. H. (2018). Furosemide. *StatPearls,* 29763096.

Kumar, S., & Kumar, M. (2016). Preformulation study of furosemide. *Der Pharmacia Lettre, 8*(13), 214-222.

Kumorek, M., Minisy, I. M., Krunclová, T., Voršiláková, M., Venclíková, K., Chánová, E. M., Kubies, D. (2020). pH-responsive and antibacterial properties of self-assembled multilayer films based on chitosan and tannic acid. *Materials Science and Engineering: C, 109*, 110493.

Liu, Y., Liu, Y., Han, K., Cai, Y., Ma, M., Tong, Q., & Sheng, L. (2019). Effect of nano-TiO2 on the physical, mechanical and optical properties of pullulan film. *Carbohydrate Polymers, 218*, 95-102.

Mahapatra, A. P. K., Murthy, P., Panda, N., Gupta, A., Paul, B., Malla, S., & Panigrahy, S. (2021). Extraction of lepidium sativum seed mucilage: Optimization of extraction process with maximum yield by using full factorial design. *Journal of Pharmaceutical Research International, 33*(64B), 720-735.

Malviya, V., & Pande, S. (2021). Development and evaluation of fast dissolving film of fluoxetine hydrochloride. *Research Journal of Pharmacy and Technology, 14*(10), 5345-5350.

Maske, R. R., Mahajan, V. R., & Bhalerao, S. B. (2022). Polymers used in mouth dissolving film: A review. *World Journal of Advanced Research and Reviews, 16*(3), 378-389.

Meher, A., & Dighe, N. S. (2019). An overview of fast dissolving oral film. *Journal of Drug delivery and Therapeutics, 9*(4-s), 822-825.

Orsuwan, A., & Sothornvit, R. (2018). Effect of banana and plasticizer types on mechanical, water barrier, and heat sealability of plasticized banana-based films. *Journal of Food Processing and Preservation, 42*(1), e13380.

Pattewar, S., Patil, D., & Sharma, S. (2019). Fabrication and characterization of self-microemulsifying mouth dissolving film for effective delivery of piroxicam. *Indian J. Pharm. Sci, 81*, 503-513.

Pawar, R., Sharma, R., & Darwhekar, G. (2019). Formulation and evaluation of mouth dissolving film of prochlorperazine maleate. *Journal of Drug delivery and Therapeutics, 9*(6), 110-115.

Pawar, R., Sharma, R., Sharma, P., & Darwhekar, G. (2019). A review on mouth dissolving film. *Journal of Drug delivery and Therapeutics, 9*(6), 206-210.

Perdoch, W., Cao, Z., Florczak, P., Markiewicz, R., Jarek, M., Olejnik, K., & Mazela, B. (2022). Influence of nanocellulose structure on paper reinforcement. *Molecules, 27*(15), 4696.

Pezik, E., Gulsun, T., Sahin, S., & Vural, I. (2021). Development and characterization of pullulan-based orally disintegrating films containing amlodipine besylate. *European Journal of Pharmaceutical Sciences, 156*, 105597.

Rabe, A., Gesell Salazar, M., Michalik, S., Fuchs, S., Welk, A., Kocher, T., & Völker, U. (2019). Metaproteomics analysis of microbial diversity of human saliva and tongue dorsum in young healthy individuals. *Journal of oral microbiology, 11*(1), 1654786.

Raza, S. N., Kar, A. H., Wani, T. U., & Khan, N. A. (2019). Formulation and evaluation of mouth dissolving films of losartan potassium using 32 factorial design. *Int. J. Pharm. Sci. Res, 10*(3), 1402-1411.

Sahu, H., Alexander, A., Gupta, S., Yadav, P., Thapa, H., Banjare, T., Diwedi, S. D. (2018). Formulation and evaluation of risperidone loaded mouth-dissolving film. *Research Journal of Pharmacy and Technology, 11*(7), 2922-2925.

Serrano, D. R., Fernandez-Garcia, R., Mele, M., Healy, A. M., & Lalatsa, A. (2019). Designing fast-dissolving orodispersible films of amphotericin B for oropharyngeal candidiasis. *Pharmaceutics, 11*(8), 369.

Shakya, A. K., Al-Najjar, B. O., Deb, P. K., Naik, R. R., & Tekade, R. K. (2018). First-pass metabolism considerations in pharmaceutical product development. In *Dosage Form Design Considerations* (pp. 259-286): Elsevier.

Sheikh, F. A., Aamir, M. N., Shah, M. A., Ali, L., Anwer, K., & Javaid, Z. (2020). Formulation design, characterization and in vitro drug release study of orodispersible film comprising BCS class II drugs. *Pakistan journal of pharmaceutical sciences, 33*.

Singh, P., Mishra, G., & Dinda, S. C. (2021). Natural excipients in pharmaceutical formulations. *Evidence Based Validation of Traditional Medicines: A comprehensive Approach*, 829-869.

Soukoulis, C., Gaiani, C., & Hoffmann, L. (2018). Plant seed mucilage as emerging biopolymer in food industry applications. *Current Opinion in Food Science, 22*, 28-42.

Vimalson, D. C., Anbazhagan, S., Gokul, L., Idhayadharani, S., Ranjith, S., Sabarinathan, J., & Surya, R. (2019). Enhancement of solubility and dissolution characteristics of furosemide by solid dispersion. *World Journal of Pharmaceutical Research,11 (8),* 696-719.

Vishvakarma, P. (2018). Design and development of montelukast sodium fast dissolving films for better therapeutic efficacy. *Journal of the Chilean Chemical Society, 63*(2), 3988-3993.

Wang, B., Fu, X., Song, S., Chu, H. O., Gibson, D., Li, C., Wu, Z. (2018). Simulation and optimization of film thickness uniformity in physical vapor deposition. *Coatings, 8*(9), 325.

Zayed, G. M., Abd-El Rasoul, S., Ibrahim, M. A., Saddik, M. S., & Alshora, D. H. (2020). In vitro and in vivo characterization of domperidone-loaded fast dissolving buccal films. *Saudi pharmaceutical journal, 28*(3), 266-273.

Zhang, F., Mao, J., Tian, G., Jiang, H., & Jin, Q. (2022). Preparation and characterization of furosemide solid dispersion with enhanced solubility and bioavailability. *AAPS PharmSciTech, 23*(1), 65.