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Formulation and In-Vitro Evaluation of Fast Dissolving Oral Film of Furosemide Using a Combination of Natural and Synthetic Disintegrant.

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ABSTRACT

This study attempts to formulate and characterize *Lepidium sativum* and synthetic disintegrant based oral films of Furosemide prepared by solvent casting method. The films were prepared using HPMC as film-forming polymer, *Lepidium sativum* mucilage as a natural disintegrant, Crospovidone as synthetic disintegrant, and PEG-400 as plasticizer. Furosemide is a loop diuretic that prevents our body from absorbing too much salt. It is used to treat fluid retention (edema) in people with congestive heart failure, liver disease, or a kidney disorder such as nephrotic syndrome and also used in high blood pressure. It has poor solubility and oral bioavailability of 60%. Furosemide was formulated as a fast-dissolving film to optimize bioavailability by solubilizing fast. Evaluation parameters such as thickness, weight uniformity, folding endurance, drug content uniformity, in-vitro disintegration time, in-vitro dissolution test, surface pH were carried out and the results were found to be satisfactory. The surface pH of films was found to be 7.8±0.5. The in-vitro disintegration time of optimized formulation was found to be 16.7±4.80 (n=10) seconds and in-vitro dissolution studies of optimized formulation showed a drug release rate of 79.3504% within 20 minutes.

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Introduction

The oral route of drug delivery is the most preferred and accepted route by medical practitioners, manufacturers, and patients and it requires some advancement to increase compliance for a particular group of patients. Recent trends are shifting toward designing and developing innovative drug delivery systems for the already existing drugs. Out of those, the drug delivery system which is very eminent among pediatrics and geriatrics is orally disintegrating films (ODFs) (Bhyan *et al.*, 2015). Oral disintegrating film or strip can be defined as "A dosage form that employs water dissolving polymer which allows the dosage form to quickly hydrate by saliva, adhere to the mucosa, and disintegrates within few seconds, dissolves and releases medication for oromucosal absorption when placed on the tongue or oral cavity" (Bilal *et al.*, 2016).

Generally, super disintegrants are added to decrease the disintegration time which in turn enhances the drug dissolution rate (Bhusnure *et al.*, 2015). Many synthetic super disintegrants such ascrospovidone(Sumaiyah, Mentari and Suryanto, 2019), sodium starch glycolate, and croscarmellose sodium(Heer, Aggarwal and Kumar, 2014) (Jadhav and Chaudhari, 2016), have been used in the formulations of fast dissolving oral film. Similarly, natural super disintegrants such as *Musa paradisiaca* powder (Jain and Mundada, 2015) in oral films, *Plantagoovata* mucilage (Ghenge*et al.*, 2011), *Ocimum Sanctum* seeds (Malik and Singh, 2012) in fast dissolving oral tablets have been used earlier. Too little evidence was found regarding the use of natural disintegrants for the preparation of ODF. Moreover, a comparative study on the effect of natural and synthetic super-disintegrants in the formulation of fast dissolving oral films was lacking in earlier researches. Mucilage of natural origin is advantageous over semi-syntheticand synthetic substances due to its cost-effectiveness, non-toxic and non-irritable property, easy availability, eco-friendly, bio-degradable, and biocompatible nature.

Thus, in the present study, an attempt was made to examine the disintegrant property of the *Lepidium sativum* seed mucilage in the formulation of the thin film of furosemide. We compared the disintegration property of *Lepidium sativum* seed mucilage with that of synthetic disintegrant (crospovidone) and formulated the fast dissolving oral films by solvent casting method. Further, we evaluated the drug-loaded ODFs of Furosemide for physical appearance, weight variation, thickness, folding endurance, content uniformity, in-vitro disintegration, and dissolution studies.

The selected drug; Furosemide is a loop diuretic that acts on the kidney to ultimately increase the water loss from the body.It is most commonly used in edema secondary to various clinical conditions such as congestive heart failure, high blood pressure. Furosemide ODF is beneficial as there is ease of administration in patients who have difficulty in swallowing like the elderly, pediatric, bedridden patients, stroke victims, and psychiatric patients. Also, the absorption of drugs is prompt from the pre-gastric area like mouth, pharynx, and esophagus which show the rapid onset of action. Stability is enhanced for a longer duration of time since the drug remains in solid dosage form until it is consumed. So, it combines the advantage of the solid dosage form in terms of stability and liquid dosage form in terms of bioavailability (Bhyan *et al.*, 2015).

Materials and Methods

Materials and Equipments

Furosemide was received as a gift sample from 'Lomus Pharmaceuticals Pvt. Ltd.', (Kathmandu, Nepal). Crospovidone was kindly gifted by Nepal Pharmaceuticals Pvt. Ltd. (Jeetpur, Parsa, Nepal). *Lepidium sativum* was bought from the local market. HPMC E5 and PEG400 were provided by Kathmandu University (Dhulikhel, Nepal). All other chemicals and reagents used were of analytical grade. All the instruments used in the research were made available by Kathmandu University.

Extraction of *Lepidium sativum* Mucilage

100 gm of seeds of *Lepidium sativum* was soaked in 1000ml of water for 24 hours and homogenized for 5 minutes using a homogenizer at 2000 rpm. The concentrate was squeezed through muslin cloth for filtering and separating the seeds.The filtrate was isolated with acetone

by forming a yellowish-brown precipitate. The precipitate was filtered using a sieve and dried in a hot air oven (Hicon instruments); at a temperature of about 45˚C till it was completely dried. Hard mucilage cake was obtained which was ground and sieved through sieve size 60, stored in desiccators (Bhatia *et al.*, 2014).

Preparation of Fast Dissolving Film

Furosemide films were prepared by using the solvent casting method. Water-soluble polymer was soaked in water along with the plasticizer and disintegrant. The active pharmaceutical ingredient was dissolved in 0.2 N NaOH. Both the mixture were mixed for 4 hours using a magnetic stirrer(Spectralab), in300 RPM and then allowed to stand for about 1 hour to remove the entrapped bubbles. The bubble-free solution was cast on the Petri plate and then left for air drying for 48 hours. The film was carefully removed from the surface of the Petri dish and cut into a dimension of (2×2) cm in size. The amount of drug added was calculated based on the area of the Petri dish so that each dosage consists of 20 mg of Furosemide.

Dose Calculation for Fast Dissolving Oral Film of Furosemide (Per Petri plate)

Radius of petriplate = 8.9/2 = 4.45 cm

Area of petriplate = $\pi r^2 = \pi*(4.45)^2 = 62.18$ cm^2

Area of the film= 4 cm^2

Number of 4 cm^2 film present in whole film= 62.18/4= 15.55

Each film contains 20 mg furosemide

15.55 film contains= 15.55*20mg= 311 mg drug

Identification and Characterization of Mucilage

Characterization of *Lepidium sativum* mucilage (Bhatia *et al.*, 2014)

1. Solubility- Solubility of the extracted mucilage was determined qualitatively by stirring 100 mg of *Lepidium sativum* mucilage powder in 50ml of water and acetone(Malviya, 2011)
2. pH determination- 1gm of *Lepidium sativum* mucilage powder was dissolved in 100 ml water and pH was determined using pH meter (Hanna instruments PH211).(Malviya, 2011).
3. L.O.D- 1gm of the sample was dried at 105˚C for 2 hours in a hot air oven.
4. Swelling index- 0.5 gm of dried mucilage was placed in 50 ml of measuring cylinder and initial bulk volume was noted. Water was added up to 50 ml mark and left for 24 hours at room temperature. Then the sediment volume of swollen mass was noted.

Identification tests of mucilage(Bhatia *et al.*, 2014)

1. Molisch Test- 100mg of *Lepidium sativum* mucilage powder was dissolved in a few ml of water and few drops of Molisch reagent were added along with H_2SO_4. Violet color indicates the presence of carbohydrates.
2. Iodine Test- 0.5 gm of powder was dissolved in 25ml of water.1ml of Iodine solution was added to it. Blue color indicates the presence of starch.
3. Ninhydrin Test- About 2-3 drops of Ninhydrin solution was added tothe sample solution. Violet color indicates the presence of protein.
4. Biuret Test- About 2-3 drops of Biuret solution was added on sample solution. The absence of colour indicates the presence of protein.

Evaluation of films

Physical appearance and surface texture-

This character was checked simply with a visual inspection of films and evaluation of texture by feel or touch.(Sarojini *et al.*, 2016)

Thickness-

It was measured using by using the calibrated digital Vernier Calipers (Mitutoyo) at four corners and center then mean value was calculated that determines the thickness of the film.(Bala *et al.*, 2013)

Weight Variation-

It was measured by taking 20 films of each formulation. The weights of films taken by weighing in an electronic digital balance (BEL Engineering/ Model:M214Ai). Mean weight and standard deviation were calculated.(Dova *et al.*, 2018)

Folding Endurance-

This is measured by taking ten films of each formulation and subjected test by folding the film at the same place repeatedly several times until a visible crack was observed. Then the mean and

standard deviation were calculated (Surendran and Vidyapeetham, 2018).

Content Uniformity and Assay

The films were tested for drug content uniformity by using UV visible spectrophotometric method. 10 films were taken from each formulation in different 100 ml volumetric flask and was dissolved using 0.1N NaOH and the resultant solution was filtered. 2.5 ml from each filtered solution was transferred to 50 ml volumetric flask and the volume was made up to mark. The absorbance of the resulting solution was measured at 270 nm against the blank in the UV spectrophotometer (Shimadzu UV-1800/SN-A 11454806352). The percentage of drug content was determined using the standard graph. The mean and standard deviation were calculated.

***In-Vitro* Disintegration time**

This test was carried out by checking the disintegration time of the film in the Petri plate of 10 ml phosphate buffer of pH 6.8 by swirling for every 10sec. The obtained time is the time film starts to break or disintegrate. Mean and standard deviation were calculated.(Joshi *et al.*, 2012)

***In-Vitro* Dissolution Study**

Dissolution studies of films were performed by using the USP I apparatus (Electrolab TDT-08L) in phosphate buffer of pH 6.8. The amount of phosphate buffer taken was 900 ml kept at the temperature of (37±0.5˚C) at 50 RPM. 5ml sample from each vessel was taken at 2 minutes time interval for 20 minutes and absorbance was measured using a UV spectrophotometer. (Pednekar *et al.*, 2012)

Result and Discussion

***Lepidium sativum* mucilage**

Mucilage of *Lepidium sativum* was soluble in water and has good swelling property with a swelling index of 11.5. pH of mucilage was found to be 5.52 and LOD was found to be 11%. Molisch test, Iodine test, Ninhydrin test, and Biuret test were performed and the results are given in Table 1.

Table 1: Identification tests for mucilage

Test	Observation	Result
Molisch	Violet color was observed	Presence of carbohydrate
Iodine	Blue color was not observed	Absence of starch
Ninhydrin Test	Violet color was observed	Presence of protein and nitrogen compound
Biuret Test	No color was observed	Presence of protein

Optimization of Film

Placebo films containing film-forming polymer HPMC E5 and plasticizer PEG 400 was prepared. Various trials were taken to formulate the fast-dissolving film where the polymer and plasticizer at different concentrations (Table 2).

Table 2: Optimization of Polymer and Plasticizer

Formulation	HPMC E5(mg)	PEG-400(ml)
F01	295	0.19
F02	295	0.19
F03	295	1.25
F04	295	1.25
F05	235.6	1.25
F06	235.6	1.25

Fig. 1: Dried Mucilage Powder

Fig. 2: Film Loaded with Drug

Fig.3: Film Cut into 2x2 cm^2

The formulation F01 and F02 have good appearance and is peelable than other formulation and hence selected. Thirteen formulations with natural and synthetic disintegrant were designed using the two-factor two levels central composite design. Thirteen formulations were prepared incorporating natural disintegrant *Lepidium sativum* and synthetic disintegrant; crospovidone was prepared and then disintegration time, folding endurance, and thickness were evaluated as shown in table 3.

Table 3: Optimization of Placebo Film incorporating Disintegrant

Formulation	Natural DT (%)	Synthetic DT (%)	Disintegration time (sec)	Thickness	Folding endurance
1	0.343145751	6	80	0.1993± 0.05957	50
2	10	10	362.33	0.182 ± 0.04003	290
3	6	6	325.67	0.1646 ± 0.0309	380
4	6	6	344.33	0.158 ± 0.02426	>300
5	11.65685425	6	336	0.1586 ± 0.02559	>300
6	10	2	367.67	0.132 ± 0.02596	>300
7	6	0.343145751	302	0.1153± 0.02099	>300
8	6	6	248.67	0.1873 ± 0.02789	236
9	2	10	188.6	0.1933 ± 0.03618	91
10	6	6	254	0.1733 ± 0.02468	>300
11	6	11.65685425	221.33	0.252 ± 0.034267	143
12	2	2	93	0.1646 ± 0.02294	160
13	6	6	236	0.1993 ± 0.02186	>300

These formulations didn't show a desirable result. The optimized quantity of natural and synthetic disintegrant was obtained from the contour plot. Based on the contour and surface plot, the optimized quantity of natural and synthetic disintegrant was found to be 0.4% (0.0049mg) and 0.3%(0.0037mg) respectively. The optimized quantity of natural and synthetic disintegrant was used alone as well as in combination to prepare a film and disintegrant time was evaluated (Table4).

Table 4: Disintegration time of Natural and Synthetic Disintegration

	Disintegration time (Sec)			Average(Sec)	SD
Natural Disintegrant	44	51	41	45.3333	5.131601
Synthetic Disintegrant	17	21	14	17.3333	3.511885
Combination	23	23	20	22	1.732051

The disintegration time of combination was less than 30 secs but the disintegration time of film incorporating natural disintegrant is more than 30 secs. *Lepidium sativum* mucilage also acts as a binder and hence retard the disintegration time. Hence, to obtain enhanced disintegration time, oral film was prepared by using both the natural and synthetic in combination (Table 5).

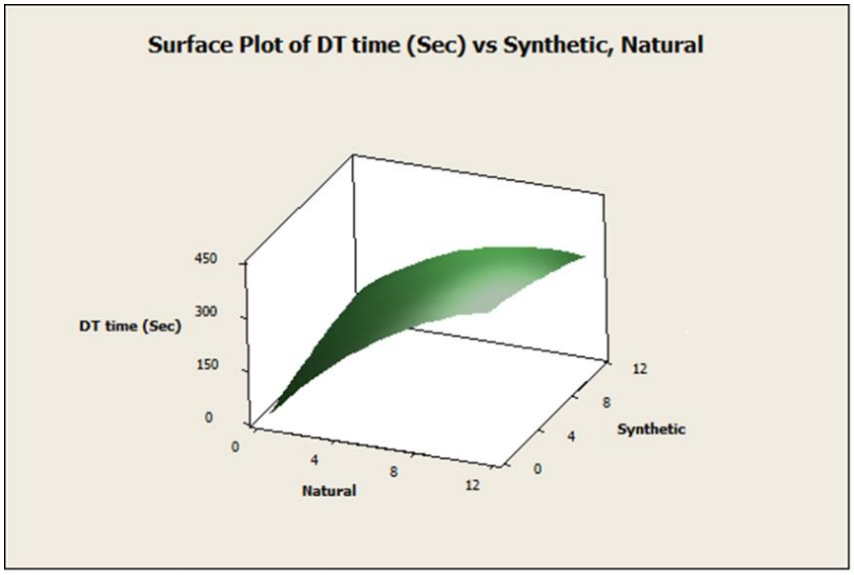

Fig.4: Surface Response curve of DT time (sec) vs. Synthetic, Natural Disintegrants

Table 5: Optimized Formulation

S.no	Materials	Quantity
1	HPMC	326.26 mg
2	PEG 400	0.19 ml
3	Furosemide	311mg
4	*Lepidiumsativum*	0.0049mg
5	Crospovidone	0.0037mg
6	0.2 N NaOH	5ml
7	Water	Q.S

Evaluation of Film

The thickness of the optimized film was measured using a digital vernier caliper given in table 6. All the films were almost uniform with very low deviation in the thickness and values ranges from 0.104 mm to 0.120 mm.

Table 6: Data for the Thickness of the Optimized Film

	Thickness(mm)						
S. No.	Corner 1	Corner 2	Corner 3	Corner 4	Center	Average	SD
1	0.12	0.10	0.11	0.10	0.11	0.108	
2	0.10	0.11	0.11	0.11	0.12	0.110	
3	0.12	0.12	0.13	0.11	0.12	0.120	
4	0.10	0.11	0.11	0.10	0.10	0.104	
5	0.12	0.11	0.11	0.10	0.13	0.114	0.004971
6	0.11	0.11	0.10	0.09	0.12	0.106	
7	0.11	0.09	0.10	0.11	0.12	0.106	
8	0.12	0.11	0.10	0.13	0.11	0.114	
9	0.11	0.12	0.09	0.09	0.12	0.106	
10	0.11	0.09	0.11	0.11	0.12	0.108	

The weight of the prepared films was determined using the analytical balance given in table 7. All the films are within range of 45.5 mg to 56.7 mg indicates that all the films are uniform in weight with minimum standard deviation.

Table 7: Data of Weight Variation of Optimized Films

Weight of 20 films (2×2 cm^2) (mg)			
49.9	48.8	49.2	51.2
49.3	51.4	50.8	52.2
55.9	48.4	56.7	52.5
51.5	51.1	48.1	45.5
50.4	55.8	47.6	47.7

Average = 50.7

Standard Deviation = 2.923048157

The in-vitro disintegration time was determined using 10 randomly selected films in phosphate buffer pH 6.8 and tabulated in table 8. The disintegration time of the formulations was within the range of 10 to 26 sec fulfilling the requirement.

The surface pH was found to be 7.8±0.5 which is near to neutral pH. This suggests that it doesn't irritate the mucosal lining of the oral cavity.

Folding endurance was found to be greater than 300.

Table 8: Data of Weight Variation of Optimized Films

No. of films	**Disintegration Time(sec)**	**Average**	**SD**
1	26		
2	21		
3	16		
4	15		
5	10	16.7	4.8085
6	11		
7	18		
8	13		
9	19		
10	18		

Content Uniformity of the optimized film was determined using the method validated in 0.1 N NaOH and results are tabulated in Table 9. The drug content ranges from 96.221 ± 0.913 to 106.005 ± 1.575.

Table 9: Data for content Uniformity of Optimized Film

No of film	**% content (n=1)**	**% content (n=2)**	**%content (n= 3)**	**Average**	**SD**	**Average SD**
1	100.264	99.385	98.682	99.443	0.792	
2	101.494	103.954	103.954	103.134	1.421	
3	99.209	99.209	97.276	98.565	1.116	
4	95.694	95.694	97.276	96.221	0.913	
5	99.561	100.439	99.209	99.736	0.634	1.154
6	96.924	97.979	98.858	97.920	0.968	
7	99.561	100.615	105.185	101.787	2.989	
8	105.009	105.185	107.821	106.005	1.575	
9	98.330	98.330	98.506	98.389	0.101	
10	99.385	97.803	97.452	98.213	1.030	

Assay of the optimized film was determined by the method validated in 0.1 N NaOH and results are tabulated in Table 10. The assay percentage was found to be 97.686% to 97.803%.

In-vitro drug release of the fast dissolving film was carried out. The plot of %cumulative drug release vs. time plotted is shown in fig.5. From the in vitro dissolution data, it was found that drug release of the fast dissolving film formulation was found to be 42.23% at 2 minutes and 79.35% at 20 minutes. However approximately 80% drug release within 20 minutes.

Table 10: Data for assay of Optimized Film

No.of film	%content (n=1)	%content (n=2)	%content (n=3)	Average	SD	Average SD
1	97.452	98.330	97.627	97.803	0.465	0.892
2	99.209	96.924	96.924	97.686	1.319	

Table 11: Data for Dissolution test of Optimized Film

Time	% cumulative drug release
2	42.2250
4	55.0720
6	57.5766
8	60.7344
10	70.1139
12	70.2795
14	75.3341
16	76.5190
18	76.5392
20	79.3504

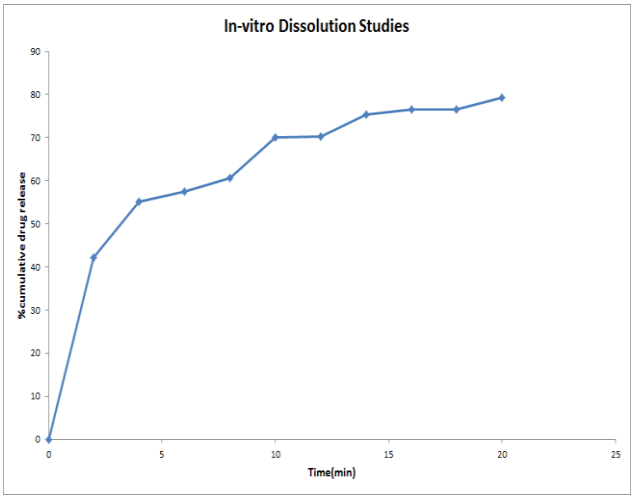

Fig. 5: *In-vitro* Dissolution Studies

Conclusion

In the present study, the disintegrant properties of *Lepidium sativum* mucilage, Crospovidone, and the combination of both disintegrants in the oral film have been explored. The main drawback of synthetic superdisintegrants is that they are usually toxic, expensive, come with various environmental issues and incompatibility problems (Khawnekar *et al.*, 2014). The film with the combination of *Lepidium sativum* mucilage and Crosspovidone disintegrated much faster compared to the use of natural disintegrant alone. Therefore, the study concludes that a combination of *Lepidium sativum* mucilage and Crosspovidone can be successfully used in the preparation of fast dissolving oral film of furosemide.Through our study, we found that the mucilage of *Lepidium sativum* has good swelling property and is water-soluble.

Conflict of Interest

The authors declare no conflict of interest.

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