Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Research Article

Design, Development and Evaluation of Fast Dissolving Buccal Film of Norethisterone.

Pratima Pokale*, Prashant Khade, Ashok Bhosale.

Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune-14, Maharashtra, India.

Received 07 June 2019; received in revised form 23 July 2019; accepted 27 July 2019

***Corresponding author E-mail address:** *pratima.tengle@gmail.com*

ABSTRACT
The aim of the present investigation was to design, develop and evaluate, fast dissolving buccal film of Norethisterone with an objective to enhance the bioavailability, avoid loss and improve therapeutic efficacy. The films were prepared using HPMC E5 and PVP as a polymer, Propylene glycol as a plasticizer, by solvent casting method. Prepared films were evaluated for their weight uniformity, thickness, surface pH, drug content uniformity, disintegration test, folding endurance, in vitro drug release and in vitro permeability studies.

KEYWORDS
Buccal, Bioavailability, Therapeutic efficacy, HPMC E5, PVP, Norethisterone.

1. INTRODUCTION

The oral route of drug administration is the most important method of administering drugs for systemic effect. The parenteral route is not routinely used for self administration of medication. It is probable that at least 90 % of all drugs used to produce systemic effects are administered by the oral route [1].

The oral cavity is an attractive site for the delivery of drugs either locally or directly into the systemic circulation [2]. The use of the oral cavity membranes as sites of drug administration has been the topic of increasing interest for the past decade. It is well known that the absorption of therapeutic compounds from the oral mucosa provides a direct entry of the drug into the systemic circulation, thereby avoiding first-pass hepatic metabolism and gastrointestinal drug degradation, both of which are associated with per oral administration [3].

For drug delivery purpose, the term bioadhesion implies attachment of a drug carrier system to a specific biological location. The biological surface can be epithelial tissue. If adhesive attachment is to a mucus coat, the phenomenon is referred to as mucoadhesion. Hence a bacterial attachment is to tissue surfaces, and mucoadhesion can be modelled after the adherence of mucus on epithelial tissue. Mucoadhesion is the relatively new and emerging concept in drug delivery. Mucoadhesion keeps the delivery system adhering to the mucus membrane.

Out of the various sites available for mucoadhesive drug delivery, buccal mucosa is the most suited one for local as well as systemic delivery of drugs. Its anatomical and physiological features like presence of smooth muscles with high vascular perfusion, avoidance of hepatic first pass metabolism and hence can potentially improve bioavailability are the unique features which make it as an ideal route for mucoadhesive drug delivery. Moreover these dosage forms are economic and patient friendly [5].

Norethisterone or Norethindrone, 19-nor-17α-ethynyltestosterone, Fig. 1, is a progesterone used to treat premenstrual syndrome, painful periods, abnormal heavy bleeding, irregular periods, menopausal syndrome (in combination with estrogen), or to postpone a period. It has very low bioavailability (46-65%) due to hepatic first pass metabolism [6]. Hence to improve its therapeutic efficacy and bioavailability the drug may be administered by buccal route through buccal film. Buccal delivery of norethisterone may circumvent hepatic first pass metabolism and improve bioavailability. Hence the present work deals with the formulation and characterization of fast dissolving buccal film of norethisterone using polymers hydroxypropylmethyl cellulose E5 (HPMC E5) and polyvinyl pyrrolidone (PVP).

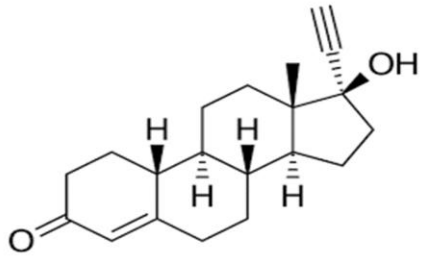

Fig. 1. Structure of Norethisterone.

2. MATERIALS AND METHODS

2.1. Materials [7-12]

Pharmaceutical grade of Norethisterone was procured from Horster biotech pvt. ltd., Indore. HPMC E5 was obtained as Gift sample from Omni Active Health Technologies PVT.LTD, Thane. PVP was procured from Sample from Research- lab Fine Chem Industries, Mumbai.

2.2. Calibration curve of NE in 6.8 pH phosphate buffer solution

UV scanning and standard calibration of Norethisterone was performed by using Shimadzu double beam UV Spectrophotometer. The maximum absorption wavelength was recorded in methanol and slope was determined in phosphate buffer 6.8 and 7.4.

2.3. Preparation of standard solution

10 mg of NE was dissolved in 50 ml of methanol to give a concentration of 1 µg/ml.

2.4. Preparation of stock solution

From standard solution take 0.2, 0.4, 0.6, 0.8, 1 ml of solution in 10 ml of volumetric flask. The volume was made up to mark with methanol to produce concentration as 2, 4, 6, 8, 10 µg/ml of NE respectively. The absorbance of prepared sample of NE was measured at 240 nm in Schimadzu UV spectrophotometer against methanol as blank. By using same procedure Calibration curve of NE in PBS pH 6.8 and pH 7.4 was plotted.

Beers and Lamberts range: 2-10 µg/ ml.

The absorbance: λ max 240 nm.

2.5. Drug- Excipient compatibility studies [13-19]

The infrared absorption spectra of pure drug, pure polymer and physical mixture of polymer and drug were performed for polymer drug interaction studies between 4000 $cm^{-1}$to 400 $cm^{-1}$by KBr pellet method.

DSC study of pure drug, pure polymer and physical mixture of polymer and drug were performed.

2.6. Dose Calculation

Radius of petri plate: 3.9 cm

Area of petri plate: πr^2=3.14×3.9×3.9=47.75 cm^2 ---Equation (1)

Now, Dose is 5 mg and cut the pieces in 2 cm× 2 cm= 4 cm^2

4 cm^2 films contain 5 mg of drug

No. of patches= area of petriplate/ size of the square film = 47.75/4=11.93 ---Equation (2)

So, 47.75 cm^2 contain (?) Drug

Total amount of the drug dose= no. of patches× dose=11.93×5=59.65 mg. ---Equation (3)

Hence 11 films of 2 X 2cm dimensions can be prepared from 47.75cm^2

Each film contains 5 mg of Norethisterone
So 11.93 no. of films contains 60 mg of NE
The amount of Norethisterone can be loaded≃ 60 mg

2.7. Method of Preparation of NE buccal films

The Orally Disintegrating films were prepared by solvent casting technique. Various polymers were used as film forming polymer. Film forming polymer was dissolved in methanol and dichloromethane (1:1) solvent and API was added in to it. Mixture was stirred continuously by adding plasticizer. The solution was poured in to petri plate and dry at room temperature for 48 hrs. Then the film was carefully removed and cut into suitable size i.e. 2 x 2 cm. The composition of buccal film is shown in Table 1.

Table 1. Formula for preparation of buccal films.

Ingredients	**Formulation code**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Norethisterone(mg)	60	60	60	60	60	60
HPMC E 5(mg)	240	300	360	-	-	-
PVP(mg)X	-	-	-	240	300	360
PG (ml)	36	45	54	36	45	54
Methanol: Dichloromethane (1:1) (ml)	20	20	20	20	20	20

(PG= 15 % w/w of polymer)

The prepared buccal film were evaluated for various properties like visual inspection, Weight variation, Surface pH study, Thickness, Folding endurance, Disintegration time, Drug content uniformity, In vitro drug release study, In vitro permeability study, Stability study.

2.8. Evaluation of Fast dissolving films

2.8.1. Visual Inspection

Visual inspection of the prepared orally disintegrating film gives information about colour, homogenecity and transparency.

2.8.2. Weight Variation

All films (area 4 cm2) were weighed and weights are recorded. It was done to ascertain the uniformity of weight of films.

2.8.3. Surface pH

The pH value of a film is usually determined by putting the prepared film in petri dish and subsequently film is made wet by using distilled water and noting pH by touching the film surface with a pH meter electrode. Determination of surface pH is vital as acidic or basic pH is liable to cause oral mucosal irritation.

2.8.4. Thickness

As the thickness of a film is directly concern with drug content uniformity so it is necessary to ascertain uniformity in the thickness of the film. It can be measured by micrometer screw gauge or calibrated digital Vernier caliper at different strategic locations. The thickness of the film should be in the range 5-200 µm.

2.8.5. Folding Endurance [21]

Folding endurance is another procedure to estimate the mechanical properties of a film. It is measured by repeatedly folding a film at the same point until it breaks. Folding endurance value is number of times the film is folded without breaking. Higher folding endurance value depicts the more mechanical strength of a film. A direct relation exists between mechanical strength and folding endurance of films. As mechanical strength is governed by plasticizer concentration so it is clearly evident that plasticizer concentration also indirectly affects folding endurance value.

2.8.6. Disintegration Time

Normally, the disintegration time is the function of composition of film as it varies with the formulation. There are no official guidelines available for determining disintegration time of orally fast disintegrating films. The following method was used for determining disintegration time of film:

2.8.7. Petri dish method

A film is placed onto 2 ml distilled water taken in petri dish. Time taken by the film to disintegrate completely is considered as the disintegrating time.

2.8.8. Drug Content Uniformity [20]

One film is dropped into a 100 ml of volumetric flask Then about 50 ml of PBS (pH 6.8) is added and dissolved with sonication then volume is made up to 100 ml with PBS (pH 6.8. the solution is filtered First few ml of the filtrate is discarded. Then 0.5 ml of the filtrate is pipette out and diluted up to 10 ml of PBS (pH 6.8). So as to get 5 µg/ml final concentrations. The absorbance of final solution of test and standard NE solution were compared at 246 nm using phosphate buffer pH 6.8 as a blank using UV spectrophotometer.

2.8.9. In vitro dissolution study [21]

In vitro dissolution studies were carried out using USP type II apparatus. pH 6.8 buffer (900 ml) was used as dissolution medium. Samples were filtered through 0.45 µm whatmann filter paper and analysed spectrophotometrically at max of 246 nm. In vitro dissolution studies were performed in triplicate for all batches.

2.9. Ex vivo drug permeation [21]

In this study, sheep buccal mucosa was as a barrier membrane. Diffusion studies were carried out, to evaluate the permeability of drug across the sheep buccal mucosal membrane, by using glass surface Franz diffusion cell. Sheep buccal mucosa was obtained from local slaughter house and used within 2 hrs. of slaughter. The tissue was stored in phosphate buffer pH 7.4 solution upon collection. The epithelium was separated from underlying connective tissues with surgical scissors clamped between donor and receiver chamber of diffusion cells for permeation studies. The smooth surface of mucosa should face the donor chamber and receiver chamber was filled

with phosphate buffer of 7.4 pH. Whole assembly was placed in water bath maintained at 37±10C. Hydrodynamic in receiver chamber was maintained by stirring with magnetic bead at 50 rpm. After the stabilization of buccal epithelium, the patch was kept on buccal epithelium and 4ml of phosphate buffer of 6.8 pH was added in donor chamber. The sample of 1 ml were withdrawn at the time interval of 2 min. up to 30 min. and replaced with equal volume of fresh dissolution medium. The sink condition was maintained throughout the study. The withdrawn sample was diluted to 5ml. The amount of NE was determined by UV Spectrophotometer at 248nm.

2.10. Stability Studies

Stability of product may be defined as the capability of a particular formulation in a specific container/ closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specification. The optimized formulation packed in aluminum foil at 40±2o C/ 75±5 % RH for 45 days in stability chamber. The samples were withdrawn initially, after 15 days, 30 days and 45 days and analysed for in vitro evaluation parameters.

3. RESULTS AND DISCUSSION

3.1. Calibration curve of NE solvent methanol

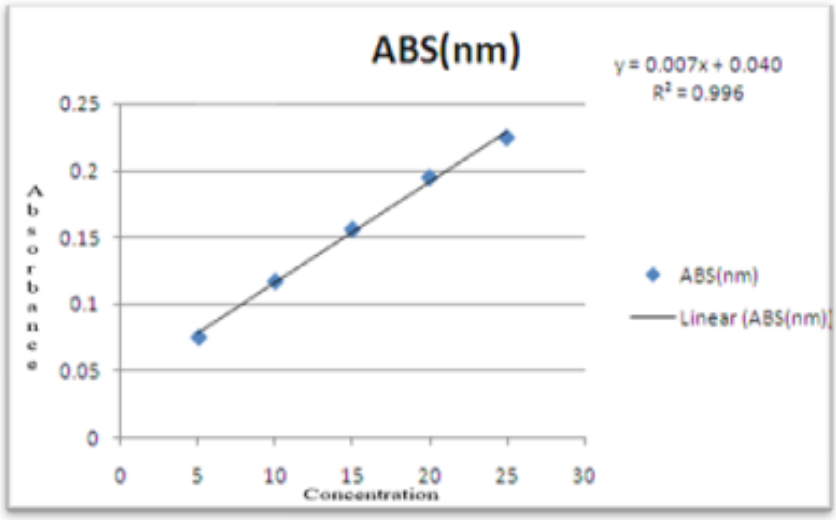

Fig. 2. Calibration curve of NE solvent methanol.

3.2. Spectral studies

The observed peak and their functional group are given in Table 2. The IR spectrum (Fig. 3) of NE showed similar characteristics peaks to that of reported IR spectrum of norethisterone. From the FTIR study the sample was authenticated. The IR spectra of pure NE drug showed the characteristic absorption bands and drug-polymer interaction not observed in the FTIR spectra of the powder mixture of drug and polymers (HPMC E5). Since it is confirmed that the drug and polymers (HPMC E5) are compatible with each others.

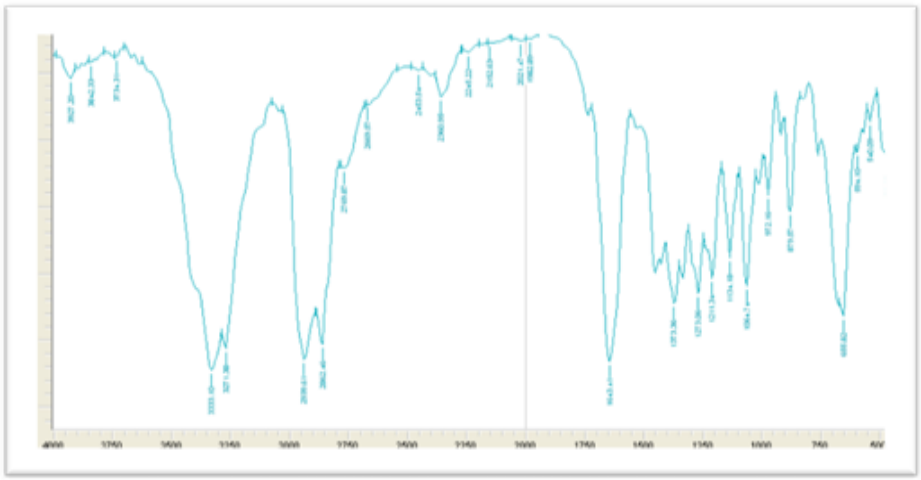

Fig. 3. IR spectra of NE.

Table 2. Functional group and observed peak value of NE.

Functional Group	Peak Range (cm^{-1})	Observed Peak (cm^{-1})
C=C Stretching (aromatic)	1680-1600	1643
C-H Stretching(aromatic)	3000-2850	2939
C-H Bending (alkyl)	1465	1373
C=O Stretching (ketone)	1350-1000	1273
N-H Stretching (amino)	3500-3100	3271

Table 3. Functional group and observed peak value of NE+ HPMC E5.

Functional Group	Peak Range (cm^{-1})	Observed peak (cm^{-1})
C=C Stretching (aromatic)	1680-1600	1651
C-H Stretching(aromatic)	3000-2850	2939
C-H Bending (alkyl)	1465	1442
C=O Stretching (ketone)	1350-1000	1134
N-H Stretching (amino)	3500-3100	3271

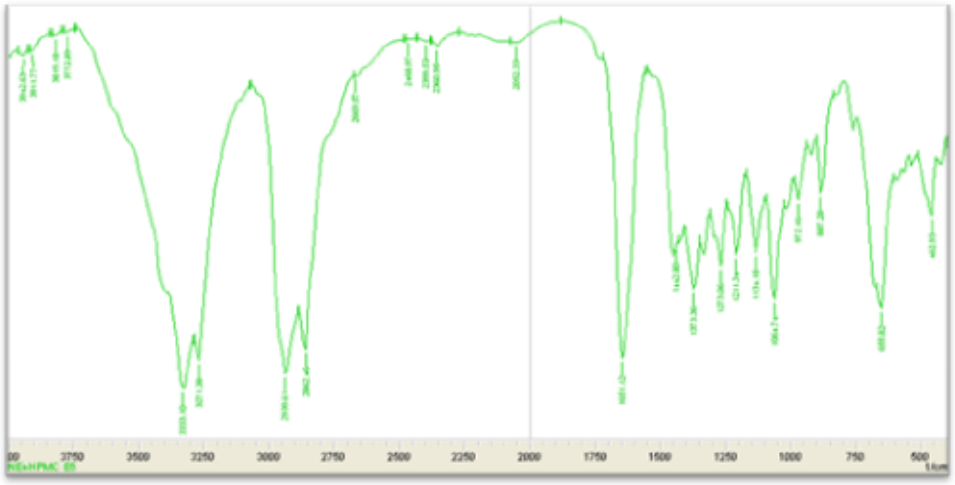

Fig. 4. IR spectra of NE+ HPMC E5.

3.3. DSC study

The DSC thermogram of norethisterone (Fig. 3) represents sharp endothermic peak at 208.2^{o}C which corresponding to melting of NE ranging from 202-208^{o}C respectively. The DSC thermogram of pure NE drug showed the characteristic melting point range and drug-polymer interaction not observed in the DSC thermogram of the powder mixture of drug and polymers (HPMC E5). Since it is confirmed that the drug and polymers (HPMC E5) are compatible with each others.

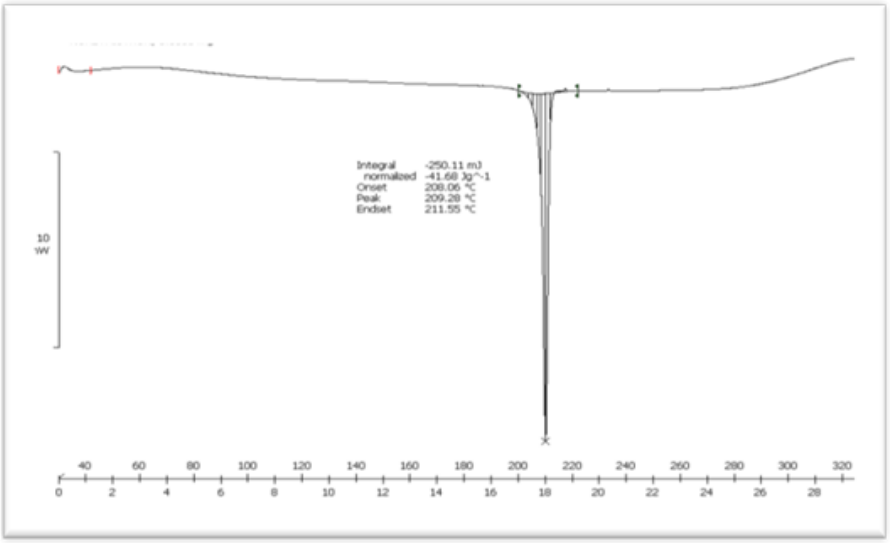

Fig. 5. DSC Thermogram of NE

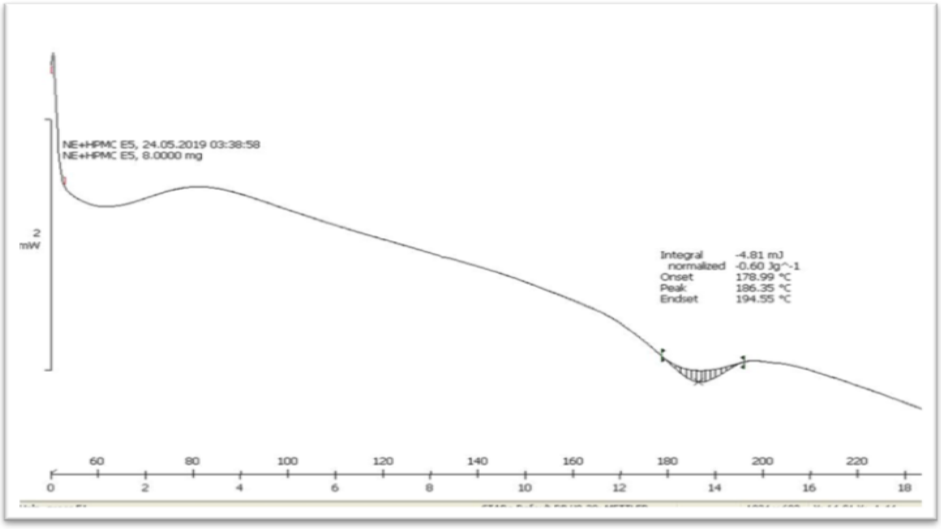

Fig. 6. DSC Thermogram of NE and HPMC E5.

3.4. Visual Inspection

Visual inspection of a prepared fast orally dissolving film gives information about color, homogeneity and transparency. The prepared film was white in color, homogenous and non-transparent.

3.5. Weight Variation

All the batches were evaluated for weight of the film. The weight of the films was found to be in range of 40-60mg. From the result it is concluded that as the concentration of polymer increases, weight of the film also increases.

3.6. Surface pH Study

The surface pH values of formulation are given in Table no. 10.5.2. All the polymers resulted in formulation that has neutral pH. Surface pH of the film was ranging from 6.8 to 7. The neutral values of surface pH of films assured that there will be no irritation on mucosal lining oral cavity.

Table 4. Physical evaluation parameters of the buccal film.

Parameters	**Formulation code**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Thickness	0.9±0.5	0.9±0.5	0.9±0.5	0.9±0.5	0.9±0.5	0.9±0.5
Folding Endurance	129±2.44	129±2.44	129±2.44	129±2.44	129±2.44	129±2.44
Disintegration Time (sec)	140±0.48	140±0.48	140±0.48	140±0.48	140±0.48	140±0.48
%Drug Content	97.8±0.32	97.8±0.32	97.8±0.32	97.8±0.32	97.8±0.32	97.8±0.32

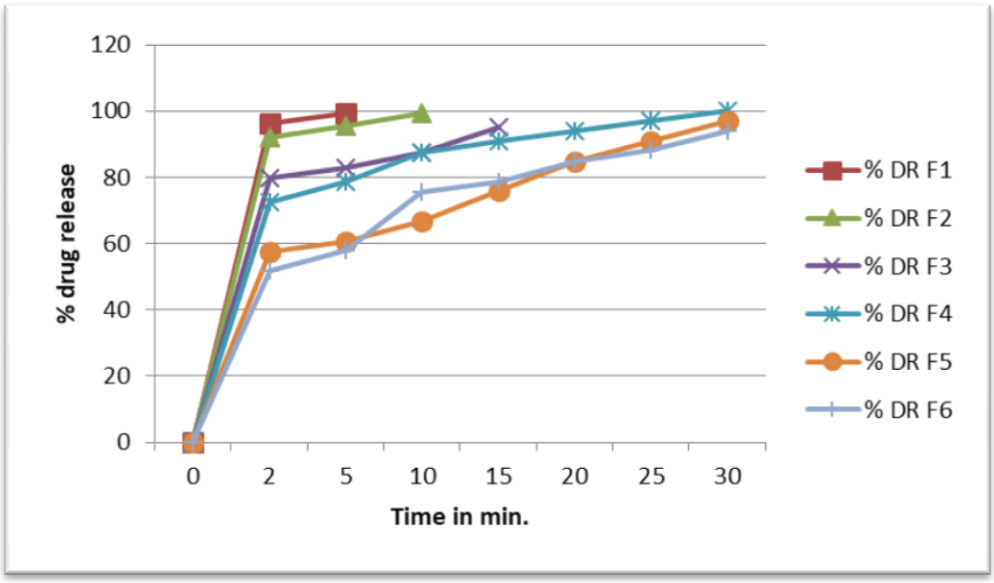

Fig. 7. % Drug release of formulations.

Table 5. Dissolution profile for formulations F1-F6.

Time (min)	% Drug release of formulations F1 to F6					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
0	0	0	0	0	0	0
2	96.147	92.204	79.880	72.486	57.698	51.738
5	99.319	95.366	83.016	78.563	60.784	57.813
10	-	99.521	87.636	87.610	68.814	75.687
15	-	-	95.224	90.761	75.855	78.813
20	-	-	-	93.920	84.896	84.902
25	-	-	-	97.084	90.998	88.047
30	-	-	-	100.56	97.114	94.156

Table 6. % permeability data of formulation F1 to F6.

Time (min)	**% Drug permeated of formulations F1 to F6**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
0	0	0	0	0	0	0
2	65.6	67.2	62.4	44	44.4	48
5	87.2	72.8	69.6	48	45.6	56.8
10	-	81.6	80	53.6	49.6	62.4
15	-	88	86.4	56.8	60	67.2
20	-	-	88	61.6	66.4	72
25	-	-	-	68	72	76.8
30	-	-	-	71.2	75.2	79.2

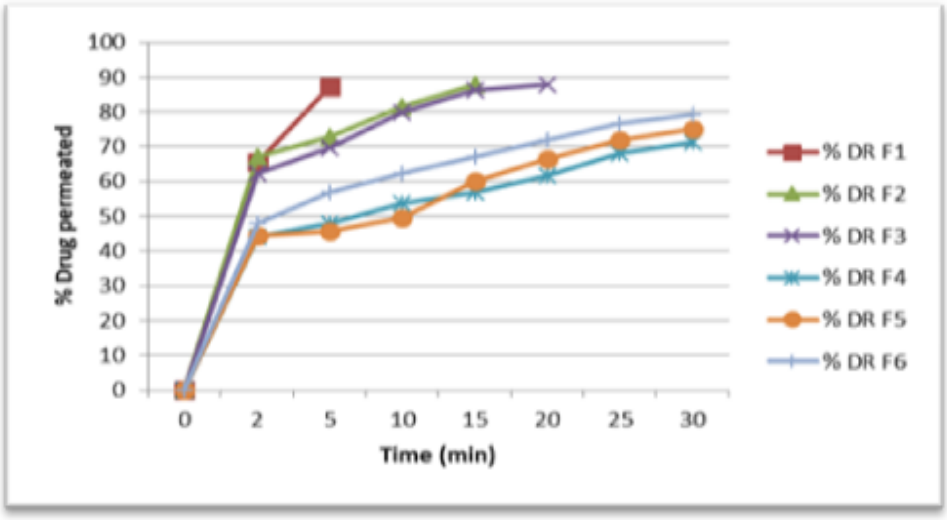

Fig. 8. % permeability data of formulation F1 to F6.

Table 7. Stability studies of optimized formulation (F1).

Parameters	**Initials**	**After 10 days Stability study**	**After 20 days stability study**	**After 30 days stability study**
Folding endurance	129±2.44	129±0.40	128±1.08	127±1.40
Surface pH	6.8±0.04	6.9±0.42	6.9±.043	6.9±0.04
Drug content (%)	97.82±0.32	97.79±0.24	96.72±0.34	96.51±0.21
% Drug release	99.31±1.58	99.02±1.32	98.90±1.13	98.82±1.15
Disintegration time (sec)	140±0.48	141±0.45	142±0.41	142±0.41

4. CONCLUSION

The 4, 5, and 6 % w/w HPMC E5 and PVP films were prepared by solvent casting method. Compatibility of NE with polymers was confirmed by FTIR and DSC studies. Six films are evaluated for weight variation and thickness showed satisfactory results. Mouth dissolving time and disintegration time of the films were increased with increase in the concentration of the polymers, as more fluid is required to wet the film in the mouth. Content uniformity study showed that the drug is uniformly distributed in the film. Present study reveals that all the six formulated films showed satisfactory film parameters.

It can be concluded that, fast dissolving film containing NE can be prepared by solvent casting method. 4% w/v of HPMC E5 film exhibited required folding endurance, disintegration time and in vitro dissolution study. Formulation F1 disintegrated in 140 sec and released 99.31 % of drug within 5 min and considered as best formulation.

From the present investigation it can be concluded that the drug NE could be successfully incorporated in the orally fast dissolving films with the help of HPMC E5 as film former and plasticizer.

5. REFERENCES

1. Hooda R., Tripathi M., Kapoor K. (2011). A review on oral mucosal drug delivery system. *The Pharma Innovation.* 1, 1, 14-21.
2. Rathbone M.J., Senel S., Pather I. (2015). Oral mucosal drug delivery and therapy. *Advances in Delivery Science and Technology.* 16, 17-22.
3. Varanasi S.N., Rani P.A., Kumar D.G. (2013). Buccal film drug delivery system- An Innovative and Emerging Technology. *Molecular Pharmaceutics & Organic Process Research.* 1, 3, 1-6.
4. Madan J., Banode S., Dangi M. (2010). Mucosal drug delivery system. *International Journal of Research in Ayurveda & Pharmacy*. 1, 1, 63-70.
5. Singh A., Sharma U.K., Prajapati S.K. (2017). A review on mucoadhesive buccal patches. *International Journal of Research and Development in Pharmacy & Life Sciences.* 6, 4, 2654-2660.
6. Sheoran R. (2018). Fast Dissolving Oral Films: A review with future prospects. *International Journal of Pharmacy & Pharmaceutical Research.* 12, 2, 15-32.
7. Squire C.A., Kremer M.J. (2001). Biology of oral mucosa and oesophagus. *Journal of the National Cancer Institute*. 29, 7-15.
8. Hearnden V., Colley H., Murdoch C., Thornhill M. (2012). Designing drugs For Transmucosal delivery in the oral cavity, what are the optimal chemical properties? School of Clinical dentistry, University of Sheffield, UK, 1-3.
9. Mathew A. K. (2015). Oral local drug delivery: An overview. *Pharmacy and Pharmacology Research.* 3, 1, 1-6.
10. Fabian T.K., Hermann P., Beck A., Fejerdy P., Fabian G. (2012). Review on salivary defence proteins: their network and role in innate and acquired oral immunity. *International Journal of Molecular Sciences*. 13, 4295-4320.
11. Mithun R., Marc T., Thejaswini B.D. (2013). Salivary biomarkers and their applicability in forensic identification. *Sri Lanka Journal of Forensic Medicine-Science & law.* 4, 1, 10-15.
12. Gupta S.K., Singhvi I.J., Shirsat M., Karwani G., Agrwal A. (2011). Buccal adhesive drug delivery system: a review. *Asian Journal of Biochemical and Pharmaceutical research.* 1, 2, 104-114.
13. Mishra S., Kumar G., Kothiyal P. (2012). A review article: recent approaches in buccal patches. *The Pharma Innovation.* 1, 78-86.
14. Shaikh S., Shaikh Z., Khan G.J., Patel S.M. (2017). A review on mucoadhesive drug delivery system. *European Journal of Pharmaceutical & Medical Research.* 4, 11, 247-252.

15. Panda B.P., Dey N.S., Rao M.B. (2012). Development of innovative orally fast disintegrating film dosage forms: a review. *International Journal of Pharmaceutical Sciences and Nanotechnology*. 5, 2, 1666-1674.

16. Muhammad I., Rabel S., Quratulain B., Farhat J., Khan A. (2016). Orally disintegrating films: a modern expansion in drug delivery system. *Saudi Pharmaceutical Journal*. 24, 537-546.

17. Patel A.R., Prajapati D.S., Raval J.A. (2010). Fast dissolving films (FDFs) as a newer venture in fast dissolving dosage forms. *International Journal of Drug Development & Research.* 2, 2, 232-246.

18. British Pharmacopoeia. (2008). Volume II: Monographs: Medicinal and Pharmaceutical Substances (J-Z); 1838.

19. Indian Pharmacopoeia. (2014). Ministry of health and family welfare, Government of India, published by Controller of publication, New Delhi, 3; 760,778,2351-2353.

20. Giradkar K.P., Channavar M.A., Kajale A.D. (2010). Design, development and in vitro evaluation of Bioadhesive dosage form for Buccal route. *International journal of Pharmaceutical Research and Development.* 2, 6, 1-20.

21. Vijaya Sri K., Ravishanker D., Rohini P. (2013). Formulation and *in vitro* evaluation of sumatriptan succinate oral thin films. *Indo American Journal of Pharmaceutical Research.* 3, 4, 3016-3025.