FORMULATION DEVELOPMENT AND PHARMACOLOGICAL EVALUATION OF MUCOADHESIVE PATCHES OF PREGABALIN IN PACLITAXEL INDUCED NEUROPATHIC PAIN

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Abstract

As oral route is the preferred route of administration of majority of drugs but it has certain limitations such as pre-systemic metabolism or first pass metabolism in liver, g.i.t upset and destructions of enzymes. Pregabalin is another synthetic atom and an underlying structure of the inhibitory synapse γ-aminobutyric corrosive. This research was based on the synthesis and in-vitro characterization of mucoadhesive patches of Pregabalin by incorporating different polymers using solvent casting method. Pregabalin (API) was obtained as gift sample from the Dr. Reddy's Pvt Ltd Hyderabad. The Ethanol, Eudragit L-100, Propylene glycol, Tween 80, Methanol and HPMCK4M & HPMCK15M and hydroxypropyl methylcellulose (HPMC) were purchased from Sigma Aldrich Pvt Ltd India. The formulation was evaluated for parameters like weight variation& thickness, flatness, folding strength, moisture content, drug content determination, diffusion cell in-vitro model, surface pH, Water Vapor Transmission rate determination, In-Vivo patch test, Pharmacological screening of formulated patch in Neuropathic pain and Stability studies. In results, an excellent in-vitro release rate of the formulation was demonstrated by the buccal patch. In order to estimate stability profile of mucoadhesive patches, it was evaluated for physical appearance. After 30 days of storage, physical appearance for different patches was found same. Pregabalin incorporated in the mucoadhesive patches may be frequently employed in the modulation of numerous CNS activities with better absorption. It concluded, a better in-vitro scattering time alongside rich appearance and other actual attributes like elasticity, % lengthening, collapsing perseverance. P2 was chosen dependent on its outcomes on performed assessment which were all ideal and furthermore had great mechanical properties. It would be very beneficial for the management of neuropathic pain as stable mucoadhesive patches consisting pregabalin.

Keywords: mucoadhesive patch, pregabalin, stability, solvent casting, neuropathic pain

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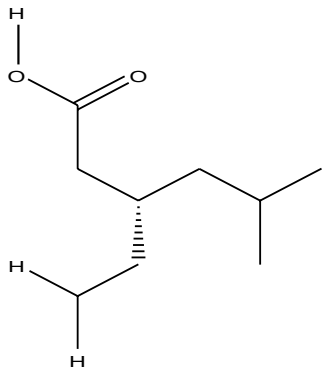
INTRODUCTION

As oral route is the preferred route of administration of majority of drugs but it has certain limitations such as presystemic metabolism or first pass metabolism in liver, g.i.t upset and destructions of enzymes (Verma et al. 2017). Advancement of new drug molecule is costly and tedious (Tiwari et al. 2012). A suitably planned Novel Drug Delivery System can be a serious step forward for taking care of the issues related towards the arrival of the medication at explicit site with explicit rate (Bhagwat and Vaidhya, 2012). Substances that work with the saturation through buccal mucosa are alluded to as saturation enhancers. Various kinds of potential penetration enhancers have been read for the buccal course to build the entrance of medications.

Pregabalin

Pregabalin is another synthetic atom and an underlying structure of the inhibitory synapse γ-aminobutyric corrosive. It is a α2-δ (α2-δ) ligand that has pain relieving, anti-convulsant, anxiolytic, and rest adjusting exercises. Pregabalin ties strongly to the α2-δ subunit of calcium channels, bringing about a decrease in the arrival of a few synapses, including glutamate, noradrenaline, serotonin, dopamine, and substance P.



Pregabalin

Fig 1. Structure of pregabalin

Pregabalin seems to create an inhibitory adjustment of neuronal edginess, especially in spaces of the focal sensory system thick in synaptic associations like the neocortex, amygdala, and hippocampus (Patel et al. 2013; Zheng et al. 2022).

Neuropathic pain results from injury to central or peripheral nerves and can be brought on by a variety of conditions, including diabetes, post-stroke, trauma, cancer, chemotherapy, autoimmune disorders, multiple sclerosis, and nerve compression. According to estimates, 30–40% of chemotherapy patients eventually experience neuropathic sensory and motor abnormalities (Guo et al. 2022).

Paclitaxel (Taxol), which is used to treat a number of malignancies including ovarian, breast, and non-small cell lung cancers, is derived from the Pacific yew tree Taxus brefolia. The emergence of a very painful sensory neuropathy, which lowers quality of life, limits its efficacy (Yu et al. 2022). Paresthesia and dysthesia, discomfort, numbness, tingling, and heightened sensitivity to touch and warmth are some of the sensory symptoms.

Based on previous researches, we found that synthesis and evaluation of Pregabalin was done in very limited manner in the form of mucoadhesive patches. So, this research is based on the synthesis and in-vitro characterization of mucoadhesive patches of Pregabalin by incorporating different polymers using solvent casting method.

MATERIALS AND METHODOLOGY

Experimental Requirements

A. Pregabalin (API), Ethanol, Eudragit L-100, Propylene glycol, Tween 80, Methanol and HPMCK4M & HPMCK15M and other types of hydroxypropyl methylcellulose (HPMC).
B. Digital weighing balance, Digital pH meter, Franz diffusion cell and UV-Spectrophotometer.

Preparation of Standard Calibration Curve

A stock solution is created by properly weighing 100 mg of pregabalin, dissolving it in 2 ml of methanol, and then adding 0.1 N HCl solution to bring the amount up to 100 ml. To create the 100 g/ml concentration solution, stock solution (10ml) is further diluted with 0.1 N HCl (pH 1.2) in 100 ml. Then, to prepare 2g, 4g, 6g, 8g, and 10g of drug/ml solution, 0.2, 0.4, 0.6, 0.8, and 1 ml of solution are taken in a 10 ml standard volumetric flask and the volume is increased to 10 ml with 0.1N HCl. The absorbance is then measured at 270 nm in a UV spectrophotometer using 0.1 N HCl as a blank. Repeating the process with phosphate buffer at pH 6.8, absorbance is measured at 271 nm (Reddy et al. 2019).

FORMULATION OF MUCOADHESIVE PATCHES

Pregabalin mucoadhesive compositions were created using the solvent casting technique. The mucoadhesive polymers hydroxypropyl methyl cellulose (HPMC K-15M), polyvinyl alcohol (PVA), and polyvinylpyrrolidone were included in

the patch formulations (PVP K-30) and cellulose acetate (CE). To prepare different polymer ratios, various stock solutions were employed. A stock solution of HPMC K-15M at 2% by weight was prepared in distilled water, and a 2 percent w/v PVA solution was also made concentration. 1% weight-to-volume (w/v) PVP K-30 and EC stock solution was also made in ethanol and water, respectively. Formulations included combinations like HPMC/PVA/PVP and HPMC/PVA/EC. The amount of plasticizer used to create patches, either 30 ml of propylene glycol (PG), or 2ml of polyethylene glycol (PEG-400) was added to the mixture of polymers above. To the alcoholic Pregabalin solution in the amount (188 mg/5 mL) estimated above the polymer combination which results in the buccal patch's 50mg per unit (3cm diameter) concentration being included. Homogenized drug and polymer solution was put into Teflon. Place a covered Petri dish (9.2 cm in diameter) carefully on a flat surface. A glass funnel was placed over the Petri dish mould to guarantee even evaporation. The contents of the Petri dishes were first dried at ambient temperature for two hours and then dehydrated for 48 hours in a hot air dryer at 50ºC. The dried patches were arranged in a sphere taken from the mould and visually checked for any distortion. next buccal from the circular disc, patch units (3cm in diameter) were cut, resulting in one patch unit per disc possibly 50 mg of pregabalin. After that, each patch was sealed in aluminum foil and kept in a desiccator.

Table 1. Compositions of the Pregabalin buccal patches

S. No.	Ingredients	F1	F2	F3	F4	F5	F6
1.	Pregabalin (mg)	50	50	50	50	50	50
2.	Eudragit L-100 (mg)	150	150	150	100	100	100
3.	Tween 80 (mg)	50	50	50	50	50	50
4.	HPMCK4M (mg)	100	-	-	50	100	-
5.	HPMCK15M (mg)	-	100	-	50	50	50
6.	HPMCK100M (mg)	-	-	100	50	-	100
7.	Propylene glycol (ml)	2.5	2.5	2.5	2.5	2.5	2.5
8.	Ethanol (ml)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

Fig 2. Dissolution of ingredients of patch

EVALUATION PARAMETERS (Tirunagari et al. 2014; Reddy et al. 2019).

Physical appearance

All the formulated mucoadhesive patches were seen for physical appearances i.e., flat, oval, thick etc.

Weight variation

The all the buccal patches will be determined for their weights and to compare among to make sure that these are under limits of weight variation.

Swelling index

Patch was weighed (W), put in a 2% w/v agar gel plate, and allowed to sit for one hour at 37°C. The patch was taken from the petri plate at regular intervals of one hour (up to 3 hours) and any excess surface water was gently blotted away with tissue paper. The swelling index was then determined using the formula after the swelled patch was reweighed (W1)- % Swelling index = W_1-W/W × 100

Folding endurance

The folding strength is measured manually for the buccal patch. A strip of the films is cut evenly and repeatedly folded at the same place until it is broken-out.

Thickness

Using a standard screw gauge, the thickness of three randomly chosen patches from each batch was measured.

Percentage moisture

Each patch is weighed before being stored for 24 hours in a desiccator. Up until a constant weight is attained, the patches are reweighed. A formula is used to determine the moisture content in percentage terms based on the difference between the beginning and constant end weights.

Drug content

Cut a small portion of the patch, and then dissolve it in a 7.4 pH PBS solution. The residual volume is then brought up to 100 ml with PBS after the solvent ethanol is added to make the polymer soluble (pH 7.4). After then, 1 ml is taken out of the solution and diluted once more up to 10 ml. At a wavelength of 270 nm, the solution's absorbance is measured, and concentration is determined. As a result, the drug content is determined (Nafee et al. 2003).

In-vitro drug release

Pregabalin from buccal patches permeates a dialysis membrane in vitro. A Franz diffusion cell was put on the membrane, and a buccal batch was created. 15.0 ml of PBS pH 7.4 are added to the receiver compartment of the diffusion cell, and the mixture is kept there over a magnetic stirrer with the temperature held at 37 °C. Every 1, 2, 3, 4, 6 and 12 hours, a sample of 3 ml is promptly removed and replenished from the receiver compartment. Before the analysis is done, they are kept in a refrigerator. The samples are subjected to UV-visible spectrophotometer analysis to determine their Atomoxetine content. The medication concentrations are measured at 270 nm wavelength (Davies & Ingham, 2015).

Surface pH

Patches are placed on an agar plate and allowed to swell for 3 hours while the agar is dissolved in simulated human saliva (NaCl, KCl, KSCN, KH2 PO4, and urea) in 1 L of distilled water with a pH of 6.8 while being stirred. The solution is then poured into a Petri dish and allowed to gel at room temperature. A pH paper was placed on the surface of the swollen area to measure the surface pH. (Ikram et al. 2015; Kopacic et al. 2018).

SEM analysis

Scanning electron microscopy was used to describe the morphology of films. Before being examined with a scanning electron microscope, samples were mounted on round brass stubs (12mm in width) using double-backed adhesive tape. Gold palladium was then sputter coated on the samples for 8 minutes at 1.1 LV under an argon atmosphere (JEOL JSM-6100 SEM, Japan). The pictures were taken using a 35mm black and white Ilford PANF 50 film.

Water Vapor Transmission Rate (WVTR)

WVTR is defined as the quantity of moisture transmitted through unit area of film in unit time. A glass bottle is used in the study having length 5cm, with internal diameter of 0.8cm that was filled with 2g anhydrous calcium chloride & an adhesive spread across its rim. The Pregabalin buccal patch is fixed over the adhesive and the assembly is placed in constant humidity chamber. It is prepared using saturated solution of ammonium chloride and maintained at 37±2°C. The difference in weight after three days is calculated (Kopacic et al. 2018).

In-Vivo patch test

Three male rabbits (of either sex) weighing approx. 5.0, 5.5, and 6.0 kg are taken for the release study of the pregabalin. The rabbits are fasted for overnight with water ad libitum. They are kept in individual cages before the experiment was carried out. The rabbits are subjected to anesthetized with phenobarbital sodium IP (1ml containing 200mg) by intra peritoneal route. Mucoadhesive patches (size 1 x 1 cm2) are cut-down and fixed on a cellophane paper- acted as a backing layer. So, the drug release is made unidirectional and threads tied to it, by which the patches can be easily removed from the buccal mucosa. After 10 minutes of the injection of anesthesia, the patches are placed (separately) in the buccal cavity one at a time. After 2 min. other patches are attached. The patches are taken out at intervals of 15, 30, 45, 60, 75, and 90 minutes. The same process is repeated for two or more times to validate the result. The patches are dissolved in 10 ml of phosphate buffer solution at pH 6.6. The unabsorbed drug is analyzed at wavelength of 241nm.

Stability

Stability studies are carried out by keeping the optimized formulations in the butter paper and covered by aluminium foil and placed in the aluminium pouch. It is sealed by heat at the end for one month at room temperature. The films are taken at different time intervals like 0 to 4th week and are analyzed for its appearance and drug content by following above mentioned protocols (Khairner at al. 2009).

Pharmacological screening of formulated patch in Neuropathic pain

Rats were randomly classified into 4 groups, containing six animals in each. Group 1 is given normal saline (1ml), Group 2 is treated with Paclitaxel (2mg/kg, *i.p.*). Group 3 is treated with Paclitaxel (2mg/kg, *i.p.*) + Pregabalin patches. Pain is being induced by intraperitoneal injection of Paclitaxel on 4 alternate days. After taking the baseline values, the group 2 is administered with respective drug patch once a day p. o. for 8 consecutive days along with paclitaxel. After

dosing, all the animals are tested for thermal hyperalgesia and cold allodynia on day 7, 14, 21 and 28 with Tail immersion test.

Tail immersion test

The primary characteristic of many neuropathic pain syndromes is pain that is induced by cold. Normal cool stimuli cause discomfort in animal models, especially in cases of cold allodynia. In the current study, two common animal models, the Acetone Drop Test and the Tail Immersion Test, were employed to test cold allodynia. The terminal portion of the tail, which is five centimetres distal, was submerged in a container of 10°C-cold water to test for cold allodynia. The experiment was conducted at a constant temperature of (10ºC). It was recorded how long it took the tail to exit the frigid water. To avoid causing tissue damage, a cut-off time of 20 seconds was maintained. For each animal, this process was carried out three times, and the mean was then computed. While longer contact duration was observed to provide an anti-allodynic effect, the decrease in tail contact time with cold water was predictive of discomfort.

RESULTS AND DISCUSSION

Standard calibration curve- Pregabalin

To examine pregabalin, the UV Spectro-photometric technique was employed. At a wavelength of 274nm, the drug's absorbance in phosphate buffered saline pH 7.4 with a little amount of methanol was recorded. Pregabalin's standard curve in PBS pH 7.4 was linear from the origin to values of 2-12µg/ml. Beer Lambert's law is observed by the curve.

Table 2. Standard calibration curve- Pregabalin

Name	Conc. (µg/ml)	Absorbance
Std 1	2	0.147
Std 2	4	0.282
Std 3	6	0.463
Std 4	8	0.589
Std 5	10	0.752
Std 6	12	0.896

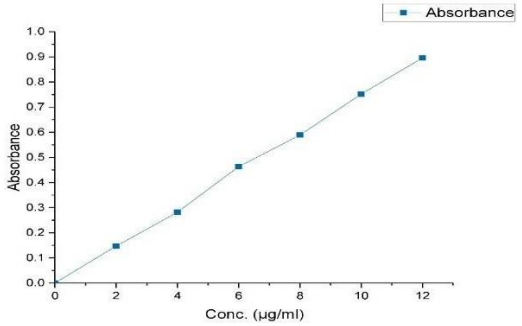


Fig 3. Std. calibration curve at pH 7.4 (PBS)

EVALUATION OF BUCCAL PATCHES

Surface pH study

The prepared patches (F1-F6) were studied for surface pH and demonstrated as slightly basic/acidic- near to neutral pH. Formulations in terms of patches- F2, F3 & F5 showed slightly acidic pH as 6.9± 0.31, 6.7± 0.16 & 6.4± 0.11, respectively. Whereas F1, F4 & F5 showed slight alkaline pH when observed as 7.3±0.29, 7.4±0.45 & 6.4±0.11 respectively. So, it may confirm that formulated patches were of optimum pH range for better tolerability and solubility in saliva.

Following table depicts the surface pH of Patches (F1-F6).

Table 4. Surface pH of Patches (F1-F6)

Patch	Time (seen after)	pH± S.D.
F1	1 hour	7.3± 0.29
F2	1 hour	6.9± 0.31
F3	1 hour	6.7± 0.16
F4	1 hour	7.4± 0.45
F5	1 hour	6.4± 0.11
F6	1 hour	7.2± 0.53

No. of evaluation: 3,
Readings were exhibited in Mean ± Std. Deviation

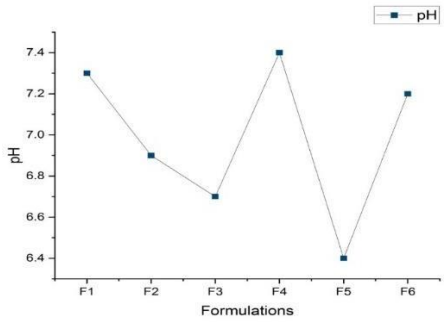


Fig 5. Graphical presentation of pH of Patches (F1-F6)

Folding endurance

Formulated mucoadhesive patches were evaluated for their folding capacity. Patches- F3, F4 & F6 showed folding endurance as 25± 0.43, 27± 0.51 & 24± 0.11 respectively. Whereas F5, F2 & F1 exhibited folding endurance as 21±0.18, 20±0.52 & 18± 0.31 respectively.

The buccal patch described above showed a rigid folding endurance in the aspect of the patch's folding ability. It can be kept under a lot of stress and intense mechanical strain. It is more stable and long lasting due to its improved folding endurance. The table below demonstrates that formulations from F1 to F6 display an ideal degree of folding power.

Table 5. Folding endurance

Patch	Folding endurance± S.D.
F 1	18± 0.31
F 2	20± 0.52
F 3	25± 0.43
F 4	27± 0.51
F 5	21± 0.18
F 6	24± 0.11

No. of evaluation: 3,
Readings were exhibited in Mean ± Std. Deviation

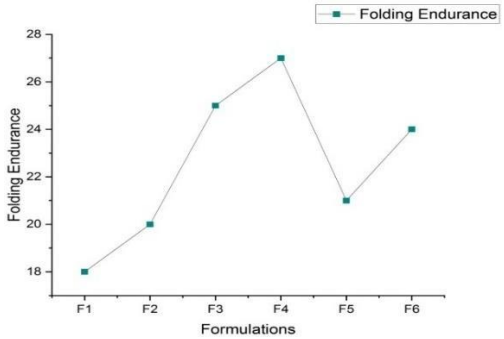


Fig 6. Graphical representation of folding endurance

Swelling index study

All the formulated patches were estimated for swelling index. For the same, they were weighed before starting the swelling index study. Prior swelling study, the weight of patch- F1 was 0.82gm and after swelling it was observed as 0.93±0.24gm that was maximum swelling index.

In F2, F3, F4, F5 & F6 swelling was observed as 0.82± 0.16 gm, 0.74± 0.34 gm, 0.81± 0.45 gm, 0.83± 0.40 gm & 0.91± 0.31 gm, respectively.

The following table shows the swelling power of the formulations-

Table 6. Swelling index

Patch	Weight (gm)	Weight after swelling (gm)± S.D.
F 1	0.82	0.93± 0.24
F 2	0.75	0.82± 0.16
F 3	0.67	0.74± 0.34
F 4	0.73	0.81± 0.45
F 5	0.75	0.83± 0.40
F 6	0.85	0.91± 0.31

No. of evaluation: 3,
Readings were exhibited in Mean ± Std. Deviation

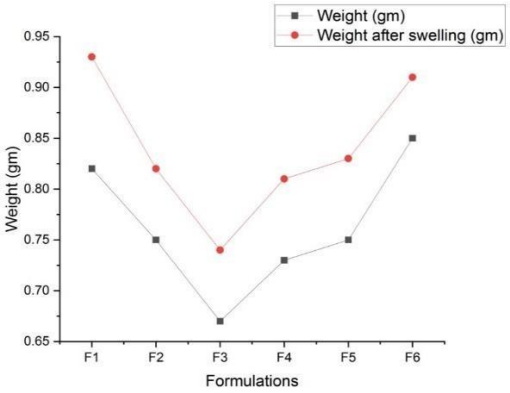


Fig 7. Graphical representation of swelling index

Measurement of muco-adhesive strength

F2 &F5 exhibited excellent mucoadhesive strength as 4.53±0.35 G & 4.71±0.38 G when observed and compared with other prepared patches. Mucoadhesive strength of F1, F3, F4 & F6 was seen as 3.35± 0.52 G, 4.37± 0.17 G, 3.81± 0.49 G & 4.27± 0.74 G, respectively. Better mucoadhesive strength is sign of better API-Excipient compatibility and bonding efficiency.

Table 7. Mucoadhesive strength

Patch	F1	F2	F3	F4	F5	F6
Muco-adhesive Strength (G)	3.35± 0.52	4.53± 0.35	4.37± 0.17	3.81± 0.49	4.71± 0.38	4.27± 0.74

No. of evaluation: 3,
Readings were exhibited in Mean ± Std. Deviation

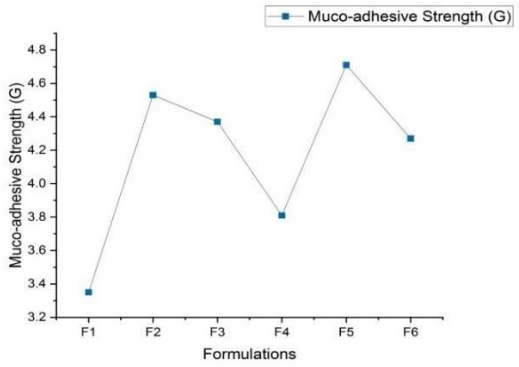


Fig 8. Graphical representation of mucoadhesive strength of patches

Determination of thickness of patches

Thickness plays an important role in drug release and its action. Thickness was found as 0.45±0.31, 0.49±0.38 in F1 & F2 respectively. Thickness of patches was estimated highest in patch- F3, F4, F5 &F6 as 0.53±0.17, 0.57±0.29, 0.59±0.69 & 0.53±0.62, respectively. Following table depicts the thickness of patches-

Table 8. Determination of thickness of Patches

Patch	Thickness± S.D.
F 1	0.45±0.31
F 2	0.49±0.38
F 3	0.53±0.17
F 4	0.57±0.29
F 5	0.59±0.69
F 6	0.53±0.62

No. of evaluation: 3,
Readings were exhibited in Mean ± Std. Deviation

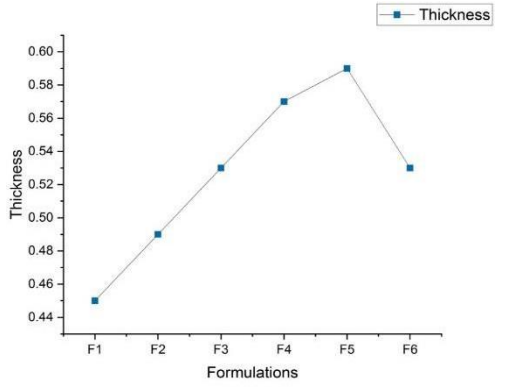


Fig 9. Graphical representation of thickness of patches

Determination of weight variation

Formulated patches were estimated for its weight variation. A negligible weight variation exhibits a better formulation. Weight was found as 0.35±0.39gm, 0.33±0.17gm & 0.33±0.62gm in F1, F3 & F6 respectively. Weight of patches was estimated highest in patch- F2, F4 & F5 as 0.39± 0.38gm, 0.29±0.20gm & 0.39±0.69gm, respectively.

Following table depicts the thickness of patches-

Table 9. Determination of weight variation of Patches

Patch	Weight± S.D.
F1	0.35± 0.39
F2	0.39± 0.38
F3	0.33±0.17
F4	0.29±0.20
F5	0.39±0.69
F6	0.33±0.62

No. of evaluation: 3,
Readings were exhibited in Mean ± Std. Deviation

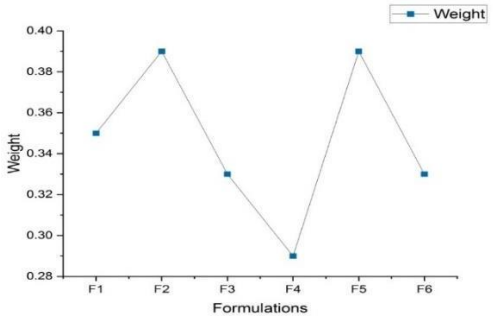
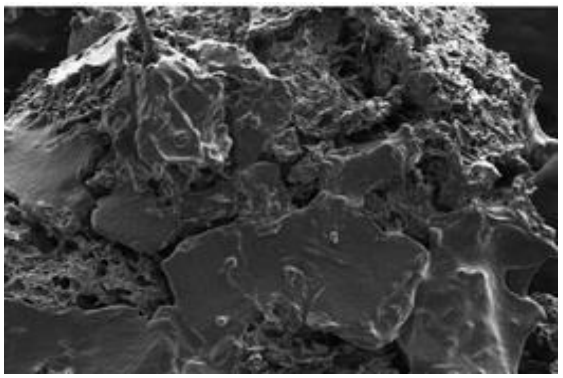


Fig 10. Graphical representation of weight variation of patches

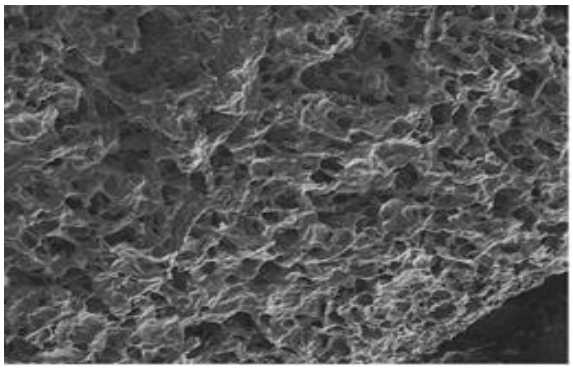
SEM analysis

The formulated patches (mucoadhesive) were evaluated in terms of scanning electron microscope (SEM). Patches (F1-F4) showed almost similar pictures when compared among them.

However, F5 and F6 demonstrated somehow different from F1-F4 patches. They all showed for better uniformity of drug with its excipients.



F 1



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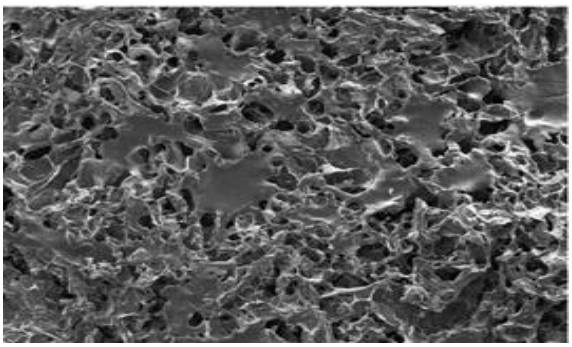

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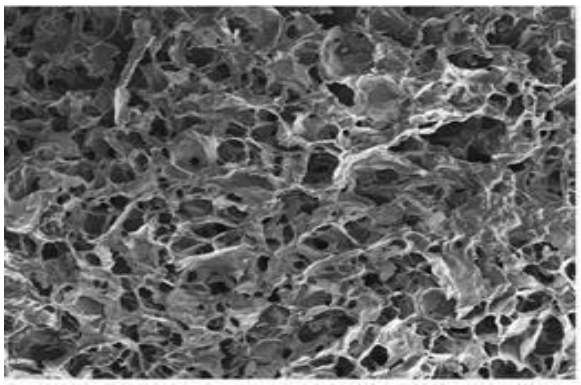

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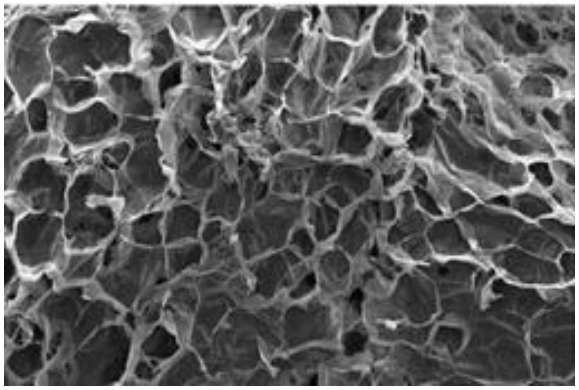
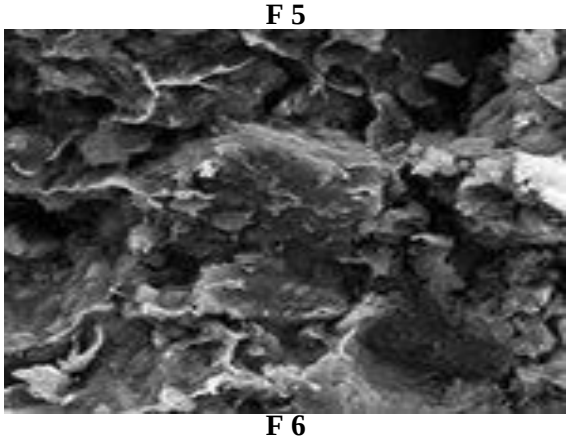


F 5



F 6

Fig 11. SEM analysis of different preparations

Estimation of % Drug release

F1, F2, F5 & F6 showed % drug release as 91.6±0.81, 85.8±0.34, 77.3±0.27 & 76.5±0.52 respectively. F 3 & F 4 exhibited % drug release as 82.4±0.33 & 78.8±0.81 respectively.

Table 10. % Drug release

Time (hr)	% drug release ± S D					
	F 1	F 2	F 3	F 4	F 5	F 6
1	33.3±0.37	27.5±0.60	23.7±0.45	23.3±0.46	18.6±0.37	19.4±0.49
2	43.7±0.55	37.8±0.72	37.5±0.34	35.7±0.65	29.8±0.58	33.9±0.55
3	57.5±0.42	42.2±0.63	46.4±0.23	41.8±0.24	36.9±0.13	47.8±0.74
4	68.4±0.89	68.0±0.86	57.8±0.92	59.7±0.92	54.8±0.67	57.5±0.53
5	83.7±0.32	73.7±0.92	71.6±0.37	65.5±0.73	67.7±0.45	64.8±0.21
6	91.6±0.81	85.8±0.34	82.4±0.33	78.8±0.81	77.3±0.27	76.5±0.52

No. of evaluation: 3,

Readings were exhibited in Mean ± Std. Deviation

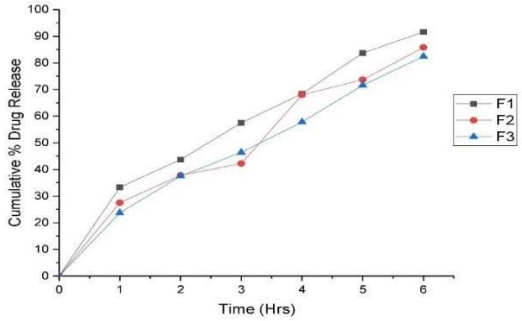


Fig 12. In-vitro drug release (F 1- F 3)

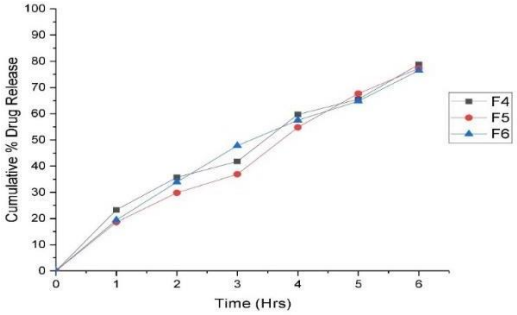


Fig 13. In-vitro drug release (F 4- F6)

Drug release kinetics

Formulated mucoadhesive patches were studies for their drug release kinetics. In this order, it showed Higuchi model & Koresmeyer Peppas model, zero order, first order kinetics in drug release kinetics. Drug release kinetics has been given as below-

Table 11. Drug release kinetics

Time (hr)	Sq. root of time	Log time	Cum. % release	Log cum. % release	Cum. % remaining drug	Log Cum. % remaining drug
1	1.00	0.00	16.42	1.29	81.78	1.91
2	1.54	0.33	32.91	1.63	67.25	1.81
3	1.78	0.46	42.56	1.69	57.29	1.72
4	2.17	0.58	53.38	1.79	47.36	1.65
5	2.26	0.68	64.27	1.86	38.46	1.53
6	2.49	0.91	72.41	1.93	27.63	1.47

Following fig. depicts the cumulative drug release profile-

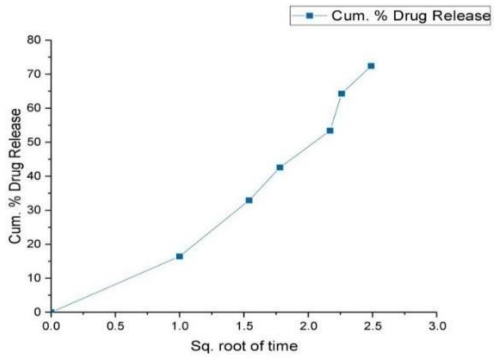


Fig. 14. Cumulative % drug release

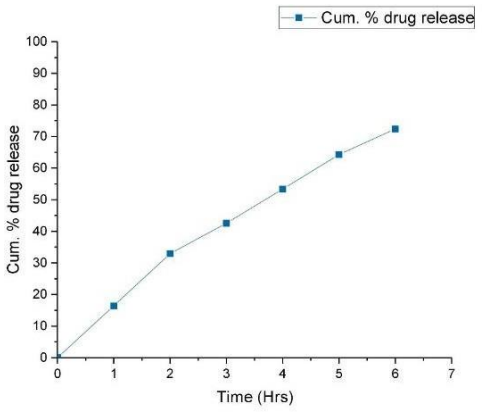


Fig 16. Cumulative % drug release (Zero Order)

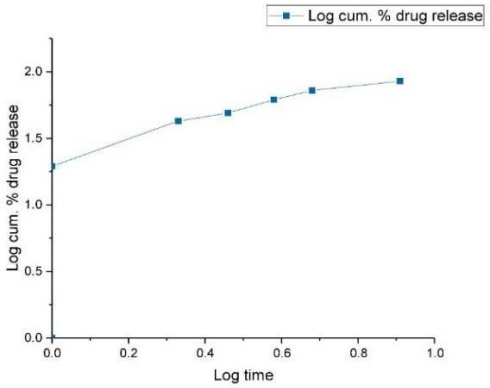


Fig 15. Log % drug release (Koresmeyer-Peppas model)

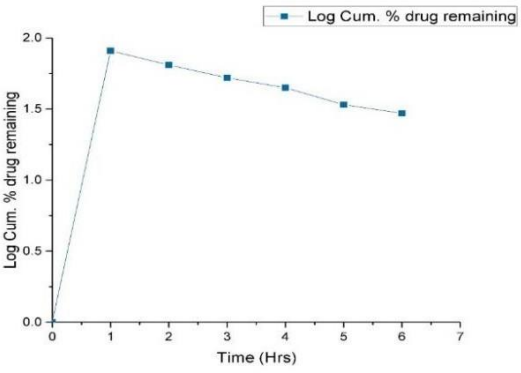


Fig 17. Log % drug release (First Order)

Water-Vapor Transmission Rate

Formulations F2, F3, F4 and F5 exhibited the WVTR as 0.294±0.37, 0.356±0.14, 0.384±0.26 and 0.392±0.64, respectively that was minimum among all. While F1 and F6 showed highest WVTR level as 0.417±0.59, 0.439±0.51, respectively.

Table 12. Determination of WVTR

Patch	WVTR± S.D.
F 1	0.417±0.59
F 2	0.294±0.37
F 3	0.356±0.14
F 4	0.384±0.26
F 5	0.392±0.64
F 6	0.439±0.51

No. of evaluation: 3,
Readings were exhibited in Mean ± Std. Deviation

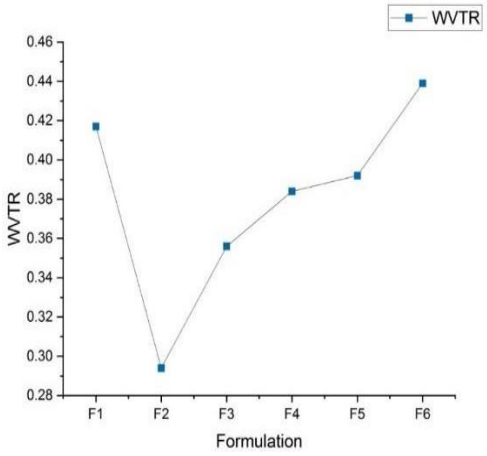


Fig 18. Graphical representation of WVTR

In vivo patch test

Table 11. Demonstrates the in-vivo test for estimation of unabsorbed concentration of mucoadhesive patches. Maximum unabsorbed conc. was observed at the time intervalof 15 min and minimum conc. was found at the interval of 90 min. The unabsorbed conc. was observed as 13.6±0.42, 14.4±0.14, 18.2±0.52, 18.8±0.26, 15.2±0.14 and 16.4±0.27 for F1-F6, respectively after 90 minutes. It demonstrated that all the patches showed a remarkable drug release and absorption with the time.

Table 13. In vivo test for estimation of unabsorbed conc. (%)

Time (min)	%Unabsorbed drug conc. ± S.D.					
	F1	F2	F3	F4	F5	F6
15	99.0±0.47	94.4±0.31	97.4±0.45	90.4±0.27	93.2±0.30	94.8±0.43
30	87.2±0.35	88.6±0.22	85.6±0.32	83.6±0.62	77.2±0.53	78.8±0.46
45	64.4±0.15	66.4±0.53	61.0±0.28	63.2±0.29	59.0±0.12	61.0±0.36
60	47.0±0.37	48.4±0.73	44.8±0.27	41.0±0.31	43.4±0.45	39.6±0.26
75	28.6±0.40	34.4±0.30	32.6±0.29	32.8±0.23	31.2±0.34	28.4±0.60
90	13.6±0.42	14.4±0.14	18.2±0.52	18.8±0.26	15.2±0.14	16.4±0.27

No. of evaluation: 3,
Readings were exhibited in Mean ± Std. Deviation

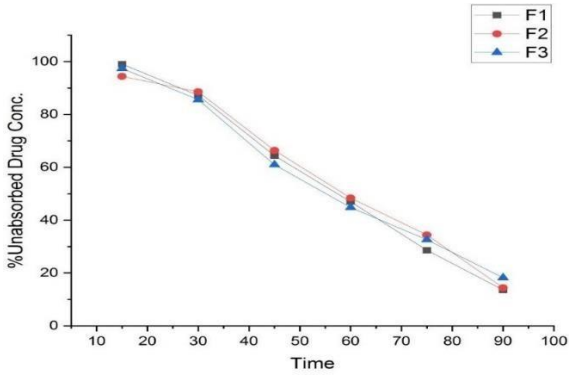


Fig 19. In- vivo test for estimation of unabsorbed conc. (F1, F2, F3)

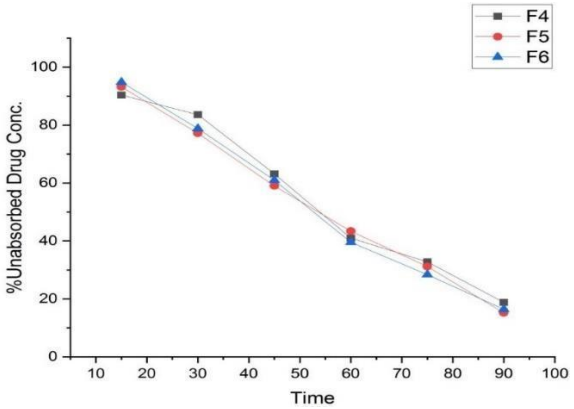


Fig 20. In- vivo test for estimation of unabsorbed conc. (F4, F5, F6)

Screening of pharmacological activity in neuropathic pain

Rabbits were grouped into 3 different groups and treated with normal saline (Group 1), Paclitaxel (2mg/kg, *i.p.*) (Group 2) & Paclitaxel (2mg/kg, *i.p.*) + Pregabalin patches (Group 3).

Paclitaxel (2mg/kg) treated rats showed the withdrawal latency as 5.79±1.82 s, 6.57±1.44 s,

3.91±1.12 s & 3.74±1.22 s at 7th day, 14th day, 21th day & 28th day, respectively. Whereas, withdrawal latency was found significantly increased in Paclitaxel (2mg/kg, *i.p.*) + Pregabalin patches treated rats. It demonstrated the increased withdrawal latency as 18.63±0.45 s, 19.12±0.63 s, 18.94±0.14 s & 19.38±0.27 s at 7th day, 14th day, 21th day & 28th day, respectively. When compared with control group, it significantly did modify (increase) the withdrawal latency (s) time that indicates for the effective role in neuropathic pain.

Table 14. Increased withdrawal latency of Pregabalin patches on 7th day

Treatment	Withdrawal latency on 7th Day [S± SEM]
Normal saline	7.83±1.25
Paclitaxel (2mg/kg, *i.p.*)	5.79±1.82
Paclitaxel (2mg/kg, *i.p.*) + Pregabalin patches	18.63±0.45

Significance level in terms of *, P<0.05
No. of evaluation: 6; readings were exhibited in Mean ± Std. Deviation

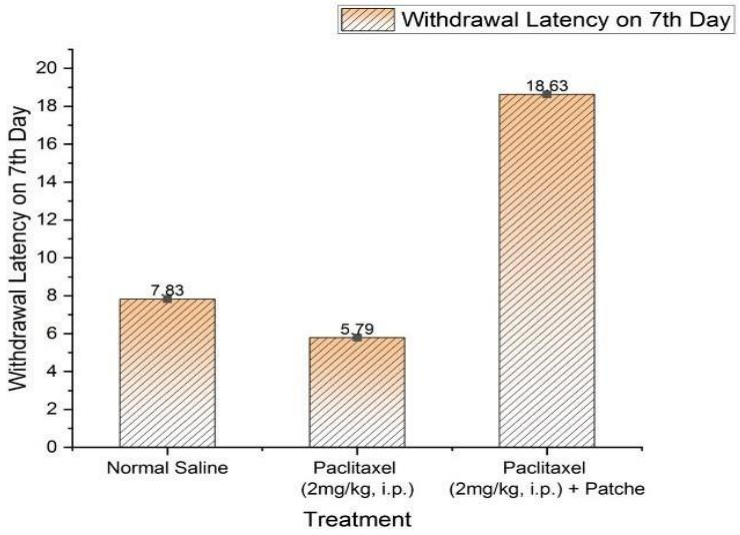


Fig 21. Increased withdrawal latency of Pregabalin patches on 7th day

Table 15. Increased withdrawal latency of Pregabalin patches on 14th day

Treatment	Withdrawal latency on 14th Day [S± SEM]
Normal saline	8.65±1.27
Paclitaxel (2mg/kg, *i.p.*)	6.57±1.44
Paclitaxel (2mg/kg, *i.p.*) + Pregabalin patches	19.12±0.63

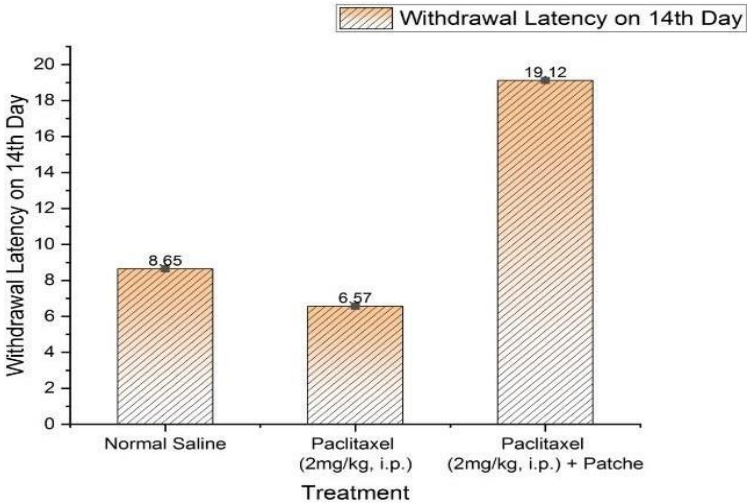


Fig 22. Increased withdrawal latency of Pregabalin patches on 14th day

Table 16. Increased withdrawal latency of Pregabalin patches on 21th day

Treatment	**Withdrawal latency on 21th Day [S± SEM]**
Normal saline	7.49±1.41
Paclitaxel (2mg/kg, *i.p.*)	3.91±1.12
Paclitaxel (2mg/kg, *i.p.*) + Pregabalin patches	18.94±0.14

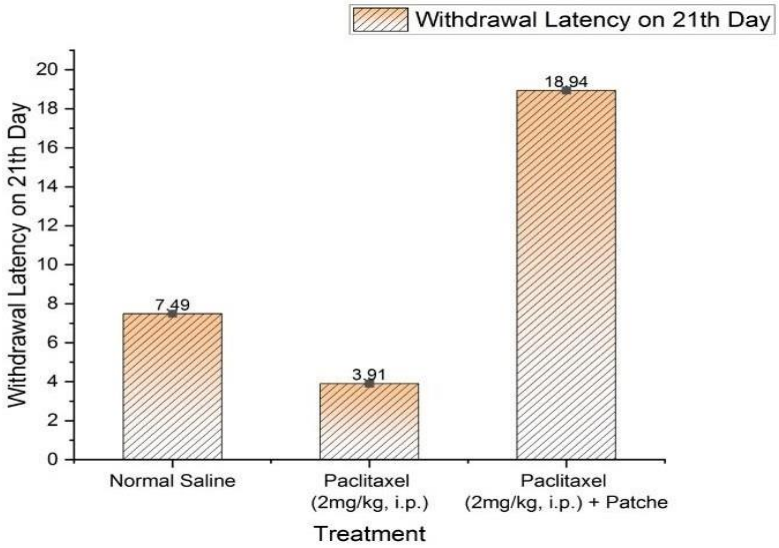


Fig 23. Increased withdrawal latency of Pregabalin patches on 21th day

Table 17. Increased withdrawal latency of Pregabalin patches on 28th day

Treatment	**Withdrawal latency on 28th Day [S± SEM]**
Normal saline	8.32±1.62
Paclitaxel (2mg/kg, *i.p.*)	3.74±1.22
Paclitaxel (2mg/kg, *i.p.*) + Pregabalin patches	19.38±0.27

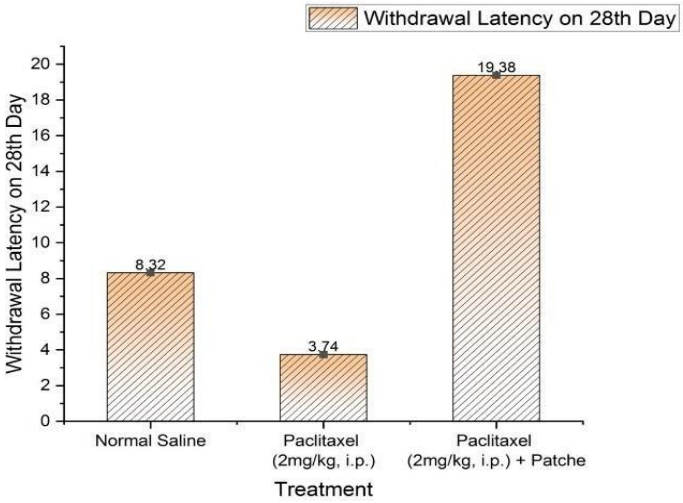


Fig 24. Increased withdrawal latency of Pregabalin patches on 28th day

The buccal patch described above displayed an appropriate swelling proportion. The physical characteristics of buccal patch are significantly influenced by swelling index. It demonstrates that saliva has a better drug release and dissolution rate. This permits the formulation to have a good bioavailability in the bloodstream after being absorbed.

Due to the inclusion of numerous mucoadhesive polymers, the formulated patch's mucoadhesive strength demonstrated a remarkable power. It aids in the formulation's stability development as well.

Additionally, it makes it easier for the Active Pharmaceutical Ingredient to release when combined with various excipients and polymers in the appropriate solvent medium.

An excellent in-vitro release rate of the formulation was demonstrated by the buccal patch. Additionally, it ensures that the contents are uniform. This formulation feature is crucial for demonstrating fast release oral films at the proper time. The uniformity of the patch and an environment that is conducive to drug release and dissolution determine how quickly a buccal patch releases its medication.

5.2.13 Stability

In order to estimate stability profile of mucoadhesive patches, it was evaluated for physical appearance. After 30 days of storage, physical appearance for different patches was found same. So, it may be concluded that mucoadhesive patch using these excipients are very much effective & stable too because it was found stable even after 3o days after the formulation.

The buccal patch mentioned above showed a proportionate amount of edoema. Swelling index has a big impact on the buccal patch's physical features. It reveals that the rate of medication release and dissolution is higher in saliva. This enables the formulation to be absorbed and have a good bioavailability in the bloodstream. Swelling ability increased, but as time passed, swelling diminished, most likely due to erosion or another process. SFD permeability was boosted in in vitro drug release assays, and the medication was destroyed in higher proportions in the majority of cases. Its most Bioadhesive formulation is F6, according to ex vivo bio adhesion. Ex-vivo permeability experiments show that just a small amount of Sulfadiazine can pass through the epidermis layer, indicating that the film can be used for topical application safely. In vivo tests show that the Sulfadiazine film (F6), which was chosen owing to its physicochemical properties, promotes epidermis layer renewal and is more beneficial on epithelization, fibrous tissue thickness, and angiogenesis than the commercial entity.

This study showed that pregabalin and gabapentin reduced thermal hyperalgesia in treated rats and had an anti-allodynic effect. When compared to gabapentin, pregabalin was the most successful at reducing neuropathic pain brought on by paclitaxel.

More thorough research with multiple doses and animal models, this study showed that pregabalin and gabapentin reduced thermal hyperalgesia in treated rats and had an anti-allodynic effect. When compared to gabapentin, pregabalin was the most successful at reducing neuropathic pain brought on by paclitaxel.

The HPMC, HEC, and CH are a few mucoadhesive polymers that have potential in the creation of film formulation because they create a swellable polymeric matrix that can regulate drug release. The plasticizer and stabilizer utilised was PEG 400. High bio-adhesion characteristics in the cellulose-derived polymers may reduce formulation leakage and disorder. The created films had good visual and formulation performance, as well as adequate mechanical characteristics and malleability. Additionally, the swelling behaviour of the polymers affects the bioadhesion of films. A suitable macromolecular mesh-like biodegradable polymer network emerged when the mucoadhesive polymers hydrated, improving the interpenetration of polymers and bio-adhesive strength.

F1, F2, F5 & F6 showed % drug release as 91.6±0.81, 85.8±0.34, 77.3±0.27 & 76.5±0.52 respectively. F 3 & F 4 exhibited % drug release as 82.4±0.33 & 78.8±0.81 respectively. In results, an excellent in-vitro release rate of the formulation was demonstrated by the buccal patch. In order to estimate stability profile of mucoadhesive patches, it was evaluated for physical appearance. After 30 days of storage, physical appearance for different patches was found same. Pregabalin incorporated in the mucoadhesive patches may be frequently employed in the modulation of numerous CNS activities with better absorption.

CONCLUSION

For patient compliance, buccal Patches drug-delivery systems were created, notably for children, seniors, people who have trouble swallowing, people who have tremors, people who are physically challenged, and people who travel. Sometimes it is not feasible to obtain the water needed to administer the medication in the form of a pill or capsule. Patients who experience swallowing issues or who are working in locations where it is difficult to access water sources. Fast dissolving tablets can help to provide the dose when patients are in situations when they are unable to swallow a pill, such as when they are unconscious, travelling, young children, or elderly. It showed a decent in vitro scattering time alongside rich appearance and other actual

attributes like elasticity, % lengthening, collapsing perseverance. P2 was chosen dependent on its outcomes on performed assessment which were all ideal and furthermore had great mechanical properties.

It concluded, a better ex-vivo release time alongside better appearances and other properties i.e., spreadibility, stability etc. While P2 was exhibited better assessment in terms of folding intensity. It would be very beneficial for the for the management of neuropathic pain as stable mucoadhesive patches consisting pregabalin.

Pregabalin incorporated in the mucoadhesive patches may be frequently employed in the modulation of numerous CNS activities with better absorption. This study recommends to fellow researchers to validate the mucoadhesive patches of pregabalin in terms of better pharmacokinetic profile in-vitro and later in human beings too. It must also emphasize on designing into dosage form and determine the efficacy in terms of lowering the neuropathic pain and also the adverse effects that will confirm for its actual and permitted dose needed to kept in dosage form.

FUNDING
Nil.

CONFLICT OF INTEREST
Authors have declared for none conflict of interest.

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