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Original Article

FABRICATION OF AN ABUSE DETERRENT AND MICROEMULSION-BASED SUBLINGUAL FILM OF BUPRENORPHINE HYDROCHLORIDE FOR BREAKTHROUGH PAIN MANAGEMENT

D. MUNDHEY[a*], N. SAPKAL[b], A. DAUD[a]

[a]Centre for Advanced Research and Innovation (CARIn), Zim Laboratories Ltd. B-21/22, MIDC Area, Kalmeshwar 441501 Dist. Nagpur (M. S.), India, [b]Gurunanak College of Pharmacy, Nari, Kamgarnagar, Nagpur (M. S.), India
Email: dmundhey1990@gmail.com

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ABSTRACT

Objective: The present research work aims to develop an abuse deterrent rapidly dissolving buprenorphine microemulsion loaded sublingual film for the treatment of breakthrough pain.

Methods: The Buprenorphine microemulsion loaded sublingual films were prepared using Capmul MCM C8 (oil), tween 20 (surfactant) and propylene glycol (co-surfactant) with different grades of film-forming polymer (HPMC) using film casting machine. The films were evaluated for *in vitro* disintegration and dissolution study, tensile strength, folding endurance, content uniformity, surface pH, thickness and weight variation, % loading of buprenorphine microemulsion in sublingual film, scanning electron microscope, *ex vivo* permeation study, droplet size and polydispersity index, Zeta potential, % moisture content, stability and abuse deterrent potential were evaluated.

Results: The optimized film formulation showed desired mechanical properties with minimum disintegration time of 21s and exhibited 34.45 % loading of Buprenorphine microemulsion. Permeation studies through goat sublingual mucosa, indicated 87% Buprenorphine release, through Buprenorphine microemulsion loaded sublingual film, whereas only 30% Buprenorphine release when it was directly added to film without microemulsion strategy.

Conclusion: The present study concludes that abuse deterrent and fast acting buprenorphine microemulsion-incorporated sublingual film of buprenorphine HCL and naloxone HCL is a promising alternative to mostly marketed buprenorphine injectable delivery systems and a non-invasive route of administration for breakthrough pain management.

Keywords: Buprenorphine HCl, Microemulsion, Sublingual film, Naloxone, Abuse deterrent

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INTRODUCTION

Buprenorphine hydrochloride (BU) is a partial agonist at mu (μ) and kappa (K) opioid receptor and antagonist at delta receptors used for the treatment of moderate to severe pain as well as chronic pain [1]. BU, being a tasteless drug and slightly acidic in nature, has two pKa values of 8.42 and 8.92 and logP of 3.4. Hence, at pH<8.4, it can be well absorbed [2]. The low oral bioavailability of BU (31%) limits its therapeutic utility and it also undergoes extensive first pass metabolism by hepatic cytochrome P-450 3A4 isozyme. Hence oral formulations of BU are not available in the market whereas parenteral, buccal and sublingual formulations are available [3-5]. With respect to buccal formulations, buprenorphine's buccal film Bunavail®is available in the market. This bilayered film increases the total bioavailability of BU to more than 40% in healthy subjects [6]. Bai *et al.*, carried out the pharmacokinetic study of BU buccal film formulation in healthy volunteers and the study revealed that bioavailability of BU was about 46 to 51% [7]. This indicates further research in the enhancement of the BU is to be carried out for better bioavailability.

Poor aqueous solubility of drug entities is today considered as a formidable challenge for pharmaceutical scientist, which is considered as an area of prime importance in the field of biomedical research. Hence lipid based formulations were chosen to overcome the above barriers and among them microemulsion (ME) as drug delivery systems have recently gained wide acceptance due to robust formulations perspectives, ease of production and practical enhancement of drug permeability [8]. The o/w microemulsion formulation enhances the sublingual and buccal bioavailability of lipophillic drug BU by facilitating transcellular (across the cell) and paracellular (between the cells) absorption. Thus literature review reveals lack of information about the bioavailability enhancement of poorly water soluble buprenorphine using microemulsion as drug delivery systems.

Also, opioid analgesics are foremost used for the treatment of breakthrough pain in cancer. To fight the transient breakthrough pain, it is necessary to achieve quick drug release from dosage form for the early onset of action for the purpose of pain management. This can be achieved with the help of a sublingual or buccal delivery of buprenorphine. The added advantage of the geometrical shape and larger surface area of thin films can be utilized to deliver an active drug sublingually [9]. Based on extensive review of literature, it revealed that controlled delivery buccal patches of buprenorphine has been developed using polyisobutylene, polyisoprene and carbopol 934P as bioadhesive polymer. Nearly 75% of the buprenorphine released after *in vitro* evaluation studies from the buccal patches following 24 h incubation period [10]. Also bilayed buccal film of BU is available with bioavailability of more than 40% in healthy subjects [11]. At the same time literature study also revealed that BU sublingual formulations are diverted and utilized outside of an established physician–patient relationship, both for self-medication of withdrawal symptoms and to produce euphoria [12, 13].

Thus, the current study was aimed to develop an abuse deterrent, fast dissolving microemulsion loaded sublingual film of buprenorphine for transient breakthrough pain. To achieve this objective, microemulsion is incorporated into the sublingual film for better sublingual bioavailability. But, since buprenorphine is having a high risk of abuse potential [14-16], naloxone is incorporated in a fixed ratio (1 mg of naloxone per 4 mg of buprenorphine) to deter abuse by parenteral route, such as nasal insufflations or injection. Naloxone hydrochloride (NA) has no therapeutic effect but still added to the formulation to prevent parental abuse. Because when patient tries to abuse this formulation parentally, naloxone binds to the receptor site in the brain and blocks the receptors, thus reducing the effect of BU and prevents the abuse potential of the formulation.

MATERIALS AND METHODS

Materials

BU and NA was purchased from Sun Pharmaceutical Industries Ltd. Propylene Glycol was purchased from Shell Chemicals, Singapore.

Capmul® MCM C8 (Mono/diglycerides of caprylic acid) was obtained as a gift sample from ABITEC Corporation, Columbus, USA. Monebat®-20 (Polyoxyethylene 20 sorbitan monolaurate) was obtained as a gift sample from Mohini Organics Pvt. Ltd. Malad (West), Mumbai.

Methods

Preparation of BU microemulsion loaded and NA sublingual films

Polymeric ME drug loaded sublingual film was prepared using glass plate and further optimized bates were casted using film casting machine at 1.5 V and wet thickness of 0.50 mm and dimensions of film was set at 20 × 22.5 mm and dried immediately. Preparation of BU microemulsion and polymeric solutions used to make the films are as follows. Weigh each ingredient accurately as described in table 1 to prepare formulations F1 to F27. Initially, BU microemulsion was prepared using Capmul MCM C8 (oil), tween 20 (surfactant) and propylene glycol (co-surfactant) using water titration method. In C2 optimized microemulsion Smix ratio was 1:1 (table 1) and in A2 it was 2:1 (table 2) [17]. This resulted into formation of clear microemulsion and to it calculated quantity of BU was added and sonicated for 30 min. Then alpha tocopherol acetate and BHA (as an antioxidant), sucralose (sweetener), sunset yellow (color) and orange flavor were added and further sonicated for 20 min to obtain solution A. Separately, HPMC E15, HPMC E50 was dissolved in water by continuous stirring and allowed to swell for 60 min until a clear solution was obtained. NA was added to aqueous solution of polymers and mixed to obtain a homogeneous mixture to obtain solution B.

Then, BU loaded ME i.e. solution A was mixed with hydrated polymeric solution i.e. solution B with continuous stirring on mechanical stirrer (Remi motors, Remi Electrotechnik Ltd., Vasai, Mumbai, India) for nearly 40 min to obtain a homogeneous mixture i.e. solution C. Finally, solution C was cast on the film casting machine and allowed to dry at 50 °C for 40 min. On removal from the release liner, the film was checked for any imperfections before being cut into 20 × 22.5 mm squares. Further Scale up trail bathes with increase in batch size was taken as shown in table 3.

Table 1: Composition of BU microemulsion loaded sublingual films with S_{mix} ratio of ME as 1:1 (optimized C2 ME)

Ingredients (%)		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Buprenorphine HCl		-	-	-	-	-	-	-	-	-	-	3.21
Naloxone HCl		-	-	-	-	-	-	-	-	-	-	0.91
C2 ME	Capmul	14.65	13.09	11.37	10.87	10.73	8.19	6.83	6.67	6.88	6.62	6.20
S_{mix} (2:1)	MCM C8											
	Tween-20	42.97	38.41	33.35	31.88	31.47	24.01	20.09	19.56	20.18	19.41	18.19
	PG	19.49	17.42	15.13	14.46	14.27	10.89	9.08	8.87	9.15	8.80	8.25
	H2O in ME	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
HPMC E5		16.96	15.16	13.17	12.58	-	-	-	-	-	-	-
HPMC E15		5.08	15.16	13.17	17.00	31.11	47.43	52.99	54.08	63.79	65.17	63.24
Xanthan gum		0.84	0.75	0.65	0.62	-	-	-	-	-	-	-
PVA		-	-	6.58	6.29	6.21	9.48	11.07	10.82	-	-	-
Glycerin		-	-	6.58	6.29	6.21	-	-	-	-	-	-
D. M. water		q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.

Table 2: Composition of BU microemulsion loaded sublingual films with S_{mix} ratio of 2:1 (optimized A2 ME)

Ingredients (%)		F12	F13	F14	F15
Buprenorphine HCl		-	-	-	3.07
Naloxone HCl		-	-	-	0.87
A2 ME	Capmul MCM C8	7.39	7.55	7.27	6.83
Smix (1:1)	Tween-20	14.54	14.86	14.30	13.43
	PG	14.54	14.86	14.30	13.43
	H2O in ME	q. s.	q. s.	q. s.	q. s.
HPMC E15		53.72	62.72	64.13	62.36
PVA		10.74	-	-	-
D. M. water		q. s.	q. s.	q. s.	q. s.

Table 3: Composition of scale up batches of BU A2 ME loaded sublingual films on film casting machine

Ingredients (%)		F16	F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27
Buprenorphine HCl		-	-	-	-	-	-	-	-	-	-	3.93	3.93
Naloxone HCl		-	-	-	-	-	-	-	-	-	-	1.11	1.11
A2 ME	Capmul MCM C8	6.85	8	9.6	10.66	10.21	10.21	8.73	8.73	8.73	9.6	8.73	8.73
	Tween-20	13.48	15.73	18.88	20.97	20.08	20.08	17.16	17.16	17.16	18.88	17.16	17.16
	PG	13.48	15.73	18.88	20.97	20.08	20.08	17.16	17.16	17.16	18.88	17.16	17.16
	H2O in ME	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
HPMC E 15		61.44	55.01	46.02	40.02	42.57	25.53	41.82	41.82	41.82	30	28.07	24.44
HPMC E 50		-	-	-	-	-	17.02	-	-	-	16	14.54	16.36
Carbopol 971 P		-	-	-	-	-	-	9.09	-	-	-	-	-
Sodium CMC		-	-	-	-	-	-	-	9.09	-	-	-	-
Polyox N80		-	-	-	-	-	-	-	-	9.09	-	-	-
Sucralose		1.43	1.66	2	2.22	2.12	2.12	1.81	1.81	1.81	2	1.81	1.81
Orange flavor		0.71	0.83	1	1.11	1.06	1.06	0.90	0.90	0.90	1	0.91	0.91
Sunset yellow color		0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.01
Alpha tocopherol acetate		2.14	2.5	3	3.33	3.19	3.19	2.72	2.72	2.72	3	5.45	7.27
D. M. water		q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.

Characterization of BU microemulsion loaded and NA sublingual film

***In vitro* disintegration study**

In vitro disintegration of film was performed as described. Initially, the film was carefully clamped from both the side and placed in a beaker in such a way that water level should be at half level of the film. The time required to break the film was recorded as disintegration time.

Mechanical characterization

Tensile strength

The prepared films were subjected for the determination of mechanical properties using using LINUX Tensile Tester (model TEN MD), LINUX Machine Incorporation, Thane, Maharashtra, India, instrument according to the procedure described as follows [18]. The films of dimensions 30×5 mm were cut and subjected for the analysis. Film specimens with physical defects were discarded. The films were carefully placed between the two vertical grips of the tester during the test. The movable grip was then driven upward with a speed of 30mV/min until the rupture of the film. From the recorded load extension profiles, the tensile strength, percent elongation at break was calculated.

Folding endurance

The folding endurance was determined by repeatedly folding one film at the same place without breaking [19]. Folding endurance is used to estimate the mechanical property of a film [20].

Content uniformity

Content uniformity of films was determined with the assay of ten individual films. Each film was transferred to a 20 ml volumetric flask and dissolved and extracted in methanol. Drug extracted in methanol was analyzed using validated high-performance liquid chromatography (HPLC) method [21]. Briefly, drug analysis was performed PrincetonSPHER-100 C18 HPLC column (250 mm × 4.6 mm, 5 µm) with the mobile phase consisting of acetonitrile and 10 $mmolL^{-1}$ potassium phosphate buffer adjusted to pH 6.0 with triethanolamine (83:17, v/v) at a flow rate of 1 ml/min. Sample injection volume was 20 µL and BU detection was performed using Shimadzu LC solution software at a wavelength of 284 nm.

Drug content

Five films were picked randomly and weighted individually. Each film was agitated in methanol and the mixture was suitably diluted and analyzed as per developed and validated HPLC method [21]. The average drug content was calculated.

***In vitro* dissolution**

The *in vitro* dissolution of the formulations was studied as per the US FDA-recommended dissolution methods [22] in 500 ml distilled water at 37.0±0.5 °C using the USP Apparatus II (Paddle) at 100 rpm. At predetermined time intervals for 30 min, 10 ml of aliquots was withdrawn and replaced with an equal volume of fresh distilled water to ensure sink condition. Aliquots were then analyzed using developed and validated HPLC as described in "Content uniformity." This *in vitro* dissolution study was performed for optimized formulation F11 and F15 (table 1 and 2), developed with proposed strategy of BU microemulsion incorporated into sublingual film. The release profile of these C2 ME (F11) and A2 ME (F15) sublingual film were compared with plain BU sublingual film in which ME was not added.

Surface pH

The surface pH of the film formulation was determined by wetting the film surface with water and the pH measured using pH probe (Contech Instruments Ltd., Navi Mumbai, India) [23].

Thickness and weight variation

The thickness of films was measured using a digital micrometer screw gage (Mitutoyo, Microvision Calibration Services, Japan). For each formulation, three randomly selected films cut into 20 mm × 22.5 mm were used. Thickness was measured at five different points in the films and mean value was expressed [23]. Each film was weighted individually on an analytical balance (Shimadzu, Japan) and average weight calculated [24].

Percent loading of BU ME in sublingual film

The percent loading of BU ME in sublingual film was determined by calculating the amount of ME (g) added to the total casting solution of hydrated polymers (g). The film has been casted till no more evidence of oiliness was observed on the surface of casting plate after removal of dried sublingual strips. The percent loading of BU ME in sublingual film was calculated from the following equation:

$$\text{Percent loading of BU ME in sublingual film} = \frac{\text{Amt of BU ME added to casting solution (g)}}{\text{Total weight of casting solution (g)}} 100$$

Scanning electron microscopy (SEM)

The morphological characteristics of the films were studied using SEM. The purpose of the morphological study was to evaluate the film samples for the presence of any deformities, microemulsion droplets and cracks. The samples were examined in a Phenom G2 Pro/G2 pure, Eindhoven, Netherlands, scanning electron microscope at an acceleration voltage of 10 kV.

***Ex vivo* permeation study**

The *ex vivo* sublingual permeation of BU through the goat sublingual mucosa was performed using a modified Franz glass diffusion cell [25]. Goat sublingual mucosa was obtained from the slaughter house and mounted between the donor and receptor compartments. The developed sublingual film was placed on the smooth surface of mucosa by gentle pressing and the compartments were clamped together. The donor compartment was moistened with 1 ml of distilled water (pH 6.2) and the receptor compartment was filled to touch the membrane with distilled water. The fluid motion in the receptor compartment was maintained by stirring with a magnetic bead at 100 rpm and its temperature was maintained at (37±0.2) °C. At predetermined time intervals, a 1 ml sample was withdrawn (replaced with fresh medium) and analyzed by developed and validated RP-HPLC method [21]. Data analysis was done to calculate steady-state fluxes (J_{SS}).

Droplet size, polydispersity index (PDI) and Zeta potential

Re-dispersion (o/w microemulsion re-dispersed from films) of optimized F27 (BU A2 ME) sublingual film was performed to measure particle size and PDI using dynamic light scattering (DLS) (Malvern Zetasizer ZEN3500, UK) and zeta potential using the nanopartica SZ-100 (Horiba Scientific Ltd., Japan). Rectangular films with an area of 4.5 cm^2 were placed in 10 ml of de-ionized water. All measurements were performed with a scattering angle of 90˚ at 25.0˚C after dilute the dispersion to an appropriate volume with dispersion medium viscosity 0.894 mPa. s. A small-volume disposable zeta cell was used to measure the electrophoretic mobility (µm/s) and converted to zeta potential by software using the Helmholtz-Smoluchowski equation.

% Moisture content (Karl Fisher titration method)

Compact volumetric KF titrator (Metrohm, 915 KF Ti-touch, Swiss made) was used for the determination of water content in sublingual film. Then film sample of 100.0 mg, was added to glass container containing sufficient quantity of KF reagent for complete standardization and analysis of sample. Then water content was calculated automatically by the apparatus.

Stability study

Stability study was performed at room condition and at 40˚ C/75% RH for 4 w. Each strip of optimized batch F11 and F15 was packed in three layered laminated aluminium pouch and ten pouches are packed in a carton. After 4 w, the films were evaluated for the physical appearance, surface pH and drug content.

Abuse deterrent potential of developed sublingual film

An important component of modern pain management includes prescription opioid products. However, abuse and misuse of these products has created a serious health problem. An important step

for creating safer opioid analgesic has been the development of opioids that are formulated to deter abuse. These developed sublingual films are an abuse deterrent opioid formulation and evaluated as per US-FDA guidance to demonstrate that a given formulation has abuse deterrent properties [26]. In order to assess the impact of a potentially abuse deterrent products, the premarket studies are performed that are discussed.

Laboratory based *in vitro* manipulation and extraction studies

The goal of laboratory based extraction studies are to evaluate the ease with which the potentially abuse deterrent properties of a formulation can be defeated or compromised. The ease with which the opioid are extracted from intact and manipulated product should be determined using a variety of solvents that are commonly available (e. g. water, vinegar, ethanol, iso-propanol, acetone, mineral spirits) and those that have potentially relevant solvent characteristics (e. g. pH, polarity etc). Accurately weight 145 mg of optimized BU A2 ME and NA sublingual film (which contains 4.0 mg BU and 1.0 mg NA) was extracted in each 25.0 ml of all above mentioned solvents by sonicating for 30 min. 1.0 ml of resulting solution was pipetted at time interval of 10 and 30 min in 10.0 ml of volumetric flasks and volume made with mobile phase (BU 40 μgml^{-1} and NA 10 μgml^{-1}). The resulting solution was filtered using 0.45 µm Polytetrafluorethylene (PTFE) filter into standard analytical glass vials and analysed using HPLC. Drug extracted in respective solvents were analyzed using developed and validated HPLC method [21].

RESULTS AND DISCUSSION

Optimization of BU microemulsion loaded and NA sublingual film formulation

In the present study BU microemulsion was incorporated into the sublingual film and for that BU C2 ME with S_{mix} ratio of 1:1 and BU A2 ME with S_{mix} ratio of 2:1 was selected and quantities of each ME was fixed as per solubility, to incorporate 2.16 mg dose of BU. Polymers with different viscosities such as HPMC E5, E15 and E50 were selected and other polymers like xanthan gum and PVA were used for development of immediate release sublingual film. These trial batches were evaluated for its film forming capacity. Films with xanthan gum have poor film forming capacity; whereas film with HPMC polymer has good film forming capacity. HPMC E15 grade has good film forming capacity as compared to E5. In each of the trial batch the concentration of HPMC E15 was increased in order to obtain the complete incorporation of microemulsion into the sublingual film.

For trial batches F5 to F10, oiliness was observed on the surface, whereas for optimized lab trial F11, 65.95% of HPMC E15 was sufficient to completely incorporate the C2 ME and in F15, 64.92% of HPMC E15 was sufficient to completely incorporate A2 ME into the sublingual film with no oiliness observed. This exhibited the complete % loading of ME in sublingual film. Further scale up trials from F16 to F27 were performed on film casting machine by keeping drying temperature at 50° C. Being the sublingual formulation the film weight should be minimum for better acceptance. Use of low viscosity HPMC E15 resulted into higher film weight, which was not acceptable for sublingual administration. HPMC E15 when used in quantity below 43.01 mg/film, not sufficient to completely load A2 ME into the film. In further scale up batches higher viscosity grade i.e. HPMC E50 was used and also carbopol 971 P, sodium CMC and polyox N80 used in combination. F27 was considered optimized scale up batch (fig. 1.), in which HPMC E15 used with HPMC E50 to sufficiently reduced the total quantity of polymer required and also achieved complete loading of A2 ME into the sublingual film without any surface oiliness observed on polyethylene base sheet after removal of the sublingual film from the base sheet.

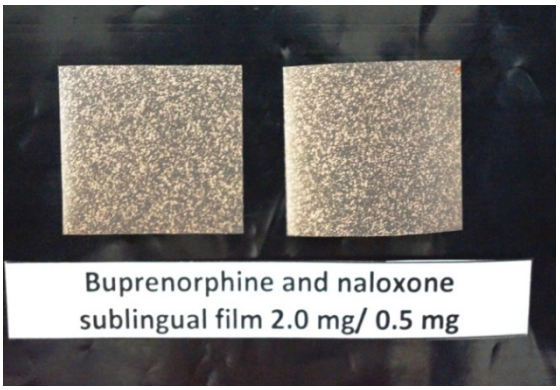


Fig. 1: Representative image for optimized buprenorphine and naloxone sublingual film (F27)

Characterization of BU microemulsion loaded and NA sublingual films

Physicochemical characteristics of the sublingual film

The physicochemical properties of the ME loaded sublingual film formulations are shown in table 4. Weight variation values (mg) of optimized films were in the range of 70.22–71.45 mg. The average thickness of all these films ranged from 61.68 to 81.75 µm. Thus there was proportional gain in weight of films with that of increase in the thickness of films. The optimized sublingual film formulation i.e. BU C2 ME sublingual film (F11) and BU A2 ME sublingual film (F15) showed the value of folding endurance>20 (table 4). This value is desirable because it would not allow easy dislocation of the films from the site of application or breaking of film during administration. The pH values of optimized films indicate that it will not produce any local irritation upon contact with the sublingual mucosa, as it is in the range of salivary pH (5.0–7.0) [27]. The slightly acidic pH of the formulation was due to the slightly acidic nature of the ME incorporated into the sublingual film. This slightly acidic pH of the film (F15) helps to deliver the BU in unionized form at the absorption site for better bioavailability. Disintegration time of both optimized sublingual film formulation was found to disintegrate in less than 30 sec and has sufficient mechanical strength to bear stress during transport and administration of the films.

Table 4: Physiochemical characteristics of the optimized BU Microemulsion loaded and NA sublingual films

Evaluation parameters	F11 (BU C2 ME)	F15 (BU A2 ME)
Thickness (µm)	61.68±1.79	81.75±1.20
Weight (mg)	70.22±0.24	71.45±0.82
Folding endurance	21.0±1.0	29.33±1.53
pH at 25° C	6.46±0.04	5.87±0.04
Tensile strength (kg/cm^2)	0.305	0.405
% Elongation at break (%)	2.133	2.066
Disintegration time (sec)	18.67±1.52	21.33±1.15
Microemulsion loading (%)	32.56	34.45
Drug content (%)	101.6±0.35	102.8 ±1.34

***In vitro* dissolution study**

BU A2 ME sublingual film (F15) showed highest drug release of 96% and BU C2 ME sublingual film (F11) showed 62% of drug release within 3 min; whereas plain BU sublingual film showed relatively less amount of drug release of 23% within 3 min (fig. 2.). This exhibited that the proposed strategy of ME incorporated sublingual

film reduces the particle size by forming o/w type of ME droplets, helps in increasing the surface area and, hence, modulates the drug release with a faster rate as compared to the release rate of plain BU sublingual film.

Key attributing factor for differential dissolution profile of F15 and F11 are varying concentration of S_{mix} ratio (T-20: PG). This is attributed due to higher solubility of BU in co-surfactant i.e. PG and its higher concentration was present in F15 formulation i. e S_{mix} ratio was 1:1 in F15, whereas less in F11 i.e. 2:1.

Hence BU A2 ME sublingual film (F15) was considered as an optimized formulation based on its *in vitro* dissolution profile and further subjected towards scale up trials and evaluated for SEM studies, content uniformity, droplet size, PDI, ZP and *ex vivo* permeation study using goat sublingual mucosa.

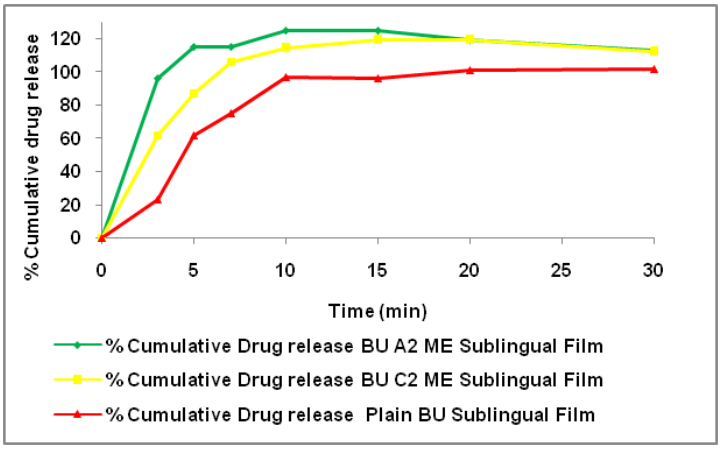


Fig. 2: *In vitro* release profile of buprenorphine through BU A2 ME sublingual film (F15), BU C2 ME sublingual film (F11) and plain buprenorphine sublingual film

Content uniformity

The content uniformity of optimized BU A2 ME sublingual film (F27) was found to be within the acceptance criteria. The % RSD was observed to be less than 2% which indicates uniformity of content.

Scanning electron microscopy (SEM)

SEM images of BU A2 ME (F15) and placebo film developed without addition of microemulsion was shown in fig. 3 and 4. Well dispersed BU microemulsion was obtained in F15, whereas microemulsion droplets were not observed in the placebo films developed without the addition of microemulsion. SEM images of vaccum oven dried microemulsion incorporated film showed spherical shaped nanoparticles were embedded in the polymeric matrix.

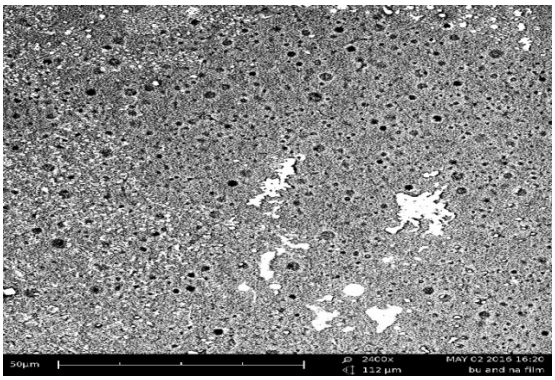


Fig. 3: Scanning electron microphotograph of buprenorphine microemulsion loaded and naloxone sublingual film BU A2 ME (F15)

***Ex vivo* permeation study**

Permeation studies through goat sublingual mucosa indicated that the % cumulative release of buprenorphine from BU A2 ME sublingual film (F15) was 87%, plain BU sublingual film was 30% and for naloxone was negligible for nearly 120 min. This indicates that the extent of permeation of buprenorphine from F15 was around 2.9 folds higher than that of plain buprenorphine sublingual film (fig. 5.). Steady state flux (J_{ss}, µgcm/h) were calculated and found 37.176 $\mu gcm^{-2}h^{-1}$ for F15, 23.056 $\mu gcm^{-2}\ h^{-1}$ for plain BU sublingual film and 5.210 µgcm/h for naloxone sublingual film. This evidenced an enhanced permeation of BU when incorporated in microemulsion form in sublingual film than over plain buprenorphine sublingual film and negligible permeation for naloxone across sublingual mucosa. Literature references are also available for negligible bioavailability of naloxone via the sublingual or oral route [28, 29]. Naloxone an opioid antagonist having as such no therapeutic effect but still added to the buprenorphine sublingual film formulation in order to only deter the intravenous abuse of this formulation, which is mainly done by patients for euphoretic effect [30].

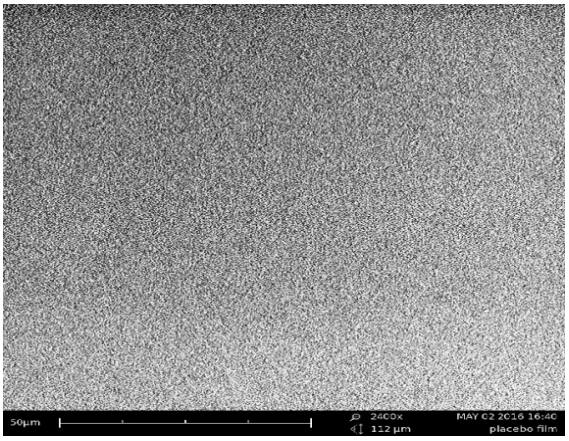


Fig. 4: Scanning electron microphotograph of the placebo sublingual film without the addition of buprenorphine microemulsion

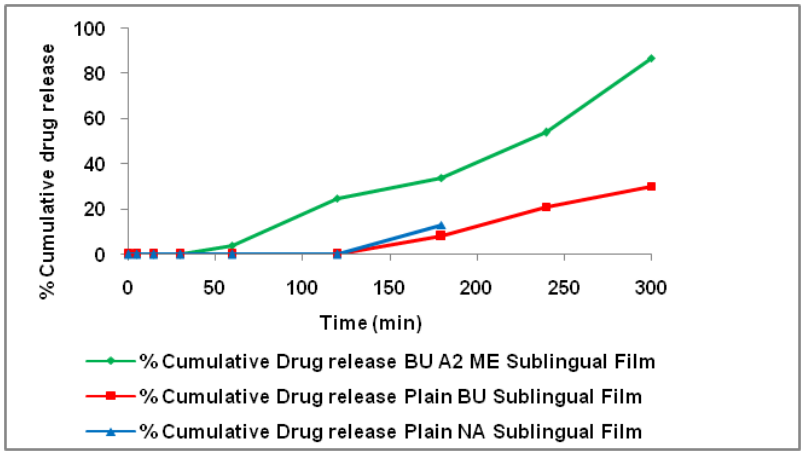


Fig. 5: *Ex vivo* release profile of buprenorphine through BU A2 ME sublingual film (F15), plain buprenorphine sublingual film and plain naloxone sublingual film

Droplet size and polydispersity index (PDI)

Droplet size of optimized F27 (BU A2) microemulsion obtained after re-dispersion of sublingual film was 151.5 nm and PDI was 0.445 which confirmed narrow size distribution of oil droplets (fig. 6.) [31, 32]. The increased particle size of BU A2 microemulsion after re-dispersion from sublingual film was may be due to covering of the embedded microemulsion with polymeric matrix of sublingual film.

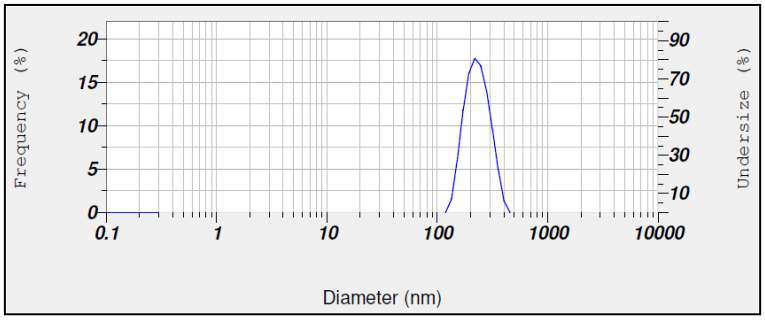


Fig. 6: Particle size distribution plot of optimized F27 (BU A2 ME) sublingual film

Zeta potential measurements

Zeta potential of microemulsion present in optimized BU A2 ME sublingual film (F27) obtained after re-dispersion of film was-21.7 mV. Despite the low ZP value the system remains stable [33]. The graph of intensity (a. u.) vs ZP (mV) of A2 ME obtained after re-dispersion from the sublingual film was exhibited in the fig. 7.

% Moisture content (Karl Fisher titration method)

The water content of the optimized F27 (BU A2 ME) sublingual film was found to be 7.16 % and its Karl Fischer titration curve graph was shown in fig. 8.

Stability study

The stability study of film formulations were carried out at room condition and at 40° C/75% RH for a period of one month. The films does not show any change in appearance and flexibility. The drug content and surface pH was found to be almost constant for upto one month.

Abuse deterrent potential of developed sublingual film formulation

Abuse deterrent studies as per US-FDA guidance for industry should be conducted in small amount of solvents i.e. 5-10 ml and sampling time should start early (e. g. 30 seconds) and continue until at least 80% of the opioid has been released or if 90% of the opioid can be extracted under a set of conditions in 10 min, then there is no need to test the same conditions for 30 min [26].

BU and NA sublingual film dissolved completely in water, vinegar, hydrochloric acid buffer pH 2.0 and phosphate buffer pH 6.0 and pH 8.0, respectively and resulted into turbid solution after 30 min of extraction in all the extraction media (fig. 9. (a, e, g, h) except in hydrochloric acid buffer pH 2, clear solution as obtained (fig. 9. (f)). However the BU and NA sublingual film during abuse deterrent studies remained intact in ethanol, iso-propanol and acetone extracting media, whereas a slight jelly type complex was formed in ethanol extracting media (fig. 9. (b, c and d)), respectively.

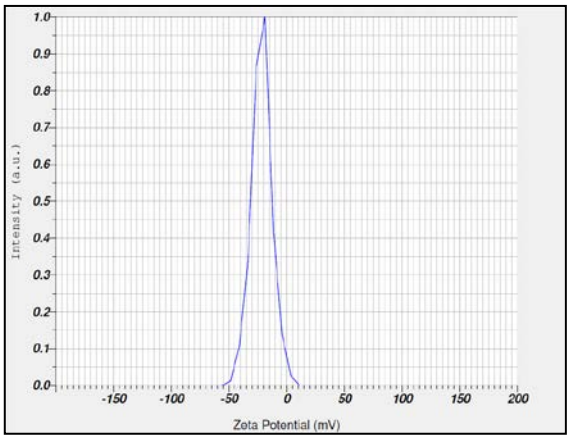


Fig. 7: Zeta potential plot of optimized F27 (BU A2 ME) sublingual film

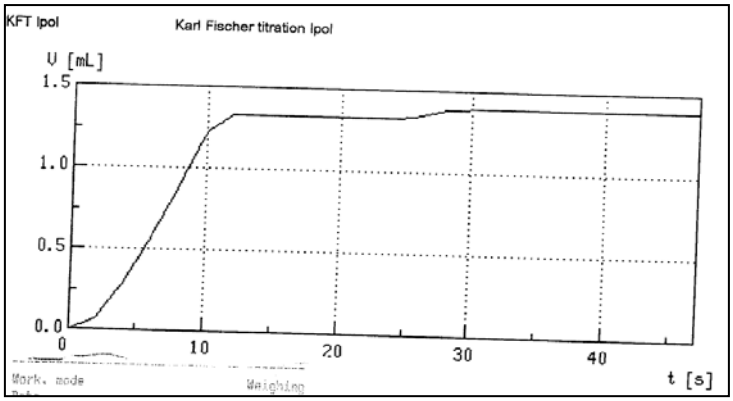
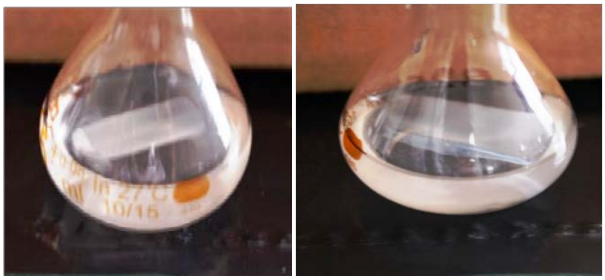
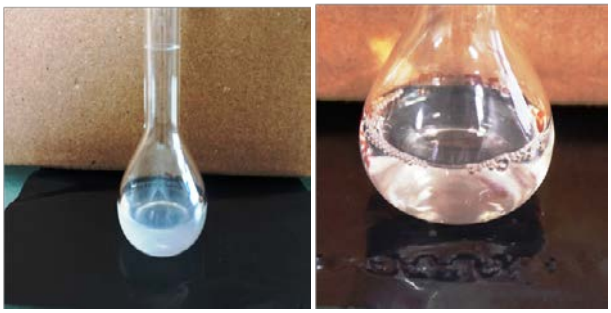


Fig. 8: Karl fischer titration curve for optimized F27 (BU A2 ME) sublingual film

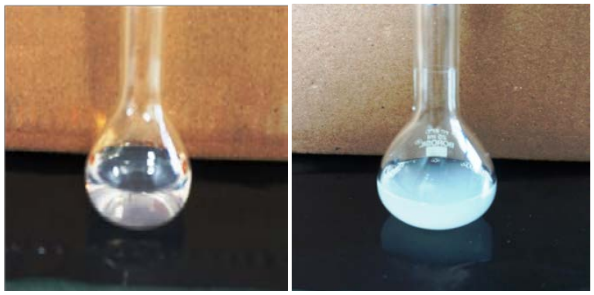
(a) **(b)**



(c) **(d)**



(e) **(f)**



(g) **(h)**

Fig. 9: Representative images for laboratory based *in vitro* manipulation and extraction studies for BU A2 ME and NA sublingual film in (a) Water (b) Ethanol (c) Iso-propanol (d) Acetone (e) Vinegar (f) pH 2.0 (g) pH 6.0 (h) pH 8.0

Thus abuse deterrent studies passes for BU and NA sublingual film in all the extracting media, as about more than 80% of opioid antagonist NA was extracted in respective extracting media in 10 and 30 min of extraction studies as per US-FDA guidance (table 5).

Table 5: Laboratory based *in vitro* manipulation and extraction studies for BU A2 ME and NA sublingual film in variety of solvents

S. No.	Extracting medium	Amount of NA extracted (%)		Amount of BU extracted (%)	
		10 min	30 min	10 min	30 min
1	Water	93.1	100.1	73.4	93.3
2	Ethanol	74.5	89.5	68.1	93.3
3	Iso-propanol	83.2	98.4	79.9	100.2
4	Acetone	80.2	94.1	88.4	100.6
5	Vinegar (5% acetic acid)	85.1	86.8	89.3	98.2
6	Hydrochloric acid Buffer pH 2.0	56.8	96.9	43.0	93.1
7	Phosphate Buffer pH 6.0	88.0	104.1	86.8	99.3
8	Phosphate Buffer pH 8.0	56.4	89.6	43.8	83.5

CONCLUSION

Two processes namely, preparation of buprenorphine microemulsion and their incorporation in sublingual film formulation was integrated successfully. SEM studies revealed that spherical o/w microemulsion droplets of buprenorphine were incorporated in the polymeric matrix of sublingual film. It can be revealed from *in vitro* dissolution and *ex vivo* permeation studies through goat sublingual mucosa that, BU microemulsion-incorporated sublingual film has enhanced the release rate of drug over the plain buprenorphine sublingual film. Further abuse deterrent studies revealed that the developed sublingual film was abuse deterrent one as 90% of opioid antagonist naloxone was extracted from the film within 30 min as per US-FDA guidelines. Hence, the present study concludes that abuse deterrent and fast acting buprenorphine microemulsion-incorporated sublingual film of buprenorphine HCL and naloxone HCL is a promising alternative to mostly marketed buprenorphine injectable delivery systems and a non-invasive route of administration for breakthrough pain management.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors report no conflicts of interest.

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