Annals of Biology **39** (1) : 30-34, 2023

Impact Score : 0.32 (Scopus)

Bioenhanced Bilayer Sublingual Films Containing Antimigraine Drugs Using Microencapsulated Bioenhancer: *in vivo* Evaluation in Humans

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(Received: October 7, 2022; Accepted: November 10, 2022)

ABSTRACT

Long-lasting migraine pain is one of the most disabling neurological disorders and requires a quick onset of action from the administered dosage form. This study aimed at providing sublingual administration of the frequently used combination of NSAIDs and triptans in order to trigger their action immediately by escaping the first pass metabolism. Sepitrap 80 and Sepitrap 4000 were used as bioenhancers to accomplish the faster systemic delivery of therapeutic agents during migraine attacks. In the present research, bilayer sublingual films were developed by joining the two loaded layers with zolmitriptan and piroxicam, respectively. Approximately 92% of zolmitriptan was released from the formed bilayer sublingual thin films within 3 min whereas 92% of piroxicam was released within 4.5 min from the best formulation. Within 30 min of the commencement of the pharmacokinetic investigation, plasma concentrations of the active component began to rise rapidly. When compared to commercial formulations, the developed films had a greater AUC and Cmax with a shorter Tmax, indicating a faster trigger of action and better bioavailability.

Key words: Bilayer films, sublingual films, pharmacokinetics, bioenhancers, migraine pain

INTRODUCTION

In the present stretch of increasing stress, neurological conditions have become the major causes of disability and death at global levels (Feigin *et al.*, 2019). Among the treatment options most commonly prescribed is symptomatic treatment of migraine pain, which includes nonsteroidal anti-inflammatory drugs (NSAIDs) as simple analgesics and triptans as migraine-specific agents. Various studies, including systematic reviews, meta-analysis and randomized placebo-controlled trials, have reflected that the combination of triptans and NSAIDs is more effective than their individual use in meeting the primary goal of acute therapy, which is aborting the pain immediately (Ong *et al.*, 2018). Many oral therapies for migraine lack effectiveness due to deficient absorption in response to migraine-induced gastric stasis, so other routes such as IV, sublingual and nasal are frequently utilized (Tfelt-Hansen, 2017). Zolmitriptan (ZOTP) is one of the top three triptans that have the highest pain-relief rates at two hours, whereas naratriptan is associated with fewer adverse effects (Jenkins, 2020). In NSAIDs, piroxicam (PRCM) with increased absorption has been shown to have a significantly greater analgesic effect than that of naproxen and is comparable with indomethacin. In another similar study of the management of acute migraine pain, sublingual piroxicam showed significant pain relief and magnificent tolerability.

MATERIALS AND METHODS

The bilayer sublingual films of ZOTP and PRCM were prepared by the solvent casting method. Finally, two separate drug-loaded layers were joined together to form a bilayer unit. Firstly, drugs were physically mixed separately with each bioenhancer Sepitrap 80 and Sepitrap 4000 in the motar pestle, which were kept undisturbed overnight, proven to enhance dissolution of various low-aqueous soluble drugs (Jagtap *et al.*, 2021). The compositions of five different bioenhanced bilayer sublingual thin films (BSTFs) are shown in Table 1. All preliminary evaluations such as appearance, surface pH, disintegration,

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Table 1. Composition of bilayer sublingual thin films (BSTF) containing piroxicam and zolmitriptan

S. No.	Ingredients	BSTF 1		BSTF 2		BSTF 3		BSTF 4		BSTF 5	
		L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
1.	Drug	2.5	10	2.5	10	2.5	10	2.5	10	2.5	10
2.	HPMCE15	15	15	15	15	15	15	15	15	15	15
3.	Pullulan	15	15	15	15	15	15	15	15	15	15
4.	PEG-400 (ml)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
5.	Mannitol	5	5	5	5	5	5	5	5	5	5
6.	Sucralose	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
7.	Citric acid	1	1	1	1	1	1	1	1	1	1
8.	Sepitrap 80	-	-	2.5	10	5	20	-	-	-	-
9.	Sepitrap 4000	-	-	-	-	-	-	2.5	10	5	20
10.	Mint (Flavour)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
11.	Ethanol (ml)	2	2	2	2	2	2	2	2	2	2
12.	Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

L1–Layer 1 (zolmitriptan) and L2–Layer 2 (piroxicam).

mechanical strength and drug release were conducted with standard procedure (Singh *et al.*, 2017).

A chicken pouch membrane was used in an *ex vivo* permeation investigation. The drugs from the BSTF-3 were tested for an *ex vivo* permeation study using a USP dissolution tester at 370.1°C. In 250 ml of phosphate buffer pH 7.4, the shafts were spun at 50 rpm. To maintain the same volume, 3 ml samples were removed at specified time intervals of 5, 10, 15, 30, 45, 60, 90, 120 and 240 min and replaced instantaneously with an equivalent quantity of new phosphate buffer pH 7.4 (El-Setouhy *et al.*, 2015). As previously stated, the drug concentration was evaluated using an approved UV-visible spectroscopic technique.

The stability test for produced sublingual bilayer films was carried out according to ICH recommendations at 40±2°C and 75±5% RH. After covering the optimal film formulations in butter paper and then putting them in an aluminium box, they were placed in the stability chamber for three months. The effect of three months of storage on the physio-chemical features of sublingual films was examined. The appearance, weight fluctuation, drug content, surface pH and drug release of the films were next assessed (Singh *et al.*, 2017).

Selection of healthy volunteers was done on the basis of the following criteria. Six healthy volunteers were enrolled in the study after getting their written consent and all were informed regarding the study of drugs. The study protocol complied with the declarations of Helsinki for humans (Holstila *et al.*, 2016) and was approved by the Teerthankar Mahaveer University Institutional Ethics Committee.

Three subjects were administered with single dose composed of 10 mg of piroxicam and 2.5 mg zolmitriptan in two different layers of sublingual film. The remaining three were administered with market formulations. The blood samples were withdrawn from the veins and collected in vacutainers at 0, 0.25, 0.50, 1, 2, 3, 5 and 8 h. Then the tubes were properly sealed, stored at -20 °C and transported for further analysis of the blood sample by using the HPLC-MS/MS method (Bhyan *et al.*, 2022). The various pharmacokinetic parameters Cmax, tmax, elimination half-life, area under the curve, area under the first moment curve and MRT were determined by using PK solver software (Zaman *et al.*, 2018).

RESULTS AND DISCUSSION

The FTIR peaks pattern of pure drugs when compared with selected formulation BSTF-3, showed that both the drugs were successfully loaded in the films without any interactions. Stability studies results as shown in Table 2 signified the stability of bilayer films. The fastest release of both drugs was observed from the formulation BSTF-3 which contained sepitrap 80 as a solubility and permeation enhancer at twice the weight of the drug. BSTF-3 that released approximately 92% of zolmitriptan was released within 3 min, whereas 92% of piroxicam was released within 4.5 min. From the analysis of *in-vitro* disintegration and dissolution tests, as formulations BSTF-2 and BSTF-4 showed longer disintegration and drug release time, they were excluded from the

Table 2. Evaluation of bilayer sublingual films (BSTF-3) during stability studies at 40°C and 75% RH

Time	Appearance	Weight (mg) Mean±S.D.	Drug content (%) Mean±S.D.		Surface pH Mean±S.D.
			Layer 1	Layer 2	
0 day	Elegant	107.20±0.55	98.63±1.93	96.01±1.31	6.82±0.036
1 month	No change	107.15±0.60	98.51±2.18	96.01±1.31	6.82±0.036
2 months	No change	107.10±0.60	98.43±1.75	96.01±1.31	6.82±0.036
3 months	No change	107.15±0.50	98.30±0.93	96.01±1.31	6.82±0.036

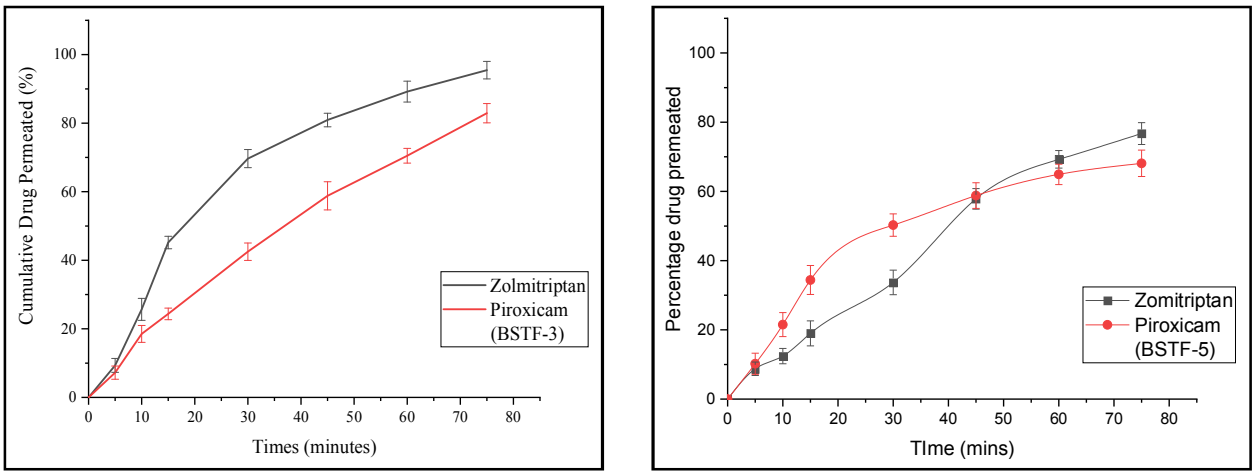

Fig. 1. The *ex-vivo* permeation of BSTF-3 and BSTF-5 containing zolmitriptan and piroxicam through used membrane.

further *ex vivo* permeability studies. Fig. 1 shows permeation profiles of bilayer sublingual films of piroxicam and zolmitriptan containing Sepitrap 80 (BSTF-3) and Sepitrap 4000 (BSTF-5) as bioenhancers. The permeation profile of BSTF-1 was used as control, which contained no bioenhancer. Thus, it showed the lowest permeation from the chicken pouch membrane. The permeation of both the drugs was found to be highest from the formulation containing Sepitrap 80 (BSTF-3) followed by BSTF-5. The greater drug penetration from Sepitrap 80 sublingual films might be attributable to a drug solubilization mechanism, a membrane contact mechanism, or both. As previously stated, all of the BSTFs showed complete drug dissolution after 9 min, indicating that the medication was totally free for absorption within 9 min. As a result, Sepitrap 80 increased drug penetration by interacting with the chicken membrane via microencapsulated polysorbate 80. Polysorbate 80 has previously been shown to improve drug penetration via buccal mucosal membranes. Sepitrap 80 increased the polysorbate 80 qualities as a bioenhancer not only by increasing membrane interaction properties and optimizing solubilization properties owing to the large surface area, but also by allowing large amounts of polysorbate 80 to be included into bilayer films. No significant changes were observed in the visual appearance such as colour, transparency, surface texture of the selected bilayer films. On completion of the storage period, the determined contents of zolmitriptan and piroxicam were observed to fall within an acceptable range. Additionally, the weight and surface pH of the investigated films were also satisfactory. Hence, the developed bilayer sublingual films presented a stable version of the sublingual dosage form. BSTF-3 had the quickest *in vitro* and *in vivo* disintegration times (26s) as well as the fastest *in vitro* dissolution rate (3-4 min). Furthermore, because Sepitrap 80 was included in the formulation, it demonstrated optimal bioenhanced absorption via the sublingual membrane. As a result, BEST-3 was chosen for *in vivo* pharmacokinetic research in comparison to the commercial formulation. Fig. 2 shows the mean plasma zolmitriptan and piroxicam concentration versus time curves after sublingual delivery of BEST-3 and a marketed formulation to six participants. However, Fig. 3 represents the initial chromatogram of plasma.

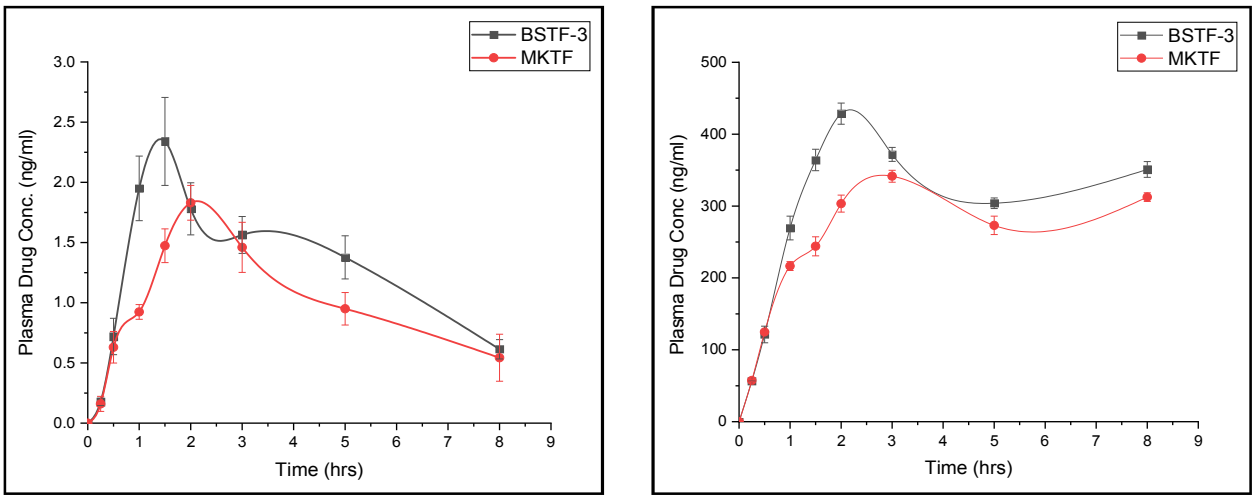

Fig. 2. The average plasma concentration of (a) Zolmitriptan and (b) Piroxicam versus time after administration of BSTF-3 and marketed formulation (MKTF) to three healthy human volunteers.

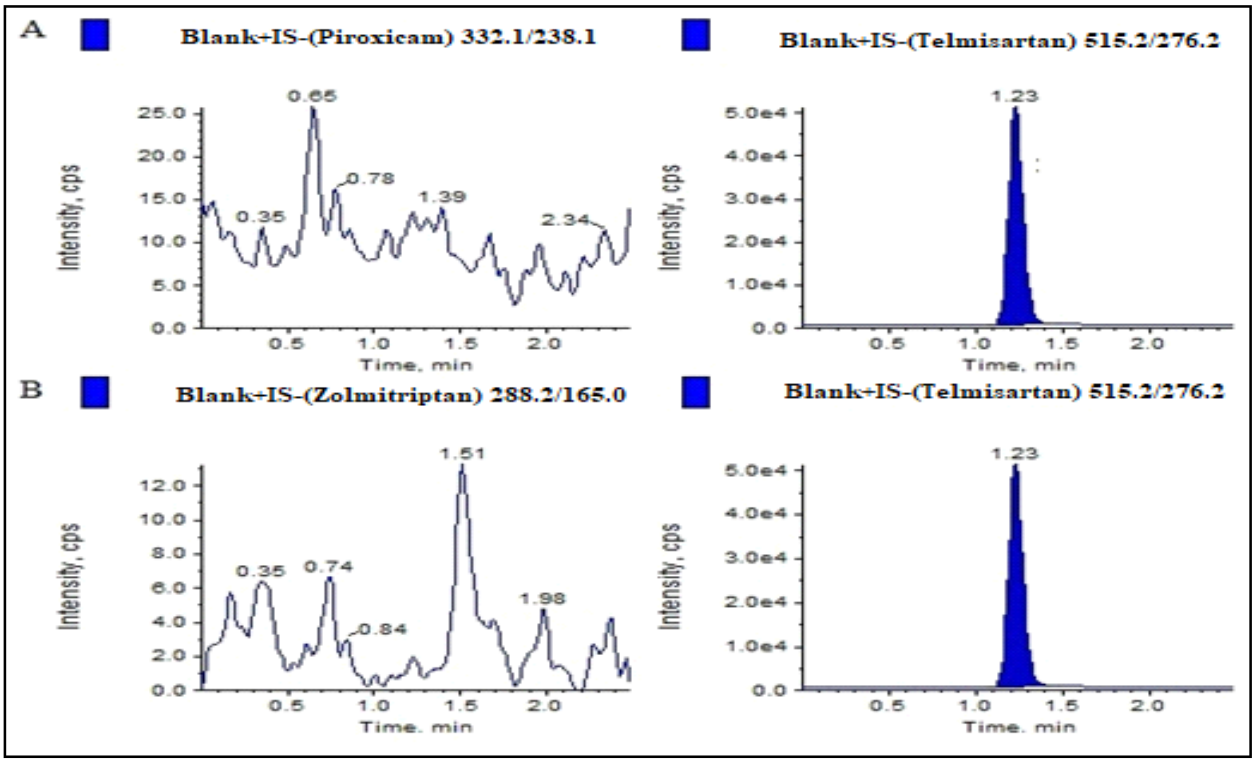

Fig. 3. Initial chromatogram of plasma with (a) Blank and (b) IS Temisartan.

The plasma concentrations of the active ingredients started to increase significantly within 30 min confirming that the developed dosage form had effectively delivered the drug into the systemic circulation. BEST-3 had a much greater Cmax and lower Tmax than the market product, indicating that the introduction of Sepitrap 80 as a bioenhancer has resulted in improved drug absorption from the sublingual mucosa. The quick drug absorption from BEST-3 was consistent with *ex vivo* permeation experiments, which demonstrated that zolmitriptan and piroxicam permeated better from bioenhanced bilayer sublingual films than from the marketed formulation. All other formulation components of the films promoted the release without hindering the absorption of zolmitriptan and piroxicam through the targeted route. Calculation of the Area Under the Curve (AUC) also supported the rationale of the study. The formulation BSTF-3 has shown its strength as

Table 3. Comparison of PK parameters of sublingual films and marketed formulation observed in humans

Parameters	Units	Zolmitriptan		Piroxicam	
		BSTF-3 Mean±S.D.	Marketed formulation Mean±S.D.	BSTF-3 Mean±S.D.	Marketed formulation Mean±S.D.
$t_{½}$	h	3.76±0.29	3.41±0.11	52.11±0.82	54.06±1.62
Tmax	h	1.33±0.28	2.0	2.0	3.0
Cmax	ng/ml	2.43±0.23	1.83±0.05	428.50±14.6	341.47±8.31
AUC 0-t	ng/ml*h	10.48±0.88	8.24±0.23	2541.84±70.6	2182.57±61.7
AUC 0-inf_obs	ng/ml*h	13.8±1.43	10.97±0.38	27736.56±86.4	26551.36±98.6
MRT 0-inf_obs	h	6.93±0.41	5.92±0.10	79.94±4.50	71.28±7.38

a valuable addition to the faster absorption of drugs for breakthrough pain. This technique could be useful for APIs other than piroxicam and zolmitriptan with which rapid action is desirable. Pharmacokinetic parameters determined after oral administration of BSTF-3 and marketed formulations of zolmitriptan and piroxicam were recorded and shown in Table 3.

CONCLUSION

The developed delivery system showed higher Cmax with lower Tmax resulted in significantly faster absorption of incorporated drugs, which helped in the rapid onset of action in the management of pain as required by migraine sufferers. Incorporation of Sepitrap 80 increased the dissolution of drugs along with the absorption of both active agents through the sublingual biomembrane. The observed increased sublingual systemic bioavailability of the drugs by Sepitrap 80 seems to happened mainly via interaction of microencapsulated polysorbate 80 with the sublingual mucosa whereas, enhanced drug dissolution could not be neglected. Although, obtained results were only considered exploratory due to small number of volunteers involved in the study hence, further investigation on the developed formulation should be conducted on large number of populations.

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