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Development of Transmucosal Patch containing Lignocaine for dental procedures

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ABSTRACT

Transmucosal patches of Lignocaine were developed and evaluated so as to achieve complete release and permeation of the drug within early minutes of application. Permeability of Lignocaine base (LB) and Lignocaine HCl salt (LH) were accessed through porcine buccal mucosa and dialysis membrane. Patches were prepared with mucoadhesive film forming polymers Sodium Carboxy Methyl Cellulose (SCMC), Hydroxy Propyl Cellulose (HPC, Grades JF, LF, LXF and MF), Hydroxy Propyl Methyl cellulose (HPMC) Labrafac PG (LPG) as permeation enhancer by solvent casting method over the dried ethyl cellulose (EC) backing. Permeation was further enhanced by addition of natural permeation enhancer clove oil and olive oil individually. Patches were evaluated for appearance, weight and thickness and content uniformity, surface pH, folding endurance, *in vitro* residence time, *in vitro* release, *ex vivo* permeation, mucoadhesive and tensile strength, FTIR, DSC, XRD, SEM. The release and permeation of LB from HPC-LF patch with clove oil was promising. Also the *in vitro* residence time, mucoadhesion and tensile strength was satisfactory. FTIR and DSC confirmed compatibility between the drug and the polymer.

INTRODUCTION

Pain is the most common manifestation of dental diseases. Despite the development of modern equipment and technical expertise, if there is one thing that dentists have been unable to control, it is the pain that presents itself in a variety of ways. In fact the nerve in the tooth or the pulp as it is called has fibers that can transmit only pain as a response to any stimulus [1]. Currently marketed dosage forms of anaesthetics namely solutions, gels, ointments and injectables have drawback of either involuntary swallowing, inaccuracy in dose, dilution due to saliva or pain due to prick of needle [2]. The two aspects of local anesthetic injections that cause pain are the needle insertions and the deposition of solution. Topical intra oral anesthetic application can be used to reduce the discomfort of intra oral local anesthetic injections [3]. It provides symptomatic relief from the pain of superficial mucosal lesions and can be used to treat toothache and post extraction pain.

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Also some soft tissue procedures can be performed more comfortably following the use of topical anesthetic alone. A lot of studies have been carried out with lignocaine where in sustained and prolonged release has been reported in literature. In the present study, we investigated the transmucosal patches of Lignocaine with various polymers and permeation enhancers for immediate release and complete permeation through the buccal tissue which is not been previously addressed. The patch can be removed after five minutes of application and dental surgeries can be carried out with patient compliance as the pain due to prick of needle is not experienced. Appropriate dose would be delivered due to presence of backing layer and prevention of dilution due to saliva. Involuntary swallowing of the dosage form would be avoided and site specificity of the drug would be maintained due to mucoadhesive polymer. Also this work was carried out to screen the best polymer and right concentration of the polymer for later incorporation of solid lipid nanoparticles (SLNs) of Diclofenac in the

same patch along with lignocaine as an extended study.

MATERIALS AND METHODS

Materials

LB, LH from Astra, Bangalore; SCMC (medium viscosity 400 cps), HPMC (15 cps) from Loba Chem; HPC-LF (75 cps), HPC-JF (150 cps), HPC-LXF (75 cps, fine powder), from Signet Chemical Corporation Pvt ltd, Mumbai; LPG (Propylene glycol dicaprylate/dicaprate) from Gattefosse, France; EC (viscosity 20cps and 50% ethoxy content) from Dow cellulosic, USA were the generous gifts. Dibutyl phthalate (DBP) from Qualigens fine chemicals, Mumbai and Clove oil, olive oil from Indus Herbs, Bangalore were purchased. Other chemicals and reagents were of Laboratory Grade. Milli Q water was utilized for all preparations. Porcine buccal mucosa was obtained from local slaughter house.

Permeability of LB and LH through porcine buccal mucosa

Animal tissue preparation

Porcine cheek tissue was obtained from a local abattoir within one hour after slaughter and transported to the laboratory in ice-cold phosphate buffer (pH 6.8). It was stored at 15°C in refrigerator for one month until required. The buccal mucosal membrane was carefully separated from the underlying tissues with the help of forceps/ surgical scissors and remnant tissue was completely removed [4].

Ex vivo drug permeation study

The buccal mucosa was placed over the Franz diffusion cell (diffusion area: 3.8028, capacity: 120ml) such that the epithelium faced the donor chamber and the connective tissue region faced the receiver chamber [5,6]. LB and LH (Dose: 40mg, half of Dose: 20mg and saturated solutions LB: 0.5g, LH: 2g) were added to the donor along with 5ml of simulated salivary solution (pH 6.8) after tissue equilibration (1 hour). The receptor cell contained physiologic phosphate buffer (pH7.4, 37°C) stirred continuously over the magnetic stirrer. Aliquots (2 ml) were withdrawn from the sampling arm at predetermined time intervals over two hour time period and replaced with equal volume of fresh buffer. Samples were analysed at 262 nm by UV-Visible spectrophotometer method. All experiments were conducted with three replicates. The cumulative amount of drug reaching the receptor compartment was determined from which the steady state flux J_s (μg h^{-1} cm^{-2}), and apparent permeability coefficient Kp (cm/s) were also calculated as per the below equations.

$$J_s = \frac{dQ}{Adt} \quad \ldots\ldots\ldots (Eq.1)$$

Where dQ is the amount of drug permeated (μg) through the mucosa during time dt (h) and A is the diffusional area (sq cm).

$$K_p = \frac{J_s}{3{,}600} \times C = \frac{(dQ/dt)}{3{,}600} \times A \times C \ldots\ldots (Eq.3)$$

dQ/dt is the steady-state rate of appearance of the drug in the receiver chamber (μg/h) and C is the initial drug donor concentration (μg/ml).

Diffusion of LB and LH through dialysis membrane

Pre-treatment of dialysis membrane

Dialysis membrane (MWCO 14000) was soaked in deionized water for 20 minutes at room temperature to remove glycerin added as humectant during manufacture to prevent them from drying and becoming brittle. It was again soaked in a mixture of 10mM of EDTA (heavy-metal scavenger) and 10% w/v of $NaHCO_3$ at 70°C for 20 min to remove sulfur present in sodium azide added as preservative [7,8].

After rinsing the dialysis membrane with water, diffusion of LB and LH through dialysis membrane was carried out with Franz diffusion cell similar to permeability study through porcine buccal mucosa. Amount of drug diffused, Flux and permeability coefficient were calculated.

Development of backing layer

Selection of solvent/ solvent system for EC

EC solution (5% w/v) was prepared separately in 5ml each of methanol, ethanol, isopropyl alcohol (IPA), cyclohexane, chloroform, ethyl acetate (EA), toluene and acetone and subjected to magnetic stirring to obtain a clear uniform solution meanwhile the swelling time was documented [9]. Initial and the resulting viscosities of these solvents with addition of polymer were determined at room temperature with an RPM set to obtain a stable % Torque and viscosity (Brookfield viscometer DV-III). Polymeric solution was plasticized with 10% w/w of DBP and stirred on magnetic stirrer. Complete removal of air bubbles was accomplished by bath sonication during which evaporation of solvent was prevented by covering the beaker with aluminium foil.

Polymeric gel was casted over the glass mold of 18 sq cm and drying time was noted. Individual patches were stored in self-sealing covers in a glass desiccator.

Selection of plasticizer for EC

EC (5% w/v) solution were prepared in 5 ml of EA and Acetone (1:1). After complete swelling of polymer 10% w/w of glycerin, PG, PEG 400 and DBP were added individually as plasticizer and observed for miscibility in the polymeric solution [9,10]. DBP was selected for further studies as it gave a clear transparent solution.

Patches were prepared with EC (5% w/v) and DBP (10%, 20%, 30%, 40% and 50% w/w) in 10ml ml of EA and Acetone (1:1). Patches were evaluated for drying time, integrity, thickness, brittleness and ease in pealing from the glass mold.

Selection of amount of EC

Patches were prepared with EC (2%, 3%, 4% and 5% w/w), DBP (30% w/w) in 10 ml of EA and Acetone (1:1). Patches were evaluated for drying time, integrity, thickness, brittleness and ease in pealing from the glass mold.

Preparation of bilayered transmucosal patch of LB and LH

Buccal patch of LB and LH was prepared by solvent casting method [11]. The mucoadhesive layer of the bi-layered patch was prepared by dissolving polymer, drug and Labrafac PG (20% w/w of polymer) in solvent (10ml) over a magnetic stirrer (1h). The polymers employed were SCMC, HPC (grades LF, JF, LXF) and HPMC. LB (1.869% w/v) was dissolved in water: ethanol (1:1) whereas LH (2.16% w/v) was dissolved in water. The beakers were covered with aluminium foil to prevent solvent evaporation. Air bubbles were allowed to escape overnight and also by bath sonication, the solution was casted over the backing layer and dried in a hot air oven (50°C).

Briefly the backing layer was prepared by casting a solution of ethylcellulose (4% w/v), DBP (1.2% w/v) in ethyl acetate: acetone (1:1, 10ml) over the glass mould (18 cm^2) and allowed to air dry. Placebo patches were prepared in the same manner without the drug. Dried patches were carefully pealed and stored in self-sealing covers at room temperature.

Preparation of bi-layered transmucosal patches of LB and LH with natural permeation enhancers

LB and LH patches with HPC-LF were additionally improved by incorporation of 10%, 20% and 30% natural permeation enhancer clove oil and olive oil individually [12]. The polymeric solution containing the drug, Labrafac PG and either clove oil or olive oil (added as % w/w of polymer) was prepared by solvent casting method as described earlier. Patches containing clove oil were prepared in ethanol: water (1:1) and that containing olive oil was prepared in chloroform: water (1:1).

Evaluation of bi-layered transmucosal patches of LB and LH

Appearance

The prepared patches were evaluated for appearance on the basis of color, elegance, texture and surface stickiness

Weight Uniformity

Patch (one sq cm) was cut from corner, centre and side of each batch and weighed over an electronic balance in triplicate.

Thickness Variation

Thickness of patch was measured with a screw gauge micrometer at three different spots from each batch.

Surface pH

Patch (one sq cm) was left to swell in 5 ml of distilled water in a petriplate [11]. The pH was measured by placing the electrode of a Digital pH meter directly in contact with the microenvironment of the swollen patch.

Folding endurance

Folding endurance was determined (in triplicate) by repeatedly folding the patch (1.5 cm*2cm) at the same place till it broke or folded up to 300 times.

Swelling index (SI)

Patch (one sq cm) was weighed and placed in a pre-weighed glass cover slip which was submerged into 15 ml simulated saliva placed in a Petri dish. At pre-determined time the cover slip was removed and weighed after wiping the excess of media. The process was continued until an increase in weight of the patch was observed due to swelling or a decrease in weight was observed due to erosion. The SI (triplicate) was calculated by the equation [11].

$$SI = \frac{W_t - W_o}{W_o} \quad \ldots\ldots\ldots (Eq.3)$$

Where W_t is the weight of the patch at time t
W_o is the weight of patch at time 0.

The degree of swelling was determined for three patches of each type of formulation.

In vitro residence time

The patch (1.5cm*2cm) was hydrated from the mucoadhesive surface with buffer (pH 6.8), brought into contact with the mucosal membrane of the porcine buccal mucosa which was glued to a glass slide with cyanoacrylate adhesive and vertically attached to the modified USP disintegration apparatus [11]. The glass slide was allowed to move up and down so that the patch was completely immersed in the 800 ml of simulated saliva (pH 6.8, 37° C) at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the patch from the mucosal surface was recorded.

Drug Content uniformity

Three patches (one sq cm) from different batches were weighed and dissolved in 100 ml of methanol, filtered and after suitable dilution analyzed at 261 nm using UV-Visible spectrophotometer against a similarly treated placebo patch.

In vitro drug release

Patch (1.5cm* 2cm) was glued to a glass slide (specially cut 2.5cm*3cm size) with cyanoacrylate adhesive such that the mucoadhesive surface faced the dissolution medium (50ml, simulated saliva pH 6.8, 37±0.5°C, 50 rpm) and placed at the bottom of the Jar of USP dissolution apparatus type II (Electrolab TDT-08L, India) [13]. The release study was carried out for 30 min. At predetermined time, 2ml samples were withdrawn from each station, filtered, diluted suitably and analyzed at 261 nm using UV-Visible spectrophotometer.

Ex vivo permeation study

Patch (one sq cm) with 2 ml of phosphate buffer (pH 6.8) was placed in intimate contact of pre-equilibrated porcine buccal mucosa mounted over Franz diffusion cell, effective surface: 4.9107 cm^2, receptor media: phosphate buffer (pH 7.4, 37±1°C, 50 ml 50 RPM) [14]. Samples were analyzed spectrophotometrically.

Differential scanning calorimetry (DSC)

Thermograms of pure drugs, DDEA-SLN and TP were taken by DSC (Mettler Toledo Star System). Weighed (7-10 mg) samples were placed in sealed aluminum pans under liquid nitrogen as coolant and scanned at 10°C/min from 40° C to 400°C.

Fourier Transform Infrared Spectrophometer (FTIR)

Pure drugs, DDEA-SLN and TP were analysed by KBr disc method over wave number range of 4000-400 cm^{-1} by Perkin Elmer Spectrum BX spectrophotometer.

X-ray diffraction

Powder X-ray diffraction patterns of the pure drugs, DDEA-SLN and TP determined using Rigaku Smartlab® diffractometer equipped with a rotating target X-ray tube and wide angle goniometry. X-ray source was Kα radiation from a copper target (λ=1.5418). X-ray tube was operated at a potential of 40 kV and a current of 30 mA. Scan range (2θ) was from 0 to 50° with speed of 2° per minute at increments of 0.02°.

Scanning electron microscopy (SEM)

Surface morphological study was carried using electron microscope (Carl Zeiss Ultra 55-FSEM) for pure drugs, DDEA-SLN and TP. A small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to aluminium stubs. These sample stabs were coated with a thin layer ($30A^0$) of gold by employing Polaron-E 3000 sputter coater. Samples were examined and photographed under various magnifications with direct data capture of the images onto a computer.

RESULTS AND DISCUSSIONS

Permeability study

Permeability study of LB and LH was carried out through porcine buccal mucosa and dialysis membrane for Dose (40mg), half of Dose (20mg) and for saturated solution for a period of 2 hrs. The permeability of LB was higher than LH through porcine buccal mucosa (Fig. 1). The epithelium of the buccal mucosa is lipidic in nature and more permeable to unionised lipophilic drug [4-6,15-17]. This explains rapid permeability of LB than LH. The diffusion of LH was higher than LB through the dialysis membrane (Fig. 2). LH is readily soluble in phosphate buffer pH 6.8 whereas LB is soluble with prolong sonication.

Table 1: Flux and Permeability coefficient of LB and LH through porcine buccal mucosa

Sl No	Time (min)	Flux (µg/sq.cm.h)						Permeability coefficient X10^{-7} (cm s^{-1})					
		Dose		Saturated solution		Reduced dose		Dose		Saturated solution		Reduced dose	
		LB	LHCl	LB	LHCl	LB	LHCl	LB	LHCl	LB	LHCl	LB	LHCl
1	0	0	0	0	0	0	0	0	0	0	0	0	0
2	5	18.58	5.51	27.66	10.21	9.07	3.33	6.45	1.91	9.61	3.54	3.15	1.15
3	10	10.07	4.99	15.04	5.84	4.92	5.31	3.49	1.73	5.22	2.02	1.71	1.84
4	15	7.45	4.14	10.94	4.96	3.65	4.35	2.58	1.44	3.8	1.72	1.27	1.51
5	30	4.07	2.59	10.87	2.94	2.00	2.62	1.41	0.902	3.78	1.02	0.69	0.912
6	60	2.25	1.90	6.71	1.77	1.11	1.59	0.78	0.663	2.33	0.615	0.38	0.553
7	90	1.56	1.60	5.88	1.34	0.77	1.14	0.54	0.556	2.04	0.466	0.26	0.396
8	120	1.36	1.40	4.97	10.21	0.67	0.96	0.47	0.489	1.73	0.546	0.23	0.333

Table 2: Flux and Permeability coefficient of LB and LH through dialysis membrane

Sl No	Time (min)	Flux (µg/sq.cm.h)						Permeability coefficient X 10^{-7} (cm s^{-1})					
		Dose		Saturated solution		Reduced dose		Dose		Saturated solution		Reduced dose	
		LB	LHCl	LB	LHCl	LB	LHCl	LB	LHCl	LB	LHCl	LB	LHCl
1	0	0	0.00	0	0.00	0	0.00	0	0	0	0	0	0
2	5	0.26	4.82	6.44	6.03	0.01	1.48	0.0892	1.67	1.07	2.09	0.00	0.51
3	10	0.31	5.72	4.06	5.00	0.05	2.17	0.107	1.98	0.8	1.73	0.01	0.75
4	15	0.33	4.36	3.36	4.15	0.17	1.72	0.115	1.51	0.74	1.44	0.05	0.59
5	30	0.21	2.36	1.92	2.60	0.11	0.94	0.07	0.82	0.44	0.9	0.03	0.32
6	60	0.13	1.24	1.08	1.91	0.07	0.50	0.0448	0.42	0.26	0.66	0.03	0.17
7	90	0.12	0.86	0.89	1.60	0.07	0.35	0.0423	0.29	0.23	0.55	0.03	0.12
8	120	0.12	0.67	0.80	1.41	0.07	0.27	0.0414	0.23	0.21	0.48	0.03	0.09

Table 3: Physical properties of Ethyl cellulose backing layer

Solvent	Swelling time (h)	Viscosity of solvent (cps)	Viscosity polymeric solution	Drying time (h)	Physical appearance of patch
Methanol	0.5	0.59	19.13	24	White opaque, Brittle, Discontinuous
Ethanol	0.5	1.2	38.92	24	White opaque ,Brittle, Continuous
Isopropyl alcohol	4	1.96	63.57	-	-
Cyclohexane	1	1.02	33.08	-	-
Chloroform	0.5	0.56	18.16	6	Transparent, Uniform
Ethyl Acetate	0.5	0.42	13.62	5	Transparent, Uniform
Toluene	0.5	0.59	19.13	8	Transparent, Uniform
Acetone	0.18	0.33	10.70	4	Transparent, non-uniform

Due to comparatively higher solubility of LH in Phosphate buffer 6.8 it could diffuse speedily through dialysis membrane. The permeability through the porcine buccal mucosa and diffusion through the dialysis membrane increased with increase in concentration of LB and LH in the donor cell. The flux and permeability coefficient (Table 1 and 2) of LB and LH decreased with time and concentration in donor compartment and remained constant after 50 min.

Table 4: Codes of LB and LH patches

Polymer concentration (% w/v)	Patch code of LB				
	SCMC	**HPC JF**	**HPC-LF**	**HPC-LXF**	**HPMC**
2.5	L1a	L2a	L3a	L4a	L5a
3.0	L1b	L2b	L3b	L4b	L5b
3.5	L1c	L2c	L3c	L4c	L5c
4.0	L1d	L2d	L3d	L4d	L5d
	Patch code of LS				
2.5	S1a	S2a	S3a	S4a	S5a
3.0	S1b	S2b	S3b	S4b	S5b
3.5	S1c	S2c	S3c	S4c	S5c
4.0	S1d	S2d	S3d	S4d	S5d

Codes of LB and LH patches with natural permeation enhancers

Permeation enhancer	Base /Salt of Lignocaine	Amount of Permeation enhancer		
		10%	**20%**	**30%**
Clove oil	LB	C1-L3d	C2-L3d	C3-L3d
Clove oil	LH	C1-S3d	C2-S3d	C3-S3d
Olive oil	LB	O1-L3d	O2-L3d	O3-L3d
Olive oil	LH	O1-S3d	O2-S3d	O3-S3d

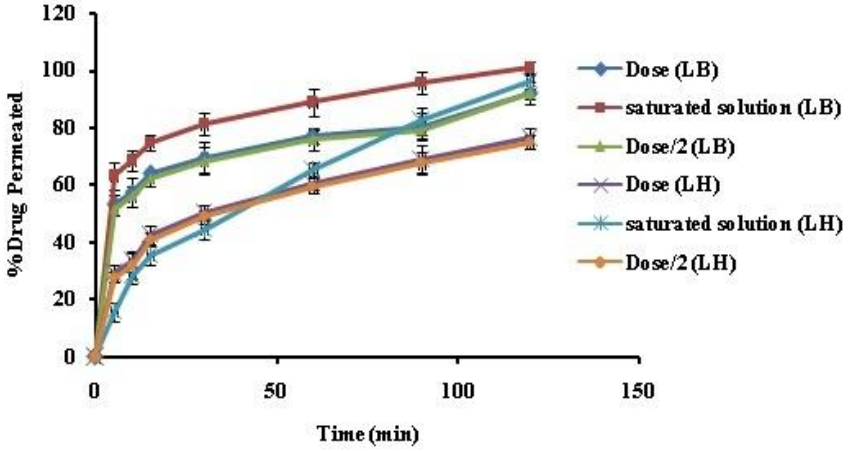

Figure 1: Permeability of LB and LH through Porcine buccal mucosa

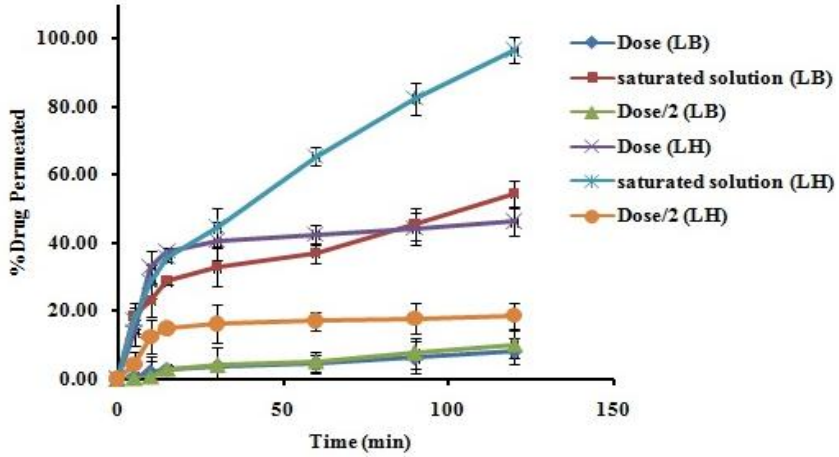

Figure 2: Permeability of LB and LH through dialysis membrane

Development of backing layer

Various solvents belonging to class 1 and class 2 were screened for preparation of backing layer with EC (Table 3). Swelling time of the polymer and drying time of the patch increased with increase in viscosity of the polymer solution. Swelling time of EC in methanol, ethanol, chloroform, toluene, EA, cyclohexane and IPA was greater however it was least in acetone (10min). EC formed lumps and did not form a continuous solution or gel with cyclohexane instead a translucent solution was obtained which turned to clear transparent upon heating (60°C) thus delaying the process. Resulting viscosity of EC in IPA was very high (63.57 cps) for subsequent preparation of patch. Patches with methanol and ethanol had the longest drying time followed by toluene, chloroform, EA and acetone. Patches prepared individually with methanol and ethanol was opaque and brittle whereas those prepared from chloroform, EA and toluene were transparent. Thickness of the patches prepared from acetone was uneven due to rapid evaporation of the solvent. Ultimately in view of the influence of solvent on swelling time and patch drying time, it was decided to utilize a combination of EA and acetone (1:1) as the solvent system for preparation of backing layer of ethyl cellulose. There was no solvent detected as evident from the graph obtained. Glycerine, propylene glycol, polyethyleneglycol 400 and DBP were evaluated as plasticizer in the preparation of the backing layer with EC. As only DBP was completely miscible giving a clear transparent solution it was selected as plasticizer whereas the other three plasticizers were immiscible in EC gel. Various concentrations of selected plasticizer were evaluated to arrive at the optimum concentration by observing the drying time and integrity of the patches. Patches with 30% w/w of DBP were clear, smooth, even and flexible with least drying time and hence was settled for further studies. Next the polymer concentration was investigated on the basis of thickness, mechanical strength, ease of pealing, flexibility, clarity and drying time and 4% w/v of EC was confirmed to be most satisfactory.

Preparation of bilayered buccal patch of LB and LH

Patches of LB and LH were prepared with four different concentrations of patch forming mucoadhesive polymers SCMC, HPC-JF, HPC-LF, HPC-LXF and HPMC by solvent casting method over the dried EC backing layer. Labrafac PG (20% w/w of polymer) was added as a plasticizer cum permeation enhancer. Permeation of LB and LH with HPC-LF patches was further improved by incorporation of natural permeation enhancer clove oil and olive oil individually (Table 4).

Evaluation of bi-layered transmucosal patches of LB and LH

Appearance

All the prepared patches were transparent except SCMC patches which were opaque. The patches were elegant and non sticky. They were free of air bubbles and undissolved drug or polymer particles. The surface of backing layer facing the glass mould was slightly more smoother then the surface of the mucoadhesive layer.

Weight and Thickness variation

The weight and thickness of the patches increased with increase in the concentration of the mucoadhesive polymer (Table 5). The weight of patch (one sq cm) was in the range of 37.51±0.07 (L5a) to 54.20±0.08 (L3d) for LB patches and from 37.66±0.76 (S) to 54.39±0.11 (S3d) for LH patches. Patch thickness varied from 0.16±0.50 (B5a) to0.36±0.032 (L2d) for LB and from 0.17±0.19 (S5a) to 0.36±0.86 (S4d) for LH.

Folding endurance and surface pH

Folding endurance of all the prepared patches was found to be greater than 300 displaying satisfactory flexibility lest brittleness and surface pH in the salivary pH range assuring no irritation to the mucosa at the site of application of the patch [11].

Swelling index and content uniformity

Wetting, subsequent swelling of the polymer is responsible for good mucoadhesion as well as release of drug [18]. The SI of patches containing LB was less than those of LH (Table 5). Water soluble drug LH contributed to increase SI as compared to water insoluble LB. The SI was in the range of 5.07±0.41 (L4a) to 9.76±0.55 (L1d) for LB patches and from 5.13±0.53 (S5d) to 9.77±0.71 (S1d) for LH patches at 15 min. With respect to increase in concentration gradual increase in SI was observed with SCMC, HPC-JF and HPC-LXF patches of LB which was due to swelling. Initial increase and subsequent decrease in SI was observed with HPC-LF and HPMC patches of LB which was due to partial erosion of polymer. While swelling was observed with SCMC, HPC-JF, HPC-LF and HPC-LXF patches represented by gradual increase in SI with respect to concentration in case of LH. Content Uniformity of all the prepared patches were in the range of 92% to 98%.

Table 5: Physical evaluation parameters of bilayered transmucosal patches of LB and LH

Patch code	Weight Variation (mg)	Thickness (mm)	SI at 15 min	Content uniformity (%)	In vitro residence time (h)
L1a	37.57±0.04	0.17±0.029	7.91±0.84	93.33±0.12	8.25±0.21
L1b	42.79±0.03	0.21±0.025	8.34±0.79	93.61±0.24	8.48±0.34
L1c	47.90±0.05	0.24±0.040	8.96±0.69	93.06±0.27	8.76±0.51
L1d	52.97±0.07	0.28±0.056	9.76±0.55	92.50±0.14	8.92±0.27
L2a	37.78±0.04	0.25±0.037	5.96±0.54	93.89±0.23	7.56±0.42
L2b	43.41±0.02	0.27±0.025	6.98±0.14	96.39±0.35	7.65±0.38
L2c	48.90±0.02	0.32±0.026	7.98±0.16	96.67±0.16	7.84±0.25
L2d	53.59±0.03	0.36±0.032	6.33±0.78	95.56±0.24	7.92±0.39
L3a	38.70±0.08	0.20±0.006	6.10±0.85	97.50±0.21	10.21±0.57
L3b	43.58±0.01	0.24±0.009	6.54±0.40	96.39±0.13	10.45±0.22
L3c	49.06±0.05	0.28±0.044	5.87±0.11	97.50±0.42	10.52±0.46
L3d	54.20±0.08	0.32±0069	5.87±0.12	97.50±0.31	10.71±0.31
L4a	38.12±0.01	0.26±0.009	5.07±0.41	95.56±0.25	10.25±0.62
L4b	43.58±0.02	0.26±0.020	5.66±0.43	96.67±0.16	7.38±0.53
L4c	48.50±0.02	0.31±0.024	6.11±0.41	95.83±0.28	7.54±0.36
L4d	53.48±0.01	0.35±00.12	6.47±0.15	95.00±0.26	7.68±0.17
L5a	37.51±0.07	0.18±0.034	7.48±0.38	92.50±0.32	6.32±0.32
L5b	43.00±0.03	0.19±0.020	6.53±0.20	94.17±0.21	6.65±0.16
L5c	48.50±0.02	0.23±0.018	6.62±0.98	95.83±0.15	6.85±0.52
L5d	53.15±0.04	0.26±0.033	6.05±0.61	93.33±0.32	6.98±0.31
S1a	37.66±0.76	0.17±0.49	8.23±0.35	92.78±0.002	8.32±0.53
S1b	42.74±0.93	0.21±0.65	8.85±0.24	92.22±0.002	8.49±0.28
S1c	47.91±0.28	0.24±0.20	9.16±0.62	93.61±0.002	8.82±0.45
S1d	53.03±0.35	0.28±0.26	9.77±0.71	93.33±0.002	8.96±0.25
S2a	38.09±0.19	0.19±0.16	6.65±0.75	95.28±0.001	7.61±0.36
S2b	43.55±0.26	0.27±0.23	7.23±0.64	96.67±0.001	7.73±0.46
S2c	48.49±0.12	0.32±0.12	7.98±0.12	95.56±0.002	7.92±0.23
S2d	54.30±0.57	0.36±0.53	8.53±0.65	97.22±0.006	7.99±0.19
S3a	38.69±0.30	0.21±0.23	5.77±0.44	98.06±0.002	10.34±0.45
S3b	43.92±0.28	0.24±0.22	6.21±0.47	97.50±0.001	10.58±0.33
S3c	49.08±0.67	0.28±0.54	6.82±0.65	97.78±0.002	10.76±0.59
S3d	54.39±0.11	0.33±0.92	7.25±0.75	98.33±0.001	10.89±0.17
S4a	38.46±0.21	0.23±0.17	5.44±0.21	97.22±0.002	7.36±0.25
S4b	43.82±0.51	0.26±0.42	6.46±0.52	97.78±0.002	7.52±0.21
S4c	48.86±0.60	0.30±0.52	6.72±0.65	97.22±0.002	7.65±0.35
S4d	54.09±0.94	0.36±0.86	7.07±0.15	97.50±0.001	7.98±0.32
S5a	38.08±0.29	0.17±0.19	7.66±0.25	95.00±0.001	6.46±0.12
S5b	43.41±0.22	0.19±0.14	7.02±0.86	95.28±0.002	6.7±0.62
S5c	48.55±0.22	0.23±0.14	6.30±0.55	95.83±0.001	6.94±0.19
S5d	54.12±0.60	0.26±0.41	5.13±0.53	96.94±0.002	6.99±0.23
C1-L3d	47.61±0.22	0.29±0.44	6.60±0.95	93.33±0.002	7.25±0..25
C2-L3d	46.41±0.15	0.29± 0.25	6.65±0.68	93.61±0.001	7.37±0.52
C3-L3d	47.33±0.22	0.30±0.12	6.59±0.85	93.06±0.002	7.46±0.47
C1-S3d	47.18±0.73	0.31±0.41	7.18±0.54	92.50±0.002	7.63±0.32
C2-S3d	48.00±0.50	0.29±0.13	7.16±0.42	93.89±0.001	7.52±0.48
C3-S3d	46.90±0.84	0.30±0.27	7.24±0.65	95.83±0.001	7.43±0.16
O1-L3d	48.16±0.22	0.29±0.42	6.59±0.46	96.67±0.002	7.73±0.38
O2-L3d	46.96±0.15	0.30±0.26	6.61±0.98	94.72±0.001	7.65±0.24
O3-L3d	47.88±0.22	0.30±0.32	6.56±0.48	95.56±0.001	7.38±0.29
O1-S3d	47.74±0.73	0.31±0.24	7.14±0.75	95.28±0.001	7.45±0.82
O2-S3d	48.56±0.50	0.29±0.31	7.11±0.65	94.44±0.002	7.83±0.46
O3-S3d	47.45±0.84	0.29±0.24	7.19±0.24	92.78±0.002	7.67±0.38

Table 6: Percentage in vitro drug release of the transmucosal patches of LB and LH

LB			LH			Natural permeation enhancer		
Patch Code	***In vitro* drug release (2 min)**	***In vitro* drug release (15 min)**	**Patch Code**	***In vitro* drug release (2 min)**	***In vitro* drug release (15 min)**	**Patch code**	**% Drug Release (2min)**	**% Drug Release (5min)**
L1c	7.733 ± 0.78	23.889 ± 0.39	S1c	4.616±0.54	15.650±0.21	C1-L3d	91.26±0.88	96.93±0.47
L1d	6.806 ± 0.21	19.782 ± 0.54	S1d	3.667±0.65	12.895±0.11	C2-L3d	92.91±0.75	96.64±0.70
L2c	12.586 ± 0.40	31.993 ± 0.20	S2c	8.283±0.39	17.155±0.51	C3-L3d	94.43±0.78	98.16±0.06
L2d	10.239 ± 0.48	25.343 ± 0.24	S2d	7.217±0.84	15.965±0.54	C1-S3d	80.43±0.51	87.44±0.39
L3c	94.741 ± 0.76	99.93 ± 0.38	S3c	72.418±0.54	80.050±0.35	C2-S3d	80.20±0.22	87.07±0.86
L3d	93.121 ± 0.11	99.8 ± 0.58	S3d	67.440±0.84	75.166±0.39	C3-S3d	79.51±0.24	86.22±0.12
L4c	84.930 ± 0.37	91.774 ± 0.18	S4c	70.059±0.29	80.507±0.75	O1-L3d	90.90±0.99	94.49±0.37
L4d	79.972 ± 0.18	86.096 ± 0.94	S4d	66.174±0.15	74.021±0.84	O2-L3d	93.72±0.47	97.35±0.35
L5c	69.200 ± 0.37	78.946 ± 0.18	S5c	57.949±0.45	65.305±0.35	O3-L3d	91.02±0.68	95.59±0.24
L5d	63.352 ± 0.67	72.657 ± 0.33	S5d	54.504±0.57	62.758±0.35	O1-S3d	79.03±0.30	85.80±0.92
						O2-S3d	77.82±0.77	81.95±0.24
						O3-S3d	76.29±0.74	86.53±0.27

Table 7: Mechanism of drug release of patches of LB and LH

Sl No	Patch code	Zero order	First order	Matrix	Krosmeyer Peppas		Hixon Crowell	Higuchi	Best fit Model
					R	n			
1.	L1c	0.8540	0.9049	0.9989	0.9966	0.5293	0.8893	0.9978	Matrix
2.	L1d	0.8139	0.8576	0.9948	0.9946	0.5012	0.8438	0.9902	Matrix
3.	L2c	0.6292	0.7323	0.9692	0.9873	0.3975	0.7009	0.9551	Peppas
4.	L2d	0.6474	0.7324	0.9738	0.9886	0.3944	0.7060	0.9646	Peppas
5.	L3c	0.2287	0.8078	0.8261	0.9973	0.0286	0.5860	0.7492	Peppas
6.	L3d	0.5831	0.8782	0.7337	0.9799	0.0330	0.5328	0.7485	Peppas
7.	L4c	0.6372	0.8116	0.4784	0.9855	0.0426	0.6154	0.5683	Peppas
8.	L4d	0.8251	0.6782	0.5183	0.9349	0.0494	0.7529	0.5917	Peppas
9.	L5c	0.9388	0.9491	0.9756	0.9796	0.6767	0.9458	0.9533	Peppas
10.	L5d	0.6108	0.6396	0.9492	0.9484	0.4876	0.6303	0.9649	Higuchi
11.	S1c	0.9173	0.9378	0.9895	0.9893	0.5927	0.9314	0.9834	Matrix
12.	S1d	0.9170	0.9340	0.9908	0.9937	0.6018	0.9286	0.9861	Peppas
13.	S2c	0.8802	0.9198	0.9917	0.9746	0.4334	0.9078	0.9837	Matrix
14.	S2b	0.7132	0.3190	0.5011	0.9860	0.0481	0.0719	0.5825	Peppas
15.	S3c	0.7285	0.3236	0.5127	0.9862	0.0511	0.2625	0.5877	Peppas
16.	S3d	0.7311	0.3313	0.5282	0.9866	0.0552	0.0596	0.5968	Peppas
17.	S4c	0.6834	0.6288	0.6835	0.9330	0.0974	0.0675	0.7152	Peppas
18.	S4d	0.6725	0.5839	0.6398	0.9315	0.0827	0.4819	0.6682	Peppas
19.	S5c	0.6631	0.5716	0.6609	0.9245	0.0890	0.5438	0.6836	Peppas
20.	S5d	0.6517	0.5602	0.6766	0.9457	0.0961	0.4165	0.6958	Peppas
21.	C1-L3d	0.5968	0.4704	0.7271	0.9894	0.0291	0.8379	0.6714	Peppas
22.	C2-L3d	0.5321	0.2025	0.7098	0.9464	0.0180	0.8821	0.6536	Peppas
23.	C3-L3d	0.5476	0.4103	0.7093	0.9459	0.0176	0.8259	0.6451	Peppas
24.	C1-S3d	0.4283	0.3727	0.4907	0.9697	0.0447	0.7542	0.5745	Peppas
25.	C2-S3d	0.4301	0.3841	0.4879	0.9696	0.0441	0.7413	0.5732	Peppas
26.	C3-S3d	0.4895	0.3865	0.4769	0.9921	0.0424	0.7385	0.5674	Peppas
27.	O1-L3d	0.6137	0.3526	0.7120	0.9800	0.0196	0.8736	0.6556	Peppas
28.	O2-L3d	0.6328	0.3237	0.7114	0.9799	0.0193	0.8621	0.6558	Peppas
29.	O3-L3d	0.6259	0.2738	0.7192	0.9598	0.0236	0.8527	0.6621	Peppas
30.	O1-S3d	0.3524	0.2261	0.4879	0.9696	0.0441	0.6858	0.5736	Peppas
31.	O2-S3d	0.3382	0.2593	0.3953	0.9745	0.0246	0.6719	0.5274	Peppas
32.	O3-S3d	0.3215	0.2492	0.3648	09831	0.0462	0.6638	0.6078	Peppas

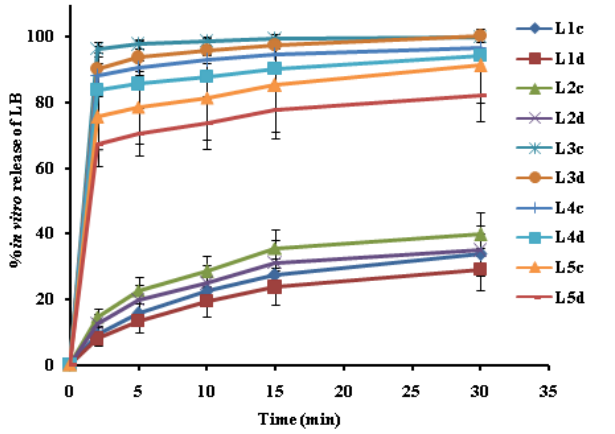

Figure 3: *In vitro* drug release of LB from various polymeric patches

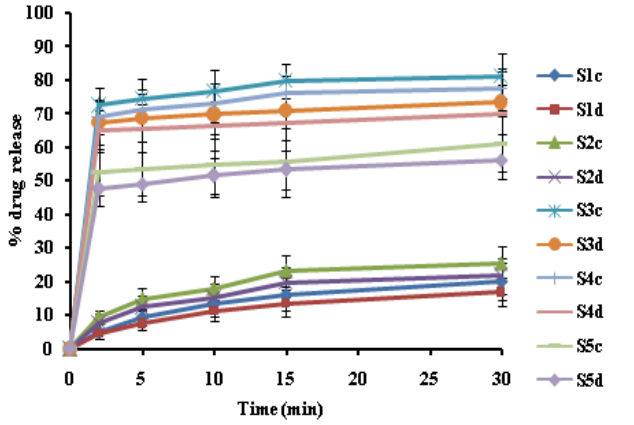

Figure 4: *In vitro* drug release of LH from various polymeric patches

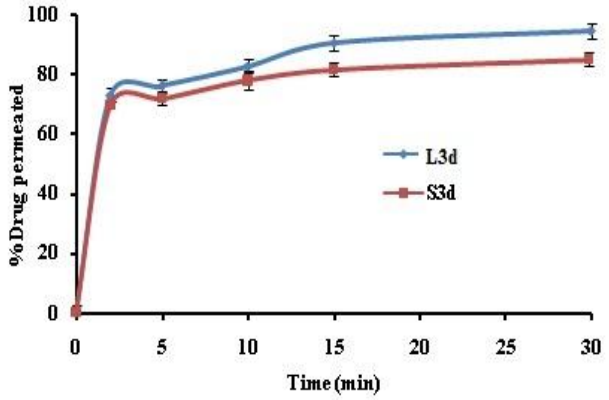

Figure 5: Percent drug permeated of B3d and S3d through porcine buccal mucosa

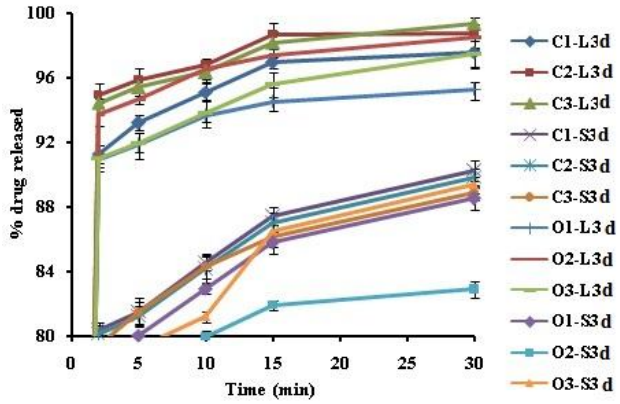

Figure 6: *In vitro* drug release of LB and LH from HPC-LF patches with natural permeation enhancer

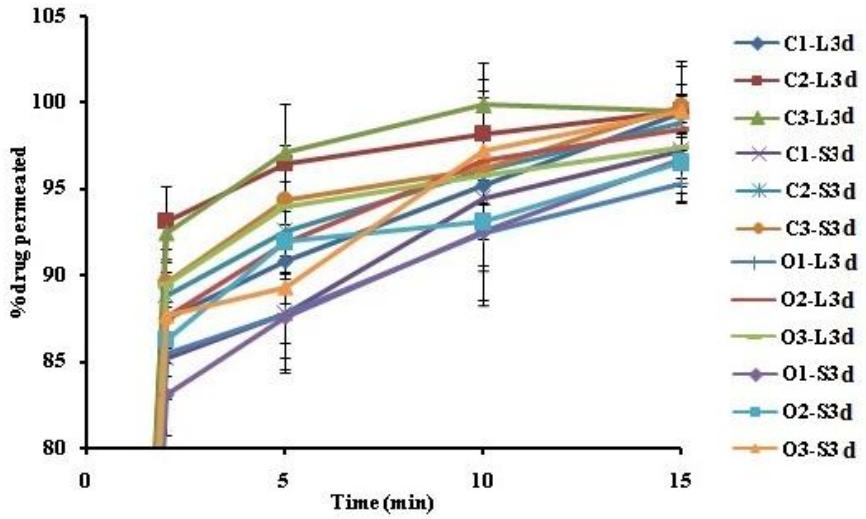

Figure 7: *Ex vivo* drug permeation of LB and LH from HPC-LF patches with natural permeation enhancer

Table 8: Permeability parameters the transmucosal patches of LB and LH

Sl No	Patch code	% drug permeated	Flux (µg/sq.cm.h)	Permeability coefficient x 10^{-7} (cm/sec)
1	L3c	76.05±0.58	26.66	9.25
2	S3c	71.95±0.71	25.23	8.75
3	C1-L3d	87.50±0.32	30.68	10.20
4	C2-L3d	91.08±0.53	31.93	11.10
5	C3-L3d	92.42±0.68	32.40	11.30
6	C1-S3d	87.77±0.68	30.77	11.10
7	C2-S3d	88.84±0.48	31.15	11.20
8	C3-S3d	89.58±0.96	31.41	11.20
9	O1-L3d	83.10±0.35	29.14	8.80
10	O2-L3d	86.22±0.95	30.23	11.00
11	O3-L3d	89.30±0.58	31.31	11.00
12	O1-S3d	86.41±0.36	30.30	10.00
13	O2-S3d	87.53±0.64	30.69	11.00
14	O3-S3d	89.46±0.42	31.37	11.20

In vitro residence time

In vitro residence time was in the range of 6-10 hours for all the patches evaluated in increasing order given polymers as HPMC>HPC-LXF>HPC-JF>SCMC>HPC-LF (Table 5). The early dislodgement of the patch from the mucosal surface was more distinct with the ionic polymer SCMC whereas enhanced erosion was observed with all other non-ionic polymers [19].

In vitro drug release

In vitro drug release study was carried out for 'c' and 'd' series of all polymeric patches after examining the physicochemical properties and also in view of capacity to further load the SLNs of Diclofenac as part of extended studies. *In vitro* drug release of HPC-LF showed release of 94% at second minute and maximum release of 99% at 15th min for patches with LB while all other polymeric patches exhibited decreased drug release (Table 6). With the increase in concentration of polymer the release of drug from the patch decreased. All the patches of LH displayed reduced release of drug when compared with patches of LB (Fig. 3 and 4). *In vitro* drug release depends on swelling of polymer and solubility of Drug in the release media [14,20,21]. *In vitro* drug release data correlates well with swelling studies of polymer. With the increase in the swelling index of polymeric patches there was decrease in release of drug. *In vitro* release data was subjected to fit the models for mechanism of release (Table 7) [13,21,22]. Most of the patches followed diffusion type of release of drug. L1c, L1d, S1c, S2c and S2c followed matrix diffusion release of drug from the polymeric patch. The best fit model of mechanism of release of drug for L5d was Higuchi. The exponent n value in the Krosmeyer Peppas equation for patches of SCMC, HPC-JF and HPC_MF were between 0.45 and 0.85 indicating ficken diffusion. The n- value of HPC-LF, HPC-LXF and HPMC were below 0.45 indicating quassi ficken diffusion.

Ex vivo permeation studies

Based on the *in vitro* release studies L3d and S3d were selected to out *ex vivo* permeation through porcine buccal mucosa (Fig. 5). There was good correlation between *in vitro* drug release and *ex vivo* drug permeation. The amount of drug permeated at second min was 74.05±0.58 % for L5d and 70.95±0.71 % for S5d prepared with HPC-LF. Labrafac PG is responsible for loosening the tight junctions of the cell membrane resulting in enhanced permeation.

Evaluation of bi-layered transmucosal HPC-LF patches of LB and LH with natural permeation enhancers

The physicochemical parameters are shown in table 5. The swelling index and in vitro residence time were not affected with the addition of clove oil and olive oil. The in vitro release was also enhanced (Fig. 6, Table 6) following Krosmeyer Peppas diffusion release (Table 7). *Ex vivo* permeation of LB still remained higher that LH (Fig. 7). Flux and permeability coefficient were significantly enhanced (Table 8).

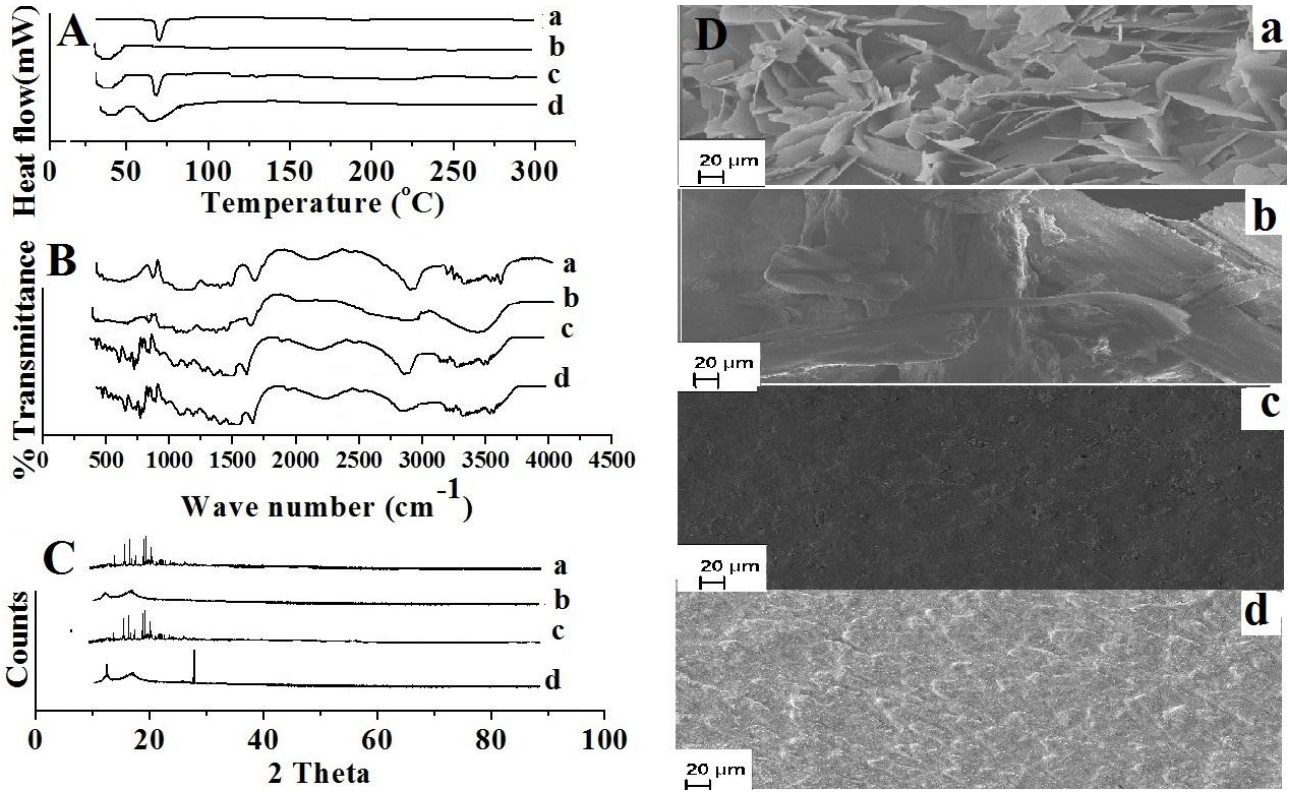

Figure 8: A) DSC thermograms **B)** FTIR spectrum **C)** XRD defractograms of a) LB b) HPC-LF c) Physical mixture of LB and HPC-LF g) patch **D)** SEM images of a) LB b) HPC-LF c) Placebo patch of HPC-LF d) patch

The permeation of LB through porcine buccal mucosa at fifth min increased and was maximum at 92.42±0.68% for C2-L3d with 20 % of clove oil as compared to olive oil. Hence clove oil was concluded to be better permeation enhancer as compared to olive oil. Further increase in percentage of clove oil to 30% did not show significant increase in permeation. Hence C2-L3d was concluded as the promising patch meeting the requirement of maximum release and permeation of the anaesthetic LB at second minute and also other satisfactory parameter of maximum *In vitro* residence time. Eugenol a phenolic compound present in clove oil reacts with phospholipids of cell membrane and increases the permeability of drug.[12] Ethyl acetate and Acetone utilized for backing layer, and ethyl alcohol utilized in mucoadhesive layer are Class 3 solvents with acceptable limit of <5000 PPM, hence Gas chromatography was not necessary to be carried out for the patch.

DSC, FTIR, XRD and SEM

The most promising patch (C3-L3d) meeting all the criteria of maximum permeation of the anesthetic within second minute was evaluated for DSC, FTIR, XRD and SEM. Pure drug (LB), polymer (HPC-LF), physical mixture of LB and HPC-LF, patch of LB prepared with HPC-LF containing Labrafac PG and Clove oil along with the backing layer were subjected DSC, FTIR and XRD. While in case of SEM, images of the placebo patch of HPC-LF was taken for comparison instead of the physical mixture.

DSC thermograms shown in Fig. 8A, the sharp endothermic characteristic peak at 68°C of pure LB was broadened in the thermogram of the patch indicating the conversion of crystalline LB to amorphous in the patch. Amorphous state of the drug is highly soluble as compared to crystalline thus increasing the rate of dissolution and permeation. The characteristic IR peaks (Fig. 8B) of LB are at 3030 cm^{-1} benzene ring range, 1450-1600 cm^{-1} (C=C), 3000-3500 cm^{-1} (H-N-C=O stretching of amide group), 3250 cm^{-1} (N-H), 1630-1690 cm^{-1} (C=O stretching of carbonyl group), 1020-1360 cm^{-1} (N attached to 3 carbons is a tertiary amine and it is not connected to H directly) which were intact in the patch and it infers that the drug is compatible with the polymer without interacting with it and also with other additives. The diffraction pattern (Fig. 8C) of LB and HPC-LF further confirms the results of DSC studies. Pure drug LB is crystalline while the crystallinity has diminished in the

patch and it exists in the amorphous form. SEM images shown in Fig. 8D, LB and HPC-LF exist in irregular shape and even distribution of LB in the patch can be observed when compared to placebo.

CONCLUSION

The results of permeation studies through porcine buccal mucosa did not match the results with dialysis membrane. On the basis of permeation studies it was found that LB permeated better than LH through porcine buccal mucosa. However studies were continued with both LB and LH. Since patches of HPC-LF had highest *in vitro* release in 15th min and satisfactory physiochemical properties, HPC-LF patches were selected for *ex vivo* permeation studies with porcine buccal mucosa. Upon incorporation of natural permeation enhancer clove oil and olive oil individually to the HPC-LF patches of LB and LH, it was found that permeation of LB increased and was maximum at second minute when compared to LH. With 20% of clove oil permeation of LB increased to 95% at second minute. Thus in conclusion patches of LB prepared with HPC-LF along with Labrafac PG and clove oil would promote rapid release and permeation of LB within second minute. Also application of patch would provide accurate dose with site specificity without any pain of needle prick with increased patient compliance.

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DECLARATION OF INTEREST

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