

Preparation and evaluation of buccoadhesive gel containing optimized Febuxostat-loaded microemulsion

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Abstract

The purpose of this study is to develop and characterize a successful colloidal nano-carrier viz. microemulsion for oral delivery as well as for topical delivery of antigout drug. Gout is a metabolic disorder which causes inflammation of the joints hence the pain. It is the most common crystal arthropathy and induced by the deposition of monosodium urate (MSU) crystal in joint and soft tissues that can produce an acute or chronic arthritis or inflammatory arthritis. The present study will conduct to investigate the microemulsion based drug delivery system of antigout drug febuxostat(FBT) in order to bypass its gastrointestinal adverse effect and to improve patient compliance and hence improve therapeutic efficacy.. Microemulsion will develop and optimize using box behnken design i.e. by using design expert software. The develop microemulsion based gels will characterized for pH, spreadability , drug content, viscosity and transdermal permeability of drug. Optimized gel formulation will then subject to in vitro antigout study in comparison to marketed available formulation of Febuxostat by using monosodium urate (MSU) crystals induced animal model.

Keywords:Febuxostat, microemulsion, microemulsion based gel, antigout, transdermal permeability and topical delivery.

1.Introduction

Introduction

Over the last decade extensive advancement in research in the direction of the development of buccal drug delivery for different diseases have been made. The oral buccal mucosa puts forward excellent opportunities to deliver drugs both locally and systemically (de Vries, Bodde et al. 1990). Since, the smooth oral mucosa surface has large number of sub-mucous capillaries amassing to the internal jugular vein. These do not connect to the liver but directly to the heart, which may circumvent the drug degradation by gastric intestinal enzymes, and also circumvent the first-pass effect of liver (Giannola, De Caro et al. 2007, Rai, Yadav et al. 2014, Singh, Kanoujia et al. 2016). Hence, buccal route is suitable for such protection enabled by muco/bioadhesive drug delivery systems (Bruschi and de Freitas 2005, Mizrahi and Domb 2008, Madhav, Shakya et al. 2009, Boyapally, Nukala et al. 2010). However, the mucus layer forms a physical barricade to the permeation of drug(s) due to short turnover time. It has been found that the muco/bioadhesive polymers on a mucous membrane might modify the turnover of mucin since of the residence time of muco/bioadhesives is generally longer than the reported mucin turnover time (Lee, Park et al. 2000). The muco/bioadhesive delivery system acquires a higher biocompatibility, allowing adhesion to the mucosa in the mouth, enabling absorption of drug, and then may be swiftly eliminated through the normal catabolic pathways, which could lessen the irritation or allergic reactions at the administration sites (Jain, Jain et al. 2008, Morales and McConville 2011).

Various drug delivery systems containing febuxostat such as chitosan microparticles (Ko, Park et al. 2002), self-emulsifying drug delivery systems (Patil, Biradar et al. 2009), hydrogel (Acharya, Shin et al. 2010), micronization and subsequent complexation with beta cyclodextrin (Sharma 2003), solid dispersion(Won, Kim et al. 2005), nano-dispersions (Karavas, Georgarakis et al. 2006), and solid self-microemulsifying tablets (Jing, Wang et al. 2016) have been developed. However, microemulsion based buccoadhesive delivery system for FEL has not been reported till date.

Drug delivery via buccal mucosa is a very intricate task, chiefly when the drug is poorly water soluble. So, the basis behind choosing microemulsion based bucco/mucoadhesive delivery system is that the lipophilic drugs cannot be incorporated into gel base directly since solubility acts as a barrier and this can influence the release of

the drug. So, to overcome this restriction, a microemulsion based approach was used, so that hydrophobic drug(s) can be efficiently incorporated and delivered through gels. Microemulsion facilitates the solubilization of lipophilic drug, thereby showing quick and proficient penetration to the application site. (Fanun 2012). The aim of the study was to develop a novel buccoadhesive gel formulation containing optimized FEL-loaded microemulsion (OPT-MEF) that possess suitable mechanical properties, adhere to the buccal mucosa for sufficient time, provide sustained action, improve buccal mucosa permeation and enhance plasma concentration of the drug. To accomplish this objective, microemulsion based buccoadhesive gels with varied concentrations of Hydroxypropyl methylcellulose (HPMC E4M) and polycarbophil (PCP) were prepared. febuxostat permeation from the prepared gel formulations was performed using goat buccal mucosa and mucoadhesive strength of gels was evaluated using mucoadhesion test. *In vivo* pharmacokinetic study was performed with a final formulation to obtain various pharmacokinetic parameters. The mechanical properties of the optimized gel formulation were examined using texture profile analysis. The rheological study of optimized gel formulation was performed with rheometer and viscosity measured using digital viscometer.

Preparation of buccal gel bases Firstly base gels were prepared. Base gel is the hydrogel without FEL-loaded microemulsion. The base gel was prepared by taking Methylparaben (0.1% w/v) and propylparaben (0.02% w/v) as preservatives and mixed properly by stirring and heating (50°C) on a hot plate digital magnetic stirrer (Tarson, Mumbai, India). Glycerine (10% w/w) and polyethylene glycol 400 (5% w/w) were added as humectants. Polycarbophil (PCP) and hydroxypropyl methyl cellulose E4M (HPMC E4M) were hydrated in sufficient quantity of double distilled water, separately for 24 h. To raise pH of PCP gel to neutral, triethanolamine was used. The final concentrations of these components were 4% w/w PCP and 4% w/w HPMC E4M.

2 Preparation of microemulsion based buccal gel As a vehicle for incorporation of microemulsion for buccal delivery, polycarbophil and HPMC E4M gels were prepared. Febuxostat-loaded optimized microemulsion (MEF: preparation and characterization reported earlier in part III) was utilized for the formulation of buccal gel. Microemulsion based buccal gels containing 1% w/w (MEF-E4M1), 2% w/w (MEF-E4M2) and 3% w/w (MEF-E4M3) HPMC K4M and 1% w/w (MEF-PCP1), 2% w/w (MEF-PCP2), 3% w/w (MEF-PCP3) PCP were prepared respectively. Different compositions were used in development of buccoadhesive gels as shown in Table 6.1.

Formulation code	Compositions of microemulsion based buccoadhesive gels (%w/w)							
	PCP	HPMC E4M	Drug conc.	Methyl paraben	Propyl paraben	Glycerol	PEG 400	MEF conc.
MEF-E4M1	-	1.0	1.0	0.1	0.02	10.0	5.0	10.0
MEF-E4M2	-	2.0	1.0	0.1	0.02	10.0	5.0	10.0
MEF-E4M3	-	3.0	1.0	0.1	0.02	10.0	5.0	10.0
MEF-PCP1	1.0	-	1.0	0.1	0.02	10.0	5.0	10.0
MEF-PCP2	2.0	-	1.0	0.1	0.02	10.0	5.0	10.0
MEF-PCP3	3.0	-	1.0	0.1	0.02	10.0	5.0	10.0

Prepared gels were assessed for homogeneity, color, texture, pH, drug content, buccoadhesive strength, viscosity, and permeation study. **Evaluation of prepared gels** **6.3.1 Physical examination** Method followed has been previously mentioned in **Section 4.3.1 of Part II. 1.2 The pH determination**

Method followed has been previously mentioned in **Section 4.3.2 of Part II 6.3.3 Drug content analysis** Method followed has been previously mentioned in **Section 4.3.3 of Part II 6.3.4 Determination of viscosity** Method followed has been previously mentioned in **Section 4.3.4 of Part II 6.3.5 Determination of mucoadhesion force** Method followed has been previously mentioned in **Section 4.3.5 of Part II 6.3.6 The *Ex vivo* drug permeation studies** Method followed has been previously mentioned in **Section 4.3.6 of Part II 6.3.7 Texture analysis** Method followed has been previously mentioned in **Section 4.3.7 of Part II 6.3.8 Rheological analysis** Method followed has been previously mentioned in **Section 4.3.8 of Part II 6.3.9 The *in vivo* pharmacokinetic studies**

The experimental protocol was approved by Institutional Animal Ethics Committee of Babu Banarasi Das Northern India Institute of Technology, Lucknow (BBDNIIT/IAEC/059/2014). The room temperature was maintained between 18 to 29 °C and relative humidity controlled between the ranges of 30% to 70% during the experiment. Animals were kept in polyacrylic cages, 12 h light/dark cycle and had free access to standard diet and water. Each group was individually housed and fasted overnight before the start of the studies while water was allowed *ad libitum*.

Study was performed as per previously reported method (Kumria, Nair et al. 2016, Singh, Kanoujia et al. 2016). Albino Wistar rats weighing between 200-220 g were divided into three groups (n=6 animals in each group) as shown in Table 6.2 i.e. Group I- kept as control, Group II- given marketed tablet oral suspension (1 mg dose) and Group III- rats were anaesthetized by intraperitoneal injection (i.p.) of phenobarbital sodium (30 mg/kg), and extra dose was given periodically to maintain anesthesia. The gel of amount equivalent to 1 mg drug was applied on both sides of the inner cheeks of group III rats.

Group	Dosage form	Route of administration
Group I Control	Given normal saline	Oral
Group II Standard	Marketed tablet suspension (1mg single dose)	Oral
Group III Test	Optimized MEF-PCP1 (1mg single dose)	Buccal

Blood samples (0.5ml) were collected in EDTA coated tubes at different time intervals through retro-orbital route after dose administration. Plasma was separated by centrifugation (at 5000 rpm for 10 min) at 4°C and stored in 2 ml vials at -20°C until the analysis.

The drug concentration in plasma supernatant was determined using HPLC by previously reported method (Sahu and Das 2014). Briefly, in this method liquid-liquid extraction was done using by gradient HPLC (Waters 2489, with UV-Visible Detector). The separation was done with analytical reverse phase column (Spherosorb C18, 250 x 4.6 mm) with a flow rate of mobile phase 1.5ml/min. The mobile phase contained a mixture of acetonitrile, methanol and phosphate buffer 0.05 M (40:20:40) adjusted to pH 3.0 with phosphoric acid and chromatographic separation was performed at 234 nm. For analysis of samples, plasma was mixed with mobile phase, vortexed and centrifuged (10000 rpm, 10 min) and supernatant collected in centrifuge tubes. The extracted supernatant was dried and reconstituted with the mobile phase, filtered through a 0.22µm membrane filter before analysis and then analyzed by HPLC. Pharmacokinetic parameters were determined using WinNonlin software. The relative bioavailability (Fr) of final optimized microemulsion (test) was calculated with respect to marketed tablet (standard) using the equation: $Fr (\%) = AUC_{test} / AUC_{standard} \times 100$

Pharmacokinetics parameters were analyzed using unpaired t-test with Welch correction to find out the statistically significant difference between the results.

Stability studies The optimized febuxostat-loaded microemulsion (OPT-MEF) based optimized buccoadhesive gel (MEF-PCP1) was kept in glass vials and stability study carried out according to ICH-guidelines viz. accelerated study (40 ± 2 °C / 75% RH ± 5% RH) for six months. The formulation was evaluated at intervals of 0, 3, and 6 months for accelerated study. During stability testing formulation was evaluated for physical appearance (color), phase separation, pH and drug content.

Results 2.1 Determination of physical parameters, pH and drug content It found that all the gel formulations were homogenous, smooth with acceptable consistency with a pH range of 6.73±0.015-7.08±0.012 and drug content of all prepared buccal gel formulations were found to be in the range of 9.57±1.18 to 9.79±1.53 mg/g of gel as shown in Table 2.3.

Table 2.3 Physical appearance, pH and drug content evaluation of buccoadhesive gels

Group	Dosage form	Route of administration
Group I Control	Given normal saline	Oral
Group II Standard	Marketed tablet suspension (1mg single dose)	Oral
Group III Test	Optimized MEF-PCP1 (1mg single dose)	Buccal

*SD-standard deviation

3.1 Viscosity

The viscosities of all the buccal gels were found to be in the range of 3788.34 ± 210.5 to $61,953.59 \pm 132.8$ cP as shown in Table 2.4

Results of viscosity, and buccoadhesive force (n=3) of gels

Formulation code	Results (n=3, mean \pm SD*)	
	Viscosity (Centipoise) \pm SD*	Buccoadhesive force (mN)
MEF-E4M1	3788.34 ± 210.5	111.12 ± 5.49
MEF-E4M2	8108.92 ± 302.3	195.92 ± 10.87
MEF-E4M3	11613.59 ± 332.4	265.79 ± 9.56
MEF-PCP1	20386.53 ± 190.6	317.84 ± 14.54
MEF-PCP2	39015.28 ± 138.3	388.76 ± 12.78
MEF-PCP3	61953.59 ± 132.8	467.63 ± 16.32

*SD-standard deviation

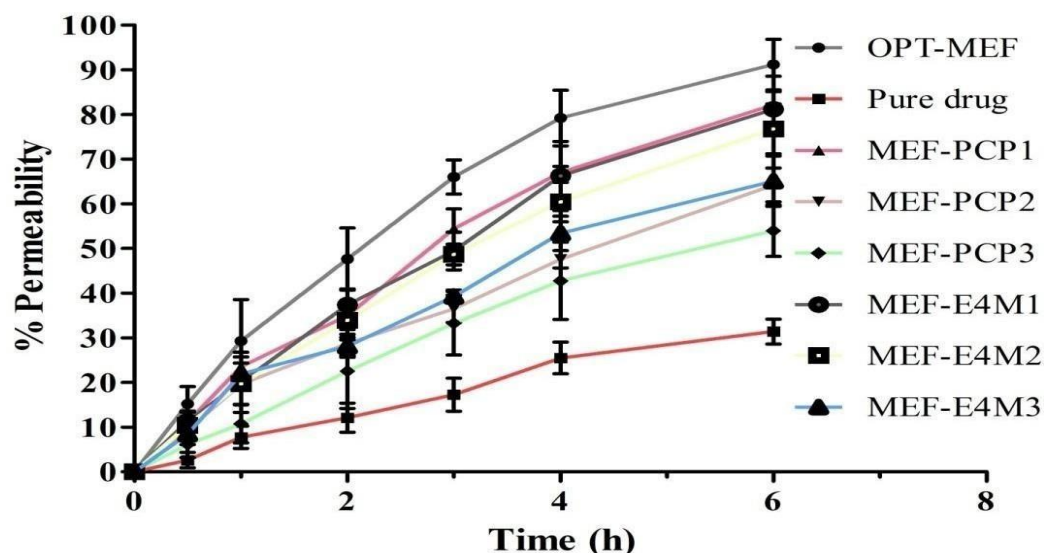
Muco/Buccoadhesive force The mucoadhesion study was executed by using the modified balance method (Fig. 2.1). Studies revealed that the buccoadhesive strength values for the buccal delivery system were between the ranges of 111.12 ± 5.49 - 467.63 ± 16.32 mN (shown in Table 2.4) at concentrations of 1% to 3% w/v HPMC E4M and PCP. **2.4.4 Ex vivo permeation** To study the effect of formulation ingredients on permeation, MEF-E4M1, MEF-E4M2, MEF-E4M3, HPMC K4M, MEF-PCP1, MEF-PCP2, MEF-PCP3, aqueous dispersion, and OPT-MEF were inspected for a period of 6h and each formulation analyzed in triplicate (Table 6.5). The MEF-E4M1, MEF-E4M2, MEF-E4M3, HPMC K4M, MEF-PCP1, MEF-PCP2, MEF-PCP3, OPT-MEF and aqueous dispersion showed 81.165 ± 3.97 , 76.797 ± 5.56 , 65.071 ± 5.64 , 82.161 ± 6.04 , 64.222 ± 3.79 , 53.961 ± 5.77 , 91.178 ± 5.67 and 31.411 ± 2.78 % drug permeation respectively at the end of 6h (Fig. 6.2 and Table 6.5).

Table 6.5 Per cent permeation of febuxostat from different formulations

Time (hr)	Per cent permeation of febuxostat (n=3, mean \pm SD)							
	OPT-MEF	Pure drug	MEF-PCP1	MEF-PCP2	MEF-PCP3	MEF-E4M1	MEF-E4M2	MEF-E4M3
0.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	15.17 ± 3.90	2.60 ± 1.72	11.09 ± 2.39	9.41 ± 1.98	6.12 ± 2.95	11.29 ± 2.35	10.44 ± 2.94	8.43 ± 2.36
1.0	29.32 ± 9.27	7.72 ± 2.52	23.53 ± 2.19	19.64 ± 1.33	10.76 ± 4.26	20.07 ± 6.74	19.72 ± 4.59	21.88 ± 0.99

2.0	47.66±6.95	12.14±3.25	35.00±2.69	28.75±3.10	22.51±8.29	37.35±3.57	33.92±4.40	28.07±2.01
3.0	66.00±3.79	17.27±3.67	54.44±4.41	36.51±3.07	33.25±7.11	49.45±4.22	48.65±2.28	39.23±1.47
4.0	79.21±6.24	25.48±3.54	67.04±1.33	47.57±1.93	42.78±8.64	66.24±7.74	60.41±4.42	53.38±3.86
6.0	91.17±5.67	31.41±2.78	82.16±6.40*	64.22±3.79*	53.96±5.77*	81.16±3.97*	76.79±5.56*	65.07±5.64*

*p<0.05 pure drug vs. MEF-PCP1, MEF-PCP2, MEF-PCP3, MEF-E4M1, MEF-E4M2, MEF-E4M3



Results of permeation studies of different formulations The steady state permeation flux (J_{ss}) and permeability coefficient (K_p) for all the formulations are shown in Table 6.6. The OPT-MEF (0.526 mg/cm²/h) and MEF-PCP1 (0.436 mg/cm²/h) was showed significantly higher flux ($p < 0.01$) than the aqueous suspension (0.168 mg/cm²/h).

Table 3.6 Comparison of permeation parameters of various formulation of februxostat

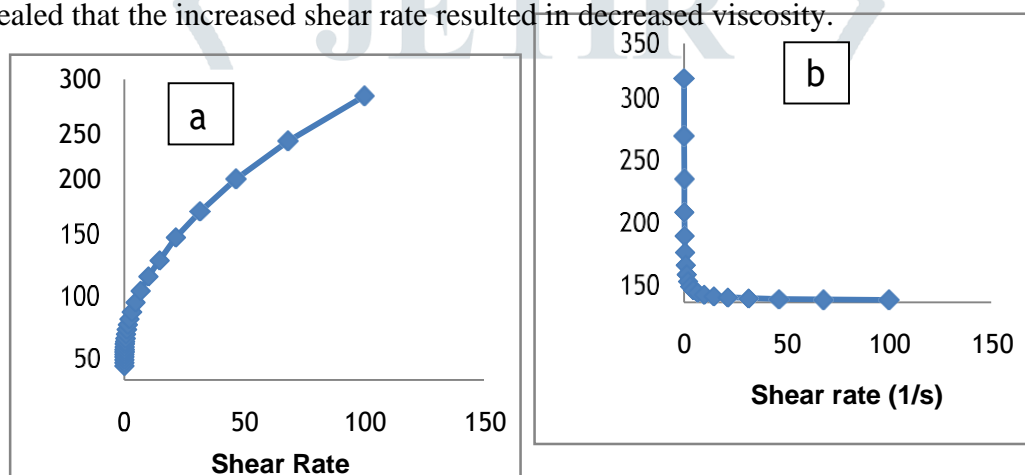
Formulation code	Permeation parameters of februxostat (n=3, mean±SD)			
	Mean per cent permeation at 6h	Flux J_{ss} (mg/cm ² /h)	Permeability coefficient K_p (cm ² /h)	Enhancement ratio
Pure drug	31.411±2.78	0.168±0.14	0.168	--
OPT-MEF	91.178±5.67*	0.526±0.13*	0.053	3.13
MEF-E4M1	81.165±3.97*	0.382±0.16	0.038	2.27
MEF-E4M2	76.797±5.56	0.332±0.12	0.033	1.97
MEF-E4M3	65.071±5.64	0.294±0.19	0.029	1.75
MEF-PCP1	82.161±6.04*	0.436±0.16*	0.044	2.59
MEF-PCP2	64.222±3.79	0.292±0.12	0.029	1.68
MEF-PCP3	53.961±5.77	0.224±0.09	0.022	1.33

$P < 0.01$, Pure drug vs OPT-MEF, MEF-E4M1 and MEF-PCP1

The correlation coefficient (r^2) values (listed in Table 6.7) for various models are calculated to determine the underlying release mechanisms. Drug suspension followed Hixon-Crowell (HC) type release mechanism, while MEF-PCP1, MEF-E4M3, OPT-MEF showed first order (FO) release, MEF-PCP2, MEF-MCP3, MEF-E4M2 followed first order as well as korsmeyer-peppas type release, and MEF-E4M1 showed korsmeyer-peppas type release mechanism (shown in table 6.7). **Table 6.7** Overview of the different kinetic models followed by various formulations

Formulation code	Permeation parameters of febuxostat (n=3, mean±SD)			
	Mean per cent permeation at 6h	Flux J _{ss} (mg/cm ² /h)	Permeability coefficient K _p (cm ² /h)	Enhancement ratio
Pure drug	31.411±2.78	0.168±0.14	0.168	--
OPT-MEF	91.178±5.67*	0.526±0.13*	0.053	3.13
MEF-E4M1	81.165±3.97*	0.382±0.16	0.038	2.27
MEF-E4M2	76.797±5.56	0.332±0.12	0.033	1.97
MEF-E4M3	65.071±5.64	0.294±0.19	0.029	1.75
MEF-PCP1	82.161±6.04*	0.436±0.16*	0.044	2.59
MEF-PCP2	64.222±3.79	0.292±0.12	0.029	1.68
MEF-PCP3	53.961±5.77	0.224±0.09	0.022	1.33

Rheological behavior of buccoadhesive gel Rheological study was performed for optimized buccoadhesive gel. The optimized buccoadhesive (MEF-PCP1) gel showed pseudoplastic behavior as shown in Fig. 6.2a and Fig. 6.2b which revealed that the increased shear rate resulted in decreased viscosity.



Rheological behavior of MEF-PCP1 gel (a) Pseudoplastic behavior (b) Effect of shear rate on viscosity

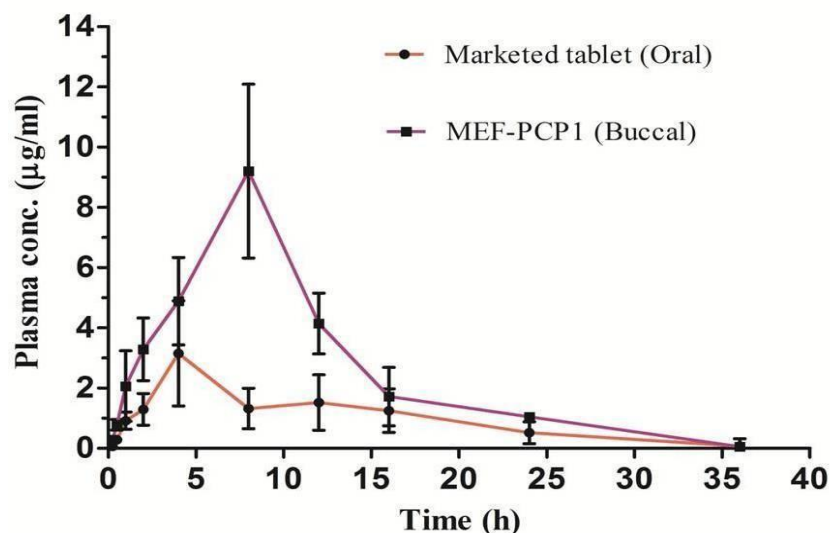
Texture analysis The texture analysis was done for the optimized buccoadhesive gel (MEF-PCP1) and texture analysis parameters are shown in Table 6.8.

Table 6.8 Texture profile analysis of blank gel (PCP1) and optimized MEF-PCP1

Texture profile parameters	Formulations	
	PCP1 blank	MEF-PCP1
Spreadability (mJ)	3.7	3.2
Extrudability (mJ)	161.2	151.8
Hardness (mJ)	18.0	13.8
Adhesiveness (g)	31.0	41.0

Pharmacokinetics study

The plasma concentration and time profile curve to contrast the plasma level profiles of febuxostat after oral administration of the marketed tablet and buccoadhesive gel (MEF-PCP1) formulations are shown in Fig. 6.4 and the various pharmacokinetic parameters are recorded in Table 6.9. Buccoadhesive gel (MEF-PCP1) was applied both sides to the cheek of albino Wistar rats. A dose of marketed tablet (1 mg) as the oral suspension was given to rats to evaluate the disparity in pharmacokinetic parameters with the buccoadhesive gel (equivalent to 1 mg drug dose).



Mean plasma concentration and time curve of febuxostat after buccal administration of MEF-PCP1 gel and oral marketed tablet

Comparative results of pharmacokinetic parameters (mean±SD, n=6)

Pharmacokinetic parameters	Marketed tablet (orally)	MEF-PCP1 (buccal)
T_{max} (h)	4	8
C_{max} (µg/ml)	3.513±1.74	9.208±2.88*
AUC_{last} (µg.h/ml)	41.87±10.41	166.39±17.65*
$AUMC_{last}$ (µg.h ² /ml)	1364.55±192.46	7203.36±588.36*

*P < 0.01, significant enhancement of C_{max} , AUC_{last} and $AUMC_{last}$ of optimized MEF-PCP1 as compared to marketed tablet

Stability study The stability study was performed to make sure the appropriateness of the developed delivery system i.e. formulations and its storage condition. The results of stability studies for developed systems MEBG5 are shown in Table 6.10.

Stability studies data of MEBG5 (n=3, mean±SD)

Test parameters	Accelerated storage conditions (40°C±2°C /75%±2% RH)		
	Time (months)		
	0	3	6
Color change	NO	NO	NO
Phase separation	NO	NO	NO
pH	7.05±0.04	6.9±0.02	6.7±0.18
Drug content (mg/g)	10.11±0.06	9.84±0.21	9.69±0.40

Discussion

The physicochemical parameters are the important parameters for the oral muco/buccoadhesive delivery of the drug. The physicochemical parameters of all the prepared gels are recorded in Table 6.2. It was found that all the gel formulations were homogenous, smooth with acceptable consistency and pH. It was found that the pH (6.73±0.015-7.08±0.012) of prepared buccoadhesive gels were similar to the oral mucosa pH 5.6-7.0 (Sudhakar,

Kuotsu et al. 2006). The pH of the developed formulations was near neutral in nature and thus could be considered as a suitable delivery system, for avoiding pain, damage or irritation to the oral mucosa tissues. The optimized MEF-PCP1 gel, having a pH of 7.08, could be administered easily through buccal mucosa, for systemic delivery of the drug. Drug content was determined to ensure the evenness of drug distribution in the developed buccal gel formulation. Drug content of all prepared buccal gel formulations was found to be in the range of 9.57 ± 1.18 to 9.79 ± 1.53 mg/g of the gel as shown in Table 6.2, showing good uniformity drug distribution in gel. The viscosity of the semi-solids formulation must be pourable from the container and spreadable on the required site. At the same time, formulations must encompass suitable retention characteristics to avert flowing and removal from the application site. Even though augmented viscosity may be more appropriate for retention, but the easiness of application on the wanted site as a thin film layer is also a significant criteria. The viscosity was found to increase by increasing the polymer concentration from 1 to 3% w/w (shown in table 6.3). This may possibly be due to the cross-linking between the polymer chains, or augmentation in intermolecular bonds thus increasing the gel network complexity (Perioli, Pagano et al. 2008, Cid, Pedrazzi et al. 2012). It was found to be maximal at about alkaline or neutral pH and was found to increase when the pH of medium elevated from acidic to neutral for PCP polymer based gel. This behavior is considerable for buccal drug delivery because it can lead to adhesive interactions and be able to improve polymer retention time over mucosal surfaces where the gel is applied.

Mucoadhesion is the binding of synthetic or natural polymer-based muco/buccal delivery system to the relevant site on mucosal tissue, providing prolonged and intimate contact between the drug delivery system and the absorption site (Salamat-Miller, Chittchang et al. 2005, Sudhakar, Kuotsu et al. 2006, Carvalho, Bruschi et al. 2010). Therefore, muco/bucco-adhesive drug delivery systems are capable of immobilizing and releasing the drug in specific regions, which contribute to upholding of therapeutic amounts of the drug at the applied site and allow for a sufficient and effectual therapeutic response (Salamat-Miller, Chittchang et al. 2005, Sudhakar, Kuotsu et al. 2006, Reddy, Chaitanya et al. 2011). It was found that an increased concentration of polymer in the buccal gel formulation resulted in an increased mucoadhesive strength (shown in Table 6.3). HPMC E4M based buccal gels showed less mucoadhesive strength compared to PCP gel formulation, which could be due to less interaction with the mucoadhesive membrane of HPMC E4M. Therefore, it can be concluded that PCP buccal gels of 1-3% w/w have good mucoadhesive strength compared to HPMC E4M gels and PCP based gels can be employed to extend the residence time of the drug at the relevance site in the oral buccal mucosa. It is reported that PCP has a high swelling ability under neutral pH condition, permitting high levels of entanglement within the mucus layer, hence providing good mucoadhesion (Robinson and Mlynek 1995). Comparisons of cumulative permeation data are shown in Fig. 6.2 and Table 6.4, in which prepared microemulsion and microemulsion based gels exhibited significantly enhanced drug permeation when compared with the conventional formulation (aqueous suspension i.e. pure drug). This increment was found approximately one to three folds when compared with aqueous suspension (shown in Table 6.4). Drug permeation from OPT-MEF ($91.178 \pm 5.67\%$) was significantly higher ($p < 0.01$) compared to the aqueous suspension of FEL (3 folds increment). Microemulsion based buccal gels (MEF-E4M1, MEF-E4M2, MEF-E4M3, MEF-PCP1, MEF-PCP2, and MEF-PCP3) showed significantly higher ($p < 0.05$) drug permeation compared to aqueous pure drug suspension at the end of 6h. All prepared buccal gels showed slower permeation than OPT-MEF which may possibly be ascribed to slower diffusion of drug through gel network. Instead of providing the optimum structure and viscosity to microemulsion for buccal application, gelling agents such as PCP and HPMC-E4M in microemulsion have a further benefit of excellent adhesive and constant releasing properties (Perioli, Pagano et al. 2008, Aggarwal, Goindi et al. 2013). The higher polymer amounts resulted in higher mean viscosities of polymeric solution which offered higher resistance to drug diffusion, therefore, slower permeation of drug (shown in Fig. 6.4). The difference in the permeation could be related to differences in gel concentrations. Results point towards diffusion of febuxostat from the buccal gel primarily reliant on the microviscosity of the water channels of the gel matrix rather than the macroviscosity of the gel formulations and the higher concentration of polymer which results in the reduction of size of water channels hence slower permeation (Attia, El-Gibaly et al. 2004). Hydration of polymer is also

Results reveal that OPT-MEF showed significant improvement in permeation of febuxostat. The permeation augmentation ratio of MEF-PCP1 buccal gel was 3.13 times higher comparing to the aqueous suspension of the FEL through oral buccal mucosa. This could be due to the presence of microemulsion in the buccal gel formulations that improved the permeation of febuxostat through the buccal mucosa (Singh, Kanoujia et al. 2016). This outcome may be due to the presence of oil and surfactant mixture in microemulsion which could be accountable for enhanced drug permeation from the buccal membranes by increasing the interaction and fluidity of the membrane. Furthermore, the presence of microemulsion in buccoadhesive gels changed the permeability of the oral buccal mucosa due to the presence of surfactant mixture (Tween 80 and Isopropyl alcohol). The surfactant mixture reduces the interfacial tension between the gel formulation and lipophilic mucosal layers, resulting in improved affinity and thus augmentation in permeability of drug across the buccal barrier. Other ingredients of the microemulsion formulations i.e. α -linolenic acid and muco/buccoadhesive polymer may be attributed to enhanced permeation due to interaction with mucosal lipids making them appropriate ingredients for such formulations. The buccal gels confirmed a slow permeation of febuxostat compared to OPT-MEF. This effect could be due to the release retarding effect of the polymer matrix, mainly due to the enhanced viscosity occurring from polymer gelation (Ritger and Peppas 1987, Zhu, Yu et al. 2008). Hence, it could be concluded that the polymer concentration and presence of microemulsion in the gel formulations plays a significant role in permeation of drug via buccal oral mucosa and gives an improved permeation of drug when compared with an aqueous suspension of pure drug. Kinetics of the release profile for various formulations was evaluated to determine the release mechanism. Drug suspension followed Hixon-Crowell (HC) type release mechanism as shown in table 6.7, which explains the release of drug from a system, where there is an alteration in diameter and surface area of the particle. It expresses the rate of dissolution based on the cube root of the particles for a drug powder containing uniform sized particles. While MEF-PCP1, MEF-E4M3, OPT-MEF showed first-order (FO) release which indicates the concentration of drug in the release media is directly proportional to time, MEF-PCP2, MEF-MCP3, MEF-E4M2 followed FO as well as korsmeyer-peppas (KP) type release, and MEF-E4M1 showed KP type release mechanism (shown in Table 6.7). KP generally demonstrates a drug's release when the release mechanism is unidentified or more than one type of release mechanism is involved. This is an amalgamation of two independent release mechanisms: one process mechanism is related to the transport of drugs in a way that obeys Fick's law, or Fickian transport, and the second process is a result of the polymer swelling/relaxation phenomenon, which is due to Case-II transport mechanism (Ritger and Peppas 1987). This model considers both mechanisms i.e. the drug diffusion as well as polymer relaxation (Korsmeyer and Peppas 1983, Korsmeyer, Gurny et al. 1983) and while in majority of cases, both diffusion and erosion occur concurrently (Gao, Skoug et al. 1996, Kim and Fassihi 1997). The drug release exponent (DRE) values (n) are employed to demonstrate diverse types of the release mechanism. According to the literature, an n value of up to 0.50 indicates that drug release from the system occurs by a Fickian diffusion mechanism, and $n = 1.00$ indicates zero order kinetics, i.e. , controlled release by the relaxation of polymer chains or erosion (Case II transport); n value higher than 1 indicates super Case-II transport; and an n value between 0.50 and 1.00 indicates anomalous transport or a combination of both diffusion mechanisms and Case II transport (Korsmeyer, Gurny et al. 1983). All the prepared microemulsion based buccal gels, drug suspension and optimized microemulsions displayed, n values as shown in Table 6.7 and were found to be between 0.50 and 1.00, indicating anomalous transport or a combination of both diffusion mechanisms. These results were similar to the previously reported studies (Baloglu, Karavana et al. 2010, Morales, Su et al. 2013).

Thus, it can be concluded that prepared gels promote the release of febuxostat and the drug release from buccal gels is dependent on the polymer concentration. Additionally, the drug release mechanism may be diffusion associated or not associated with gel matrix relaxation. The network complexity of polymer, viscosity of the prepared gel, diffusion and dissolution of the drug and the swelling/relaxation of the polymer matrix gel, constitute a barrier to the release of the drug. Therefore, drug permeation from gel system is sustained when compared with OPT-MEF. Based on the different parameters such as the physicochemical analysis, viscosity, in vitro mucoadhesion force, ex vivo permeation study of the prepared microemulsion based buccal gels, MEF-PCP1 was selected for further evaluations like rheological study, texture analysis, and in vivo pharmacokinetics study. Pharmaceutical gel formulations must include suitable rheological properties illustrated as gel formulation efficacy, stability, and the consumer's acceptability. A pseudoplastic or plastic property for a bio/mucoadhesive formulation to the buccal application is required for suitable rheological behavior (Sudhakar, Kuotsu et al. 2006). The pseudoplastic behavior (shown in Fig. 6.2a) of a gel system is due to the presence of long molecules and high molecular weights of the polymer. In solution form, these polymer molecules become entrapped with the immobilized solvent which results in high viscosity and shows flow resistance. On applying of shear, the molecules tend to become extricated and align in the direction of the flow and therefore show less flow resistance to release the entrapped water, consequently, the viscosity decreases as shown in Fig 6.2b. Upon removing the

shear stress, a structural reorganization of the system occurs and viscosity increases hence this process is reversible. For that reason, a pseudoplastic behaviour is appropriate for buccal delivery purpose, because as the shear stress increases the viscosity decreases, assisting in the spreading of formulations over tissue (Gaspar and Campos 2003).

The instrumental technology was used to emulate the human being sensorial observations in terminology of Hardness, spreadability, extrudability and adhesiveness while applying semisolid formulations such as gels, creams as topical applications. Texture analysis of the optimized gel formulation was carried out by the automatic CT3 Texture Analyzer (Brookfield Engineering Laboratories, USA). Spreadability and extrudability of semisolid preparations are the vital texture parameters of an organoleptically aesthetic formulation (Rai, Yadav et al. 2014, Singh, Kanoujia et al. 2016). Spreadability and extrudability are the sum of energy or work done (mJ) obligatory to pierce the sample of gel or cream up to a definite depth when applied to the surface and to uniformly extrude out from the packaging tube (Contreras and Sanchez 2002). Spreadability is also a simulation of human perception of spreading any semi-solid over the skin surface or application to mucosal surfaces. It generally depends on the ingredients used in the preparation of cream, gels or ointments. Spreadability of MEF-PCP1 (3.2 mJ) was observed to be better than that of PCP1 (3.7mJ). Work done needed to extrude out product from the tube i.e. extrudability of MEF-PCP1 and PCP1 gels from the tube was found to be 161.2 mJ and 151.8 mJ respectively as shown in Table 6.6. Hardness is the maximum positive force necessitated to distort the gel sample by a finger. The hardness of PCP1 and MEF-PCP1 was found to be 18.0 mJ and 13.8 mJ, respectively, in texture analysis. PCP1 was found to be practically harder than MEF-PCP1 indicating that PCP1 required higher force to deform its surface. The hardness of MEF-PCP1 was found to be lesser than the PCP1 gel, which could be attributed to the presence of microemulsion in MEF-PCP1. Adhesiveness of the gel preparation is an imperative parameter for muco/buccoadhesive quality and defined as the highest force required in surmounting the attractive force between the surface and gel formulation and it is a measure of stickiness of the sample (Inoue, Furuya et al. 2012). Adhesiveness of the MEF-PCP1 and PCP1 gels was found to be 41.0 g and 31.0 g, respectively. Finally, the texture analysis unveiled that the FEL-loaded MEF-PCP1 gel has admirable gel strength, ample adhesiveness, and ease of spreading, which is essential for application and retains the gel formulation in the buccal cavity. The plasma drug profile showed that the application of buccoadhesive gel delayed the febuxostat absorption and augmented the duration of absorption as shown in Fig. 6.3, which in turn augmented the absorption of febuxostat in the body as compared to the oral suspension of the drug. The C_{max} value (3.513±1.74 µg/ml) of MEF-PCP1 (buccoadhesive gel) was found significantly higher (P<0.01) when compared with the same dose of oral route C_{max} (9.208±2.88 µg/ml). This could be due to drug binding in the oral epithelium which might mimic the rate of drug disappearance from the oral cavity and appearance in the plasma after buccal administration. Also, permeability of the drug increased via the capillary vessels present in the oral buccal cavity hence the drug directly enters into the systemic circulation and as well because of avoidance of drug degradation through intestine and liver (Smart 2005, Giannola, De Caro et al. 2007, Patel, Liu et al. 2011). As expected, the T_{max} value was found to be significantly increased following buccal gel delivery (8 h) when compared to oral marketed tablet (4 h). This may perhaps be attributed to the presence of gelling agent which sustained the drug release in the oral buccal cavity.

The enhancement in AUC value (in the case of buccal gel) signifies the increment in rate and extent (relative bioavailability) from the buccal gel when compared to the orally administered marketed tablet. The AUClast through buccal route was found to be 3.973 folds higher when compared with oral marketed tablet. The results showed significant differences as indicated by the p values <0.01, representing enhanced bioavailability through buccoadhesive gel when compared with the marketed tablet also given orally. The relative bioavailability of the MEF-PCP1 buccal gel was 397.30% higher with respect to orally administered marketed tablet suspension. The augmentation in relative bioavailability, AUC and C_{max} may possibly be due to the fact that drug molecules, when absorbed through the buccal route due to the presence of rich blood vasculature of the buccal oral mucosa, directly enter into the systemic circulation and also due to avoidance of drug degradation through intestine and liver. Other reason for enhancement in AUC and C_{max}, may be that the buccal gel (MEF-PCP1) enhanced the permeability of the buccal mucosa due to the presence of OPT-MEF which contains surfactant mixture. The presence of mucoadhesive polymer enabled intimate contact with lipophilic buccal mucosal layers resulting in increased concentration of drug in the blood. Hence, a buccal gel containing drug loaded microemulsion could be a good choice for circumventing the first pass metabolism, intestinal degradation, and enhancement of the bioavailability of poorly water-soluble drugs. During the stability study no visible physical changes (color, phase separation) observed for MEF-PCP1. Also, no considerable changes in pH and drug content observed over 0-6 months when stored at accelerated condition.

6.6 Conclusion

The microemulsion based muco/buccoadhesive gel was gainfully developed and evaluated for systemic delivery of FEL via buccal route. Buccoadhesive gel containing febuxostat loaded microemulsion was evaluated for

various physicochemical parameters, ex vivo and in vivo performances which demonstrated the formation of a consistent, stable and effective delivery system. The results of the permeation study were established the role of the microemulsion for efficient buccal permeation of febuxostat by means of the buccal route. The pharmacokinetic studies illustrated adeptness of prepared gel toward efficient absorption of febuxostat via buccal mucosa as shown by the pharmacokinetic parameters i.e. C_{max}, AUC, and T_{max}. The future prospective includes intrication of the clinical studies for developing a microemulsion based muco/buccoadhesive gel or wafer formulation of febuxostat for commercial purpose. It was concluded that the buccoadhesive gel containing drug loaded microemulsion may possibly be

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