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Formulation and evaluation of Lornoxicam mucoadhesive buccal films

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ABSTRACT: Lornoxicam (LN) is a non-steroidal anti-inflammatory and analgesic drug of the oxicam class. As with other non-steroidal anti-inflammatory drugs, it has the same side effects of this group of drugs if they are taken orally such as gastrointestinal, renal and hepatic disorders. Besides, it binds extensively to plasma albumin (99%), has a relatively short plasma half-life (3 to 5 hrs) and undergoes first pass hepatic metabolism and gastrointestinal degradation upon oral administration. These drawbacks render LN a good candidate for local delivery via sustained release dosage forms. Therefore, LN mucoadhesive buccal films were prepared by the solvent casting method using different polymers, that is sodium carboxymethyl cellulose (Na CMC), hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), gelatin, polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP) K-30. Differential scanning calorimetry (DSC) and infrared spectroscopy (IR) studies indicated the absence of any physical or chemical interaction of the drug with any of the used polymers. The prepared films were evaluated for their weight uniformity, thickness uniformity, swelling index, surface pH, folding endurance, *in vitro* drug release as well as mucoadhesion force. On the basis of the results obtained, it was deduced that the best formula of LN mucoadhesive buccal films was that containing a mixture of Na CMC, HPC and PVP; since it exhibited a high bioadhesive strength, a high percentage of drug release, a good folding endurance and a high swelling index. It can be finally concluded that mucoadhesive buccal films can be one of the alternatives available for administration of LN in order to minimize its side effects and avoid the disadvantages of parenteral and oral routes of administration.

KEYWORDS: Lornoxicam; mucoadhesive buccal films; solvent casting method; bioadhesive strength.

1. INTRODUCTION

Buccal drug administration indicates the application of drugs to the mucous membrane in the mouth to facilitate the drug effects either locally or systemically [1]. The buccal site of drug administration allows faster medication delivery and enhanced bioavailability through bypassing the first-pass metabolism and enzymatic breakdown. Moreover, it permits self-administration and provides an effective therapy to patients suffering from dysphagia through a non-invasive means [2 - 4]. There have been numerous trials to make a variety of mucoadhesive drug delivery systems such as tablets, films, patches, disks, strips, gels and ointments, for various therapeutic substances. Mucoadhesive buccal films are thought to be the most favored form owing to their high flexibility, compact design and thinner thickness and thus they will be more likely accepted by the patient than the tablets. Additionally, they provide more precise application of the medication compared to gels and creams [1, 5]. Such films consist of many layers specified for sustained drug liberation inside the oral cavity [6]. Even with the great therapeutic value of the buccal site of application, there is a shortage of commercially available buccal formulations which may be caused by the absence of compendial and physiologically appropriate evaluation methods for the accurate in vitro characterization of developed dosage forms [7, 8]. The development of these methods needs a thorough comprehension of the physiological environment where applied buccal dosage forms exist in. Buccal films can be formulated by several ways: hot melt extrusion method, casting of solvent method, direct milling method and 3D printing technology [9-13]. The production of buccal films is most commonly achieved through solvent casting, as it is cost-effective and less complex when compared to other methods [14]. Mucoadhesive films are favorably designed to treat cardiovascular and inflammatory conditions, as they can minimize adverse reactions and improve the oral bioavailability of medications prescribed for these disorders [3].

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Lornoxicam (LN) is a powerful NSAID medication that is generally used to alleviate pain and lower levels of inflammation. However, the drug is poorly soluble in water (BCS class II), has a short half-life (3 - 4 h) and many adverse effects such as dizziness, vomiting, nausea, stomach pains, drowsiness, somnolence, headache and flushing [15]. Hence, this research aimed to use LN as mucoadhesive buccal films to give a prolonged profile of drug release and a sufficient quantity at the aimed site and to get rid of the drug drawbacks. The mucoadhesive films were made by the solvent casting technique through the use of different film making polymers and then assessed for consistency in drug content, weight, film thickness, surface pH, the endurance of folding, index of swelling, residence time, mucoadhesive force and in vitro drug release. Kinetics of the drug release was also investigated.

2. RESULTS AND DISCUSSION

Solvent casting method was used for simple and cost-effective formulation of buccal films of LN. The work in this research comprised the utilization of the following mucoadhesive polymers: HPMC, Na CMC, HPC, gelatin, PVP K-30 and PEG 4000. Different polymer content and concentrations were evaluated. DSC and IR studies were done for Lornoxicam and its physical mixture with the polymers employed in fabricating its mucoadhesive buccal films for investigating any possible chemical or physical interaction which may occur between the drug and any of these polymers. The properties of the manufactured films were assessed for their weight variation, thickness uniformity, content uniformity, swelling index, folding endurance, surface pH, mucoadhesion properties and *in vitro* drug release.

2.1. Drug-excipient compatibility study

The assessment of probable incompatibilities between the drug and different additives is an important element of the pre-formulation phase during the development of pharmaceutical dosage forms. Therefore, differential scanning calorimetry (DSC) and infrared spectroscopy (IR) studies were performed for Lornoxicam and its physical mixture with the polymers used in preparation of its mucoadhesive buccal films for investigation of any physical or chemical interaction of the drug with any of these polymers.

2.1.1. Differential scanning calorimetry (DSC)

DSC thermographic images of LN, polymers and their physical blend are graphically demonstrated in Figure 1. The incompatibilities between Lornoxicam and the different polymers, if present, could be identified by observing changes in the characteristic peaks of the drug visible on its thermogram. Lornoxicam thermogram, as shown in the figure, is characterized by an endothermic peak appearing at 222.53 °C, that corresponds to its melting point and indicates the crystallinity of the medication. Each of the DSC thermograms of Na CMC, HPMC E5, HPC, PVP K-30, PEG and gelatin is characterized by a shallow and broad endothermic peak at 103.55 °C, 101.56 °C, 74.06 °C, 101.3 °C, 60.47 °C and 90.31 °C respectively, which accurately reflects the melting points of the polymers. The distinctive melting endotherm of LN was clearly visible in the DSC thermogram of the physical mixture which advocates the absence of interaction between LN and the employed polymers.

2.1.2. Fourier transform infrared spectroscopy (FT-IR)

Infrared spectroscopy was employed to verify any incompatibility between Lornoxicam and the previously mentioned polymers. Figure 2 shows the FTIR spectra for LN, polymers and their physical mixture. IR spectrum of Lornoxicam demonstrates a characteristic absorption peak for primary amines at 1621 cm^{-1} which falls within the normal range of amine absorption. The spectrum demonstrates a noticeable peak for the carbonyl group of the non-conjugated carboxylic acid at 1645 cm^{-1}, whereas a second band which is expected to shift to a lower frequency (owing to conjugation) appears as an overlapping band. The O=S=O and acyclic amide both show a peak at 1379 cm^{-1}. Additionally, the C-H band stretching was viewed in the extent of 1532 cm^{-1} to 1501 cm^{-1}. The identifying peaks of LN did not change and were still visible in the infrared spectrum of the physical mixture of the drug with the employed polymers as shown in the figure. This confirms the absence of interaction between the drug and polymers.

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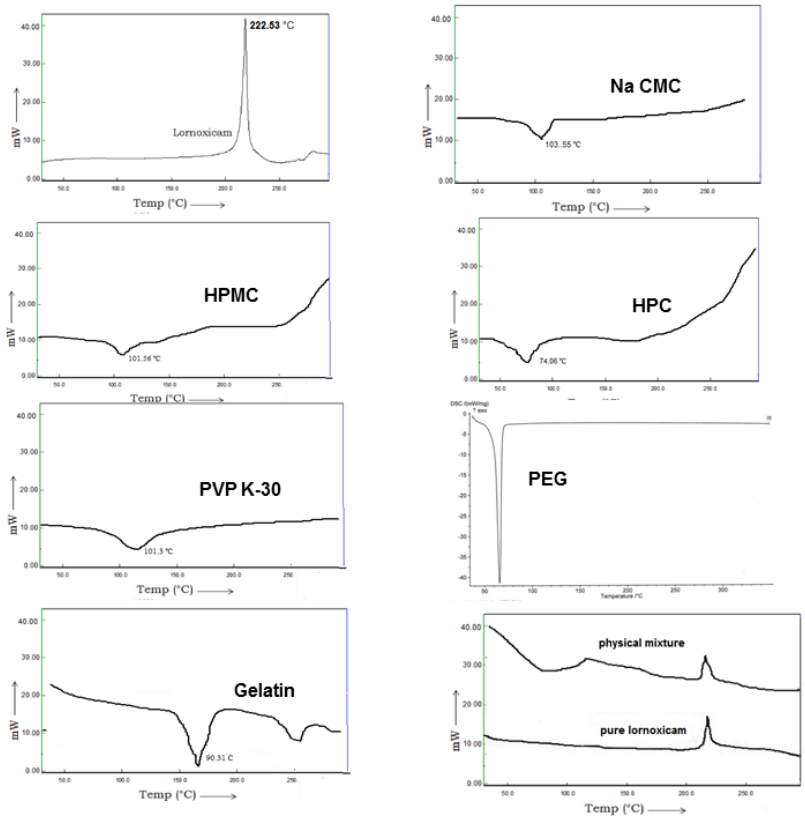

Figure 1. DSC thermograms of Lornoxicam, polymers and their physical mixture.

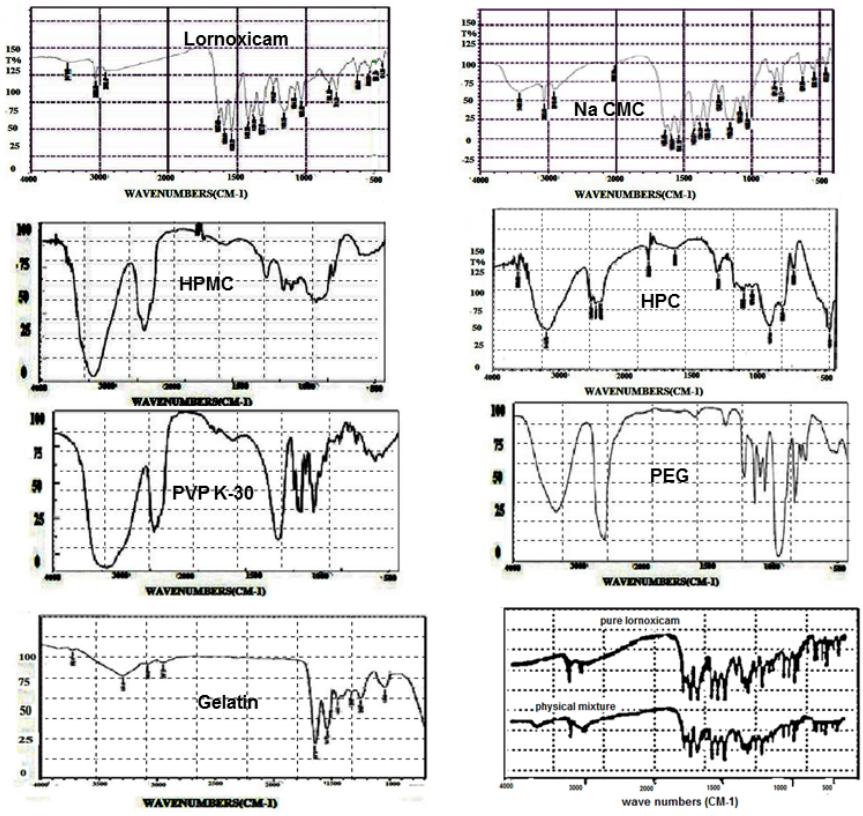

Figure 2. FTIR spectra of Lornoxicam, polymers and their physical mixture.

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2.2. Evaluation of LN mucoadhesive buccal films

2.2.1. Drug content uniformity

The content of LN in all films was accepted ranging from 95.17% to 102.5% as shown in Table 1. This outcome suggested that the medication was evenly distributed throughout the films, which demonstrated the effectiveness of the solvent casting technique for preparing the medicated mucoadhesive buccal films.

2.2.2. Weight uniformity

Drug loaded films had uniform weight. The mean weight of the films varied from 105.857 mg for formula FM_{25} to 167.743 mg for formula FM_{24} as shown in Table 1. The average weight determined reflects the appropriateness of the formulated films for buccal use [16].

2.2.3. Thickness uniformity

All the films had consistent thickness ranging between 0.217 mm for formula FM_{24} and 0.403 mm for the two formulations FM_{27} and FM_{36} (Table 1), which could be a result of the differences in the polymer content of each formula. The measured thickness of the prepared films will be suitable for application into the buccal cavity without any feel of irritation or bulkiness for the patients [17].

2.2.4. Folding endurance

All film formulations exhibited good folding endurance ranging from 272 to > 300 without showing any cracks except formula FM_{21} which had a relatively low folding endurance of 156 as exhibited in Table 1. This indicates that these films had high elasticity and good mechanical strength. Folding endurance provides valuable information about how will a film perform when used in the buccal cavity as well as during storage. Inflexible films may break up during storage leading to loss of some of the drug content. Additionally, the firm consistency of these films can make them difficult to adapt to the buccal cavity upon use and they may cause discomfort, irritation and distress upon application [18, 19]. The low folding endurance of formula FM_{21} may be due to the presence of gelatin which has strong cohesive properties, thus the gelatin films obtained tend to be fragile and easily prone to fissures [20].

2.2.5. Surface pH

The oral administration of a dosage form with a highly acidic or alkaline pH can lead to irritation of the buccal mucosa and may furthermore affect hydration of the polymers, which in turn can influence the drug release. Therefore, the surface pH of the films was investigated to prevent mucosal irritation and optimize mucoadhesion and drug release [21]. Surface pH measurement of the films indicated that all the formulations had a surface pH that ranged from 6.31 for formula FM_{37} to 6.92 for formula FM_{19}. These pH levels of the buccal films were close to that of human saliva, which typically has a pH range of 6.2 to 7.6 [22]. Therefore, it was expected for these buccal films to be non-irritant when applied to the mucosa. Values of the surface pH of the mucoadhesive films are displayed in Table 1 and graphically illustrated in Figure 3.

2.2.6. Swelling index

The swelling index values of the medicated mucoadhesive buccal films are shown in Table 1 and clearly illustrated in Figure 4. Results of the swelling study revealed that films containing 3% HPMC (FM_{19}, FM_{26}, FM_{27} and FM_{36}) had the lowest swelling index where its value ranged between 244 and 255%. On the other hand, films containing HPC or gelatin (FM_{20}, FM_{21} and FM_{37}) had the highest swelling index where its value ranged between 688.277 and 763.24%. These results may be attributed to the higher hydrophilicity of HPC and gelatin when compared to HPMC, and also to the higher content of hydroxyl groups in HPC molecules which play an important role for improvement of water absorption and maintenance of matrix integrity of swollen polymer [23].

Based on the results of folding endurance and swelling index studies, five medicated formulations were selected for assessment of their *in vitro* mucoadhesion, *in vitro* residence time and *in vitro* release of the drug. These five formulations were FM_2, FM_{20}, FM_{25}, FM_{35} and FM_{37}, which had folding endurance values of 279, > 300, 280, > 300 and > 300 respectively, while their percentage swelling values were 480.60, 688.277, 456.20, 346.40 and 692.433 respectively.

Table 1. Physicochemical evaluation of LN mucoadhesive buccal films.

Formulation number	Drug content (%) (Mean ± SD)	Weight (mg) (Mean ± SD)	Thickness (mm) (Mean ± SD)	Folding endurance (Mean ± SD)	Surface pH (Mean ± SD)	Swelling Index (%) (Mean ± SD)
FM_2	98.17 ± 0.007	111.573 ± 4.058	0.233 ± 0.012	279 ± 4.041	6.47 ± 0.02	480.60 ± 6.21
FM_{19}	98.93 ± 0.006	115.940 ± 1.421	0.235 ± 0.006	> 300	6.92 ± 0.015	255.767 ± 1.855
FM_{20}	100.50 ± 0.010	136.553 ± 1.492	0.23 ± 0.002	> 300	6.72 ± 0.012	688.277 ± 0.937
FM_{21}	95.17 ± 0.007	120.897 ± 1.305	0.327 ± 0.012	156 ± 0.577	6.64 ± 0.006	763.24 ± 2.281
FM_{24}	95.50 ± 0.002	167.743 ± 1.232	0.217 ± 0.012	280 ± 0.577	6.72 ± 0.012	387.29 ± 3.34
FM_{25}	95.83 ± 0.005	105.857 ± 2.418	0.337 ± 0.015	280 ± 1.528	6.86 ± 0.012	456.20 ± 3.35
FM_{26}	100.97 ± 0.006	130.133 ± 0.351	0.303 ± 0.006	> 300	6.43 ± 0.015	251.533 ± 1.106
FM_{27}	102.50 ± 0.003	120.833 ± 0.306	0.403 ± 0.006	272 ± 1	6.45 ± 0.015	244.233 ± 0.833
FM_{35}	100.40 ± 0.003	133.433 ± 0.723	0.230 ± 0.010	> 300	6.43 ± 0.021	346.40 ± 0.954
FM_{36}	100.30 ± 0.008	106.407 ± 1.100	0.403 ± 0.006	> 300	6.44 ± 0.012	255.817 ± 3.927
FM_{37}	96.50 ± 0.002	126.333 ± 0.896	0.397 ± 0.006	> 300	6.31 ± 0.015	692.433 ± 2.113

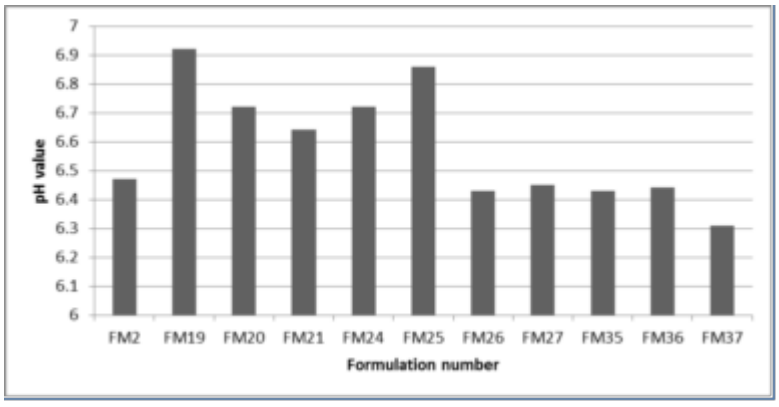

Figure 3. pH values of Lornoxicam mucoadhesive buccal films.

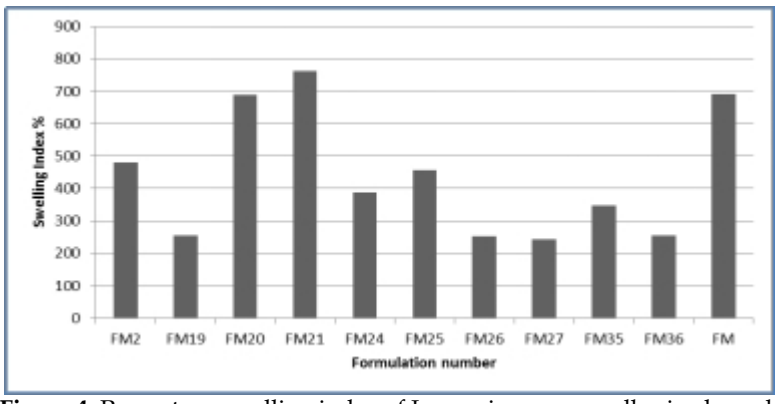

Figure 4. Percentage swelling index of Lornoxicam mucoadhesive buccal films.

2.2.7. In vitro mucoadhesion study

The efficiency of drug adhesion to the body surface is vital in ensuring sufficient drug levels at the place of application and minimizing the elimination of the formulation. Bioadhesion force and bioadhesion strength of the selected LN mucoadhesive films on rabbit buccal mucosa were measured and exhibited in Table 2 and demonstrated in Figure 5.

It has been suggested that hydrophilic polymers combine with mucus via physical entanglement and chemical bonds similar to hydrogen bonding, as stated by Pongjanyakul and Suksri [24]. The films under investigation showed bioadhesion strength ranging from 22.578 to 34.574 g and adhesion forces values ranging between 0.221 and 0.339 N. The films FM_2 and FM_{37} showed high bioadhesive strength (34.574 and 33.335 g respectively) while the other films (FM_{20}, FM_{25} and FM_{35}) showed relatively lower bioadhesive strength (22.578, 24.627 and 24.513 g respectively). It was also detected that existence of PVP improved the bioadhesive strength. Thus, formula FM_{20} containing Na CMC and HPC had a bioadhesive strength of 22.578 g, while such value was increased significantly ($P<0.05$) to reach 33.335 g in formula FM_{37} containing Na CMC, HPC and PVP. This judgment was in contrast with that stated by Shidhaye and co-workers [25] who stated that PVP K30 gave a negative impact on mucoadhesive strength; where, a decrease in mucoadhesive strength was observed with the increase in concentration of PVP K30. Conversely, inclusion of HPC decreased the bioadhesive strength of the films. Thus, formula FM_2 containing Na CMC had a bioadhesive strength of 34.574 g, while formula FM_{20} containing a mixture of Na CMC and HPC had a bioadhesive strength of 22.578 g which was significantly lower than that of formula FM_2 ($P<0.05$). Such observation was inconsistent with that of Gaber *et al*. who found that HPC, being a hydrophilic polymer, forms a thick, sticky gel that improves the adhesion of the film to the mucous membrane [21]. The slightly elevated bioadhesive force of formula FM_{35} in comparison to formula FM_{20} may be attributed to presence of HPMC. HPMC is a highly hydrophilic polymer containing a large number of OH groups which can form a strong viscous gel with aqueous media through hydrogen bonding, and such gel can strongly penetrate the mucous layer. These outcomes were in agreement with those stated by El Sharawy *et al*., Gilhotra *et al*. and Gaber *et al*. [17, 18, 21].

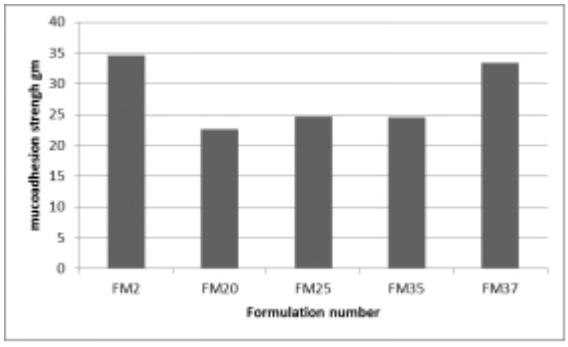

Figure 5. Mucoadhesive strength of LN mucoadhesive buccal films.

2.2.8. In vitro residence time

The time of *in vitro* residence of various LN mucoadhesive buccal films ranged from 4.14 h for FM_{37} to 5.24 h for FM_{20} as shown in Table 2. It was notable that no relationship was observed between the bioadhesion force of the polymers and their time of residence. It seems that polymers with significant bioadhesive strength might not persist for an extended time on the mucosal surface. Bioadhesion is significantly influenced by factors such as chain flexibility and surface charge density, while the time of residence is mainly governed by the rate at which the polymer dissolves [26].

2.2.9. In vitro release of the drug

Results of the in vitro release studies of the selected LN mucoadhesive buccal films are exhibited in Table 3 and graphically illustrated in Figure 6. The extent of the released drug after 6 h ranged from 75.26% to 92.3% for all studied formulations. It was noticed that inclusion of HPC had a positive effect on the dug release from its films.

Table 2. *In vitro* mucoadhesion measurement of LN mucoadhesive buccal films.

Formulation number	*In vitro* mucoadhesion measurements		*In vitro* residence time (h) Mean ± SD
	Bioadhesion strength (g) Mean ± SD	Force bioadhesion (N) Mean ± SD	
FM_2	34.574 ± 0.221	0.339	4.35 ± 0.03
FM_{20}	22.578 ± 0.195	0.221	5.24 ± 0.03
FM_{25}	24.627 ± 0.089	0.242	4.36 ± 0.02
FM_{35}	24.513 ± 0.072	0.240	5.07 ± 0.02
FM_{37}	33.335 ± 0.204	0.327	4.14 ± 0.03

Thus, the extent of drug liberated after 6 h from formula FM_2 containing Na CMC was 88.6%, while upon using a mixture of Na CMC and HPC in formula FM_{20}, a significant increase ($P<0.05$) in the percentage of drug release was observed where it reached 92.3% after 6 h.These results may be explained by the higher swelling ability of formula FM_{20} due to presence of HPC that is considered as a highly hydrophilic polymer and this will lead to an increase in the drug diffusion from the loose swollen film matrix. On the other hand, inclusion of HPMC was found to decrease the amount of drug released from its films as observed in the two formulae F_{20} and F_{35}. Thus, formula FM_{20} containing a mixture of Na CMC and HPC had a percentage of drug release of 92.3% after 6 h, while such percentage was significantly lowered ($P<0.05$) to reach only 83.8% upon addition of HPMC in formula F_{35}. These results can also be correlated to the swelling behavior of the film matrix. Thus, the low swelling capacity of formula F_{35} will reduce the drug diffusion from the compact film matrix. Addition of PVP was also found to significantly decrease the amount of drug released ($P<0.05$). This can be noted upon comparing formula F_2 with formula F_{25}, and formula F_{20} with formula F_{37}. Thus, the ratio of drug liberated after 6 h from formula F_2 containing Na CMC was 88.6%, while it was only 75.2% in formula F_{25} containing a mixture of Na CMC and PVP. Also, the quantity of the released drug after 6 h from formula F_{20} containing a mixture of NaCMC and HPC was 92.3%, while it was 87.75% in formula F_{37} containing a mixture of Na CMC, HPC and PVP. The formation of a thick gel barrier by PVP-containing films led to an increase in the diffusional drug path length , which in turn decreased the drug release.

Table 3. ***I****n vitro* release of LN from its mucoadhesive buccal films.

Time (min)	% Cumulative drug released from different formulations (Mean ± SD)				
	FM_2	FM_{20}	FM_{25}	FM_{35}	FM_{37}
30	7.0 ± 0.0008	12.11 ± 0.001	9.5 ± 0.003	17.6 ± 0.001	7.17 ± 0.001
60	14.7 ± 0.003	26.04 ± 0.007	13.4 ± 0.006	19.2 ± 0.004	19.8 ± 0.011
90	29.06 ± 0.006	32.66 ± 0.001	25.5 ± 0.006	26.6 ± 0.002	23.6 ± 0.003
120	35.5 ± 0.003	42.3 ± 0.01	35.08 ± 0.011	34.03 ± 0.001	39.3 ± 0.007
180	55.05 ± 0.006	55.4 ± 0.003	42.1 ± 0.002	56.0 ± 0.006	47.5 ± 0.003
240	63.4 ± 0.012	66.95 ± .01	53.05 ± 0.03	65.2 ± 0.002	57.01 ± 0.019
300	83.03 ± 0.0008	83.34 ± 0.01	64.5 ± 0.005	75.5 ± 0.001	73.02 ± 0.012
360	88.6 ± 0.005	92.3 ± 0.004	75.2 ± 0.002	83.8 ± 0.002	87.75 ± 0.004

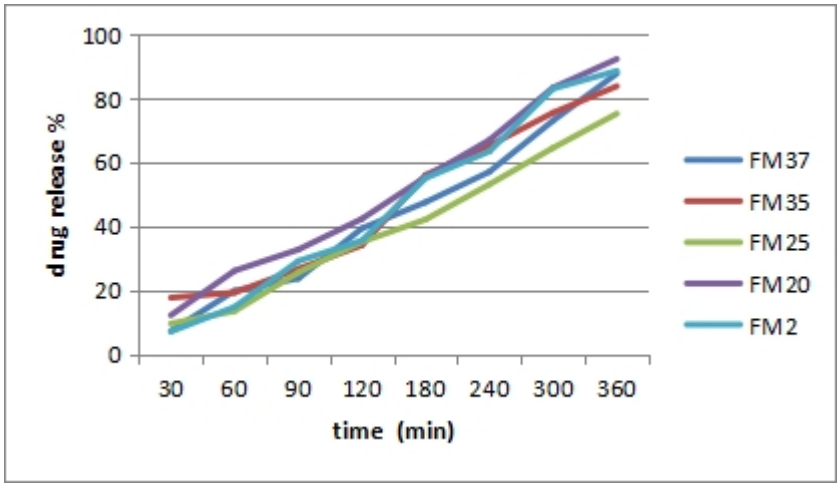

Figure 6. Release profiles for Lornoxicam from its mucoadhesive buccal films.

Based on all the aforementioned results, we can consider that formula F_{37} containing a mixture of Na CMC, HPC and PVP is the best formula for LN mucoadhesive buccal films since it showed a high bioadhesive strength (33.335 g) and a high percentage of drug release after 6 h (87.75%), in addition to its good folding endurance (> 300) and high swelling index (692.433%). Therefore, this formula of LN mucoadhesive buccal films can be introduced as a promising dosage form of LN that alleviates pain and inflammation while minimizing side effects of the drug.

2.2.10. Study of kinetics of the drug release

To study the drug release kinetics, the in vitro release data were scrutinized according to numerous models namely, zero order, first order and Higuchi diffusion models. The pattern of the drug release will closely match the model having the highest correlation coefficient (R^2) value. The drug release data were also analyzed according to Korsmeyer-Peppas equation to know the drug release mechanism. Kinetic analysis of LN release data from its mucoadhesive buccal films showed that LN release obeyed zero order kinetics in all the selected formulations except formula F_{35} which obeyed first order kinetics as displayed in Table 4. The n values of all formulations, except F_{35}, were greater than 0.85 as illustrated in Table 4, indicating that these formulations follow super-case II drug release mechanism. The super-case II drug release mechanism implies the simultaneous occurrence of drug diffusion, polymer relaxation (due to swelling) and erosion (due to dissolution) [27]. The n value of formula F_{35} was less than 0.85, which indicates that the release of drug from this formula follows anomalous (non-Fickian) diffusion mechanism.

Table 4. Kinetics and mechanism of LN release from its mucoadhesive buccal films.

Formulation number	Correlation coefficient (R^2)			Release kinetics	Korsmeyer-Peppas release exponent (n)	Release mechanism
	Zero order	First order	Higuchi Diffusion			
FM_2	0.9866	0.9603	0.9538	Zero order	1.2643	super-case II transport
FM_{20}	0.9876	0.9245	0.9767	Zero order	0.9218	super-case II transport
FM_{25}	0.9800	0.9639	0.9527	Zero order	1.148	super-case II transport
FM_{35}	0.8629	0.9483	0.7844	First order	0.8087	anomalous (non-Fickian) diffusion
FM_{37}	0.9789	0.9094	0.9630	Zero order	1.1373	super-case II transport

3. CONCLUSION

Lornoxicam has been successfully developed into mucoadhesive buccal films by the solvent casting method using various film-forming polymers, namely, Na CMC, HPMC, HPC, gelatin, PEG and PVP either separately or in combinations. The uniformity of the drug content declared the appropriateness of the solvent casting process for preparing the medicated mucoadhesive buccal films. Both DSC and IR studies indicated absence of drug - polymer interactions. Thus, these polymers can be used in preparing Lornoxicam mucoadhesive buccal films with sufficient confidence that the drug will be available in its active form for exerting its pharmacological action. It was found that the best formula of Lornoxicam mucoadhesive buccal films was that containing a mixture of Na CMC, HPC and PVP; since it showed a high bioadhesive strength, a high percentage of drug release, a good folding endurance and a high swelling index. The obtained results suggested that Lornoxicam mucoadhesive buccal films can be regarded as promising therapeutic systems for the buccal delivery of Lornoxicam for the relief of pain and treatment of inflammations to minimize side effects of the drug and avoid the disadvantages of parenteral and oral routes. However, further clinical studies are still required to endorse these outcomes.

4. MATERIALS AND METHODS

4.1. MATERIALS

Lornoxicam was kindly supplied by Global Napi Pharmaceuticals (Cairo, Egypt). Hydroxypropylmethyl cellulose E5 and hydroxypropyl cellulose were gifts from the Egyptian International Pharmaceutical Company, EIPICO (Cairo, Egypt). Polyvinyl pyrrolidone (PVP K-30) was obtained from Sigma Company (Cairo, Egypt). Sodium carboxymethyl cellulose and gelatin were purchased from EL-Nasr Pharmaceutical Chemicals Company (Cairo, Egypt). Polyethylene glycol (PEG 4000) and propylene glycol were obtained from October Pharm (Giza, Egypt). All other chemicals and solvents were of analytical grade and were used without further purification.

4.2. **Preparation of mucoadhesive buccal films**

The films were prepared by the method of solvent casting. Hydroxypropyl methyl cellulose (HPMC), hydroxypropylcellulose (HPC), sodium carboxymethyl cellulose (Na CMC), gelatin, polyvinyl pyrrolidone (PVP) K-30, and polyethylene glycol (PEG) were employed as film-forming polymers, while propylene glycol was added as a plasticizer. Different polymer solutions were prepared in accordance with the composition shown in Table 5. All the polymer solutions were prepared using 50 ml of alkalinized distilled water (5 ml of 0.15 N NaOH/100 ml water) excepting HPC which was dissolved in alkalinized methanol (5 ml of 0.15 N NaOH/100 ml methanol). The polymer dispersions were stirred on a magnetic stirrer (Gallenkamp, England) for 45 min at 50 rpm. Propylene glycol was then added and the stirring was continued for further 15 min. A sufficient amount of Lornoxicam was dissolved in 50 ml of either alkalinized distilled water or alkalinized methanol according to type of the polymer. The drug solution was added to the polymer solution under stirring. The prepared viscous solutions were kept overnight at room temperature to become clear and bubble-free. Then they were casted onto a glass Petri dishes each of 9 cm diameter and left to dry in an oven kept at 40°C until flexible films were obtained. The final volume of each formula was 100 ml which was required to prepare five Petri dishes. Films were then cut into discs of 4 cm^2 (equivalent to 5 mg of Lornoxicam), packed in aluminum foil and stored in a glass desiccator kept at room temperature and 60 ± 5% relative humidity to keep the integrity and elasticity of the films.

At first, plain mucoadhesive films were formulated with varying polymer concentrations and exposed to preliminary tests as folding endurance and index of swelling. Only eleven of these formulations, as shown in Table 5, with favorable characteristics were selected to be incorporated with the drug.

Table 5. Composition of LN mucoadhesive buccal films.

Formula number	Lornoxicam (g)	NaCMC (g)	HPMC (g)	HPC (g)	Gelatin (g)	PEG (g)	PVP (g)	Propylene glycol (ml)	Distilled water to (ml)
FM_2	0.4	3						0.5	100
FM_{19}	0.4	3	3					0.5	100
FM_{20}	0.4	3		1				0.5	100
FM_{21}	0.4	3			0.8			0.5	100
FM_{24}	0.4	3				1.5		0.5	100
FM_{25}	0.4	3					1.5	0.5	100
FM_{26}	0.4		3	1				0.5	100
FM_{27}	0.4		3				1.5	0.5	100
FM_{35}	0.4	3	1.5	1				0.5	100
FM_{36}	0.4	3	3				1.5	0.5	100
FM_{37}	0.4	3		1			1.5	0.5	100

4.3. Drug-excipient compatibility study

Differential scanning calorimetry (DSC) and infrared spectroscopy (IR) studies were performed for Lornoxicam and its physical mixture with the polymers used in preparation of its mucoadhesive buccal films for investigation of any physical or chemical interaction of the drug with any of these polymers.

4.3.1. Differential Scanning Calorimetry (DSC)

DSC studies were performed for Lornoxicam and its physical mixture with the polymers, using a differential scanning calorimeter (DSC-50, Schimadzu, Kyoto, Japan) connected with a thermal analyser TA-501 and Deskjet 500c printer. Each sample (3 - 4 mg) was put in aluminum pan and heated at a rate of 10 °C/min over a temperature range of 30 to 250 °C. Dry nitrogen gas was used as a carrier gas with a rate of flow of 25 ml/min.

4.3.2. Fourier transform infrared spectroscopy (FT-IR)

Infrared (IR) spectroscopic analysis of lornoxicam and its physical mixture with polymers was carried out using an IR spectrophotometer (IR-470, Schimadzu, Kyoto, Japan). Samples were prepared by mixing the drug with an IR-grade potassium bromide in 2:200 ratio. The mixtures were then pressed into transparent films using an IR hydraulic press (Schimadzu, Japan). The films were examined over a wavenumber range of 4000 to 400 cm^{-1}.

4.4. Evaluation of LN mucoadhesive buccal films

4.4.1. Drug content uniformity

Films of size 4 cm^2 (2 cm x 2 cm) were cut from three different areas on the casted plates. Each film was dissolved in a volume of 100 ml of phosphate buffer of pH 6.8 in a volumetric flask, and occasionally shaken for 8 h. Afterward, samples of 5 ml volume were taken and assayed for drug content using a UV spectrophotometer (Shimadzu-50-02, Kyoto, Japan) at λ_{max} 374 nm. The concentration of the drug was calculated using a pre-established standard calibration curve. Each experiment was done in triplicate and the mean ± SD was calculated.

4.4.2. Weight uniformity

Three films of the size 4 cm^2 were weighed individually using a digital balance (Sartorius, Germany) and the average weight of each formula was calculated.

4.4.3. Thickness uniformity

Thickness of the films at three different points was measured using a screw gauge micrometer (Mitutoyo Co. Ltd, Japan). Data were represented as mean ± SD (n = 3).

4.4.4. Folding endurance

The folding endurance is used to estimate the flexibility of the films quantitatively. Folding endurance of the films was measured by repeatedly folding a small strip of the film at the same place up till 300 times until it was broken. The number of times at which the film could be folded with no breaking was the value of the folding endurance [28, 29].

4.4.5. Surface pH

To assess the potential irritating effect of the films on the buccal mucosa, the pH of the surface of the prepared buccal films was measured. Three films of each formulation were allowed to be in contact with 1 mL of distilled water. The surface pH was recorded by a pH meter (Model 3510, Genway, USA) by contacting the electrode with the film surface and letting it to equilibrize for 30 s. The mean of three measurements was obtained.

4.4.6. Swelling index

The swelling index was determined to guess the swelling behavior of the films upon contact with the saliva after application into the oral cavity. Pre-weighed films (W_1) of the size 4 cm^2 were immersed in 50 ml of phosphate buffer solution of pH 6.8 maintained at 37 °C. The strips were taken out carefully at the end of 90 min, blotted with filter paper and weighed accurately (W_2) [26]. The experiment was repeated three times for each film and the average was recorded. The swelling index was determined by the equation:

$$\%\text{Swelling Index} = (W_2 - W_1) / W_1 \times 100$$

4.4.7. In vitro mucoadhesion study

In vitro mucoadhesion study is of high importance because when the films are applied, they need to adhere to the buccal mucosa in order for the drug to be absorbed [31]. The adhesive properties of various films were evaluated using rabbit buccal mucosa as a model for this study [32]. The mucosal membrane was fixed using cyanoacrylate adhesive on the bottom side of a tissue holder made from plexiglass, and the film was affixed to another similar-sized holder. The mucosal membrane surface was initially wiped with a filter paper, then wetted with 25 µl of phosphate buffer of pH 6.8. The two holders carrying the mucosal membrane and the film were placed in touch with each other and subjected to an even and constant force for a period of 5 minutes to promote adhesion between the film and the mucosal tissue. The tissue holder with buccal mucosa was hanged on an iron stand using an aluminum wire, a pre-weighed light weight polypropylene bag was hanged to the hook on backside of the film holder using a piece of aluminum wire. After 5 minutes, water was added to the polypropylene bag through an intravenous infusion set at a steady rate of 1 drop/s till the film separated from the tissue. The water gathered in the bag was measured in grams to represent the bioadhesion strength. Bioadhesion force for each film was determined using the equation:

$$\text{Force of bioadhesion (N)} = \text{bioadhesion strength (g)} \times 9.81/1000$$

The average of three determinations was calculated.

Figure 7 exhibits the apparatus utilized for in-vitro bioadhesion testing. The apparatus was as described by parodi *et al.* [33].

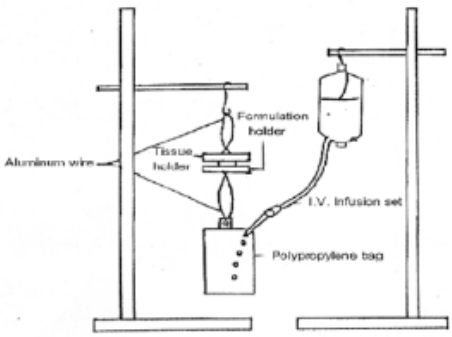

Figure 7. A schematic presentation of the apparatus used for *in-vitro* bioadhesion test.

4.4.8. In vitro residence time

The *in vitro* residence time was measured using a modified USP disintegration apparatus (Erweka, GmbH, Germany) with a disintegration medium consisting of 500 ml phosphate buffer of pH 6.8 maintained at 37 ± 0.5 °C. A piece of rabbit mucosal membrane was glued to the surface of a glass slab. The mucoadhesive film was hydrated from one surface using 50 µl of the phosphate buffer then the hydrated surface was put into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down so that the film was totally immersed in the buffer solution at the lowest point and was out at the highest point. The time required for complete dissolution or separation of each film from the mucosal surface was the residence time [34, 35]. The mean of three measurements was taken.

4.4.9. In vitro release of the drug [36]

The drug release rate from the films was studied using the USP dissolution test apparatus type II (Pharma test model PT-DT40, Germany). Films of the size 4 cm^2 (equivalent to 5 mg of LN) were cut and allowed to release the drug from only one side by placing a water-impermeable backing membrane of ethyl cellulose on the other side of the film. The assembly for release studies was prepared by sandwiching the film in a dialysis membrane. A piece of glass slide was placed as a support to prevent the assembly from floating. Closure clips were used to secure the dialysis tube with the film inside from both ends. The tube was placed in the dissolution medium composed of 500 mL of phosphate buffer of pH 6.8 rotated at 50 rpm and kept at a temperature of 37 ± 0.5 °C. Aliquots of 5 mL were collected at different time intervals for up to 6 h and replaced with 5 mL of fresh dissolution medium. The taken samples were filtered, appropriately diluted and analyzed for the drug content using a UV spectrophotometer at 374 nm. The cumulative percentage of drug released was calculated from a pre-established standard calibration curve and plotted against time. The release studies were performed in triplicates and the mean values were calculated.

4.4.10. Study of kinetics of the drug release

To study the kinetics of LN release from its mucoadhesive buccal films, the in vitro release data were analyzed according to zero order (%cumulative drug released vs. time), first order (log %cumulative drug retained vs. time) and Higuchi diffusion models (%cumulative drug released vs. square root of time). Correlation coefficients (R^2) were determined for each model by Microsoft Excel Program [37, 38]. The model with best fits was selected based on the highest correlation coefficient (R^2). To reveal the drug release mechanism, the release data of the drug from these films were also analyzed according to Korsmeyer-Peppas model (log %cumulative drug released vs. log time) which describes the drug release from polymeric systems. In Peppas model, the release exponent "n" was calculated which is indicative of drug release mechanism. As specified by Peppas theory, if $n = 0.43$ then the drug release follows Fickian diffusion mechanism, for $0.43 < n < 0.85$, the drug release follows anomalous (non-Fickian) diffusion mechanism, for $n = 0.85$, the drug release follows case II transport and for $n > 0.85$, the drug release follows super-case II transport mechanism [39].

4.4.11. Statistical analysis

All experiments were carried out in triplicate (n=3), and the results were expressed as the mean ± standard deviation (SD). Differences in the mean values were analyzed using one-way analysis of variance (ANOVA) using SPSS® software V. 22 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA). Differences were considered to be significant at p values < 0.05 [40].

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