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Clove Oil Emulsified Buccal Patch of Serratiopeptidase for Controlled Release in Toothache

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Abstract

The objective of this work was to formulate and characterize clove oil emulsified buccal patch of serratiopeptidase to offer their combinational controlled release actions in dental pain. The buccal patches were prepared by two methods 1. Adding serratiopeptidase and clove oil in alcoholic solution of hydroxypropyl methylcellulose (HPMC) and Eudragit. 2. Emulsification of clove oil in aqueous serratiopeptidase solution and then dispersed in mixture of polymeric solution of HPMC and Eudragit. Scanning electron microscopy (SEM) of patches showed uniform distribution of w/o emulsion of serratiopeptidase and clove oil; and regular surface of patches. Entrapment efficiencies of serratiopeptidase and clove oil were found to be significant. *In vitro* and *in vivo* release studies showed the controlled release of serratiopeptidase and clove oil for 24 h. Fourier transform infrared spectroscopy (FTIR) and Differential scanning calorimetry (DSC) studies confirmed no interaction of serratiopeptidase and clove oil with polymers and excipients. It showed that buccal patch of emulsified clove oil with serratiopeptidase can be used for their controlled delivery in toothache or dental pain.

Keywords: Serratiopeptidase; Clove oil; Buccal patch; Dental pain

Introduction

Buccal drug delivery shows an attractive alternative route to oral delivery systems due to its advantages like prevention of GI irritation, minimum systemic toxicity and evasion of first pass metabolism. Since buccal mucosa has rich blood supply and acts as excellent site for absorption, it is well accepted by patient and easy for self-medication [1,2]. Serrapeptase is known as serratiopeptidase and is a proteolytic enzyme isolated from the non-pathogenic *Enterobacterium serratia* sp. E-15. The serratiopeptidase acts by hydrolyzing the histamine, bradykinin and serotonin and uses for pain and inflammatory conditions such as arthritis and minor muscle trauma. It can absorb through intestines and transported directly in to blood stream [3,4]. Clove oil is an essential oil and is obtained from the dried flowers, buds, leaves, stem of the tree *Syzygium aromaticum* or *Eugenia cariophyllata* and *Eugenia aromaticum*. It has been used as anesthetic, anti-inflammatory and analgesic agents for toothaches [5]. Clove oil acts by denaturing the proteins and reacting with cell membrane phospholipids changing their permeability and inhibiting the Gram-negative, Gram-positive bacteria and yeast. To overcome first pass metabolism, gastrointestinal irritation and systemic toxicity of serratiopeptidase; and burning sensation, nerve and tissue damage of concentrated clove oil, the buccal patch was an interesting delivery system in dental pain [6]. So, the objective of this work was to formulate and characterize clove oil emulsified buccal patch of serratiopeptidase to investigate their combinational controlled release actions like analgesic and anti-inflammatory in dental pain.

Materials and Methods

Materials

Serratiopeptidase was obtained from Advanced Enzyme Technologies, Thane, India. Eudragit L100 was gifted by Evonik Roehm Pharma Polymer, Mumbai, India. Clove oil, Tween 80, ethanol and HPMC K4M were purchased from S.D. Fine Chemicals, Mumbai, India. All other chemicals used were of analytical grades.

Methods

Preparation of buccal patch

Non-emulsification method: Ten mg serratiopeptidase was dissolved in ethanol at ambient temperature under continuous stirring and then added to alcoholic HPMC solution. Later, clove oil and Eudragit L100 were added to form homogenous mixture. This mixture was poured in a petri dish and dried at room temperature for 12 h [7]. The dried film was removed and stored in a desiccator. Different patches of polymer concentrations of HPMC and Eudragit L100 were prepared in various ratios 1:1, 3:2, 2:3 and 4.5:5.5 were abbreviated as F1, F2, F3 and F4, respectively as shown in Table 1.

Emulsification method: W/o type emulsion was prepared by

Ingredient	F1	F2	F3	F4	F5
HPMC	500 mg	600 mg	400 mg	450 mg	500 mg
Ethanol	8 ml	8 ml	8 ml	8 ml	4 ml
Serratiopeptidase	10 mg	10 mg	10 mg	10 mg	10 mg
Ethanol	4 ml	4 ml	4 ml	4 ml	4 ml
Clove oil	0.06 ml	0.06 ml	0.06 ml	0.06 ml	2 ml
Eudragit L100	500 mg	400 mg	600 mg	550 mg	500 mg
Tween 80	-	-	-	-	0.05 ml
Water	-	-	-	-	4 ml
Ratio w/w (HPMC:Eudragit L 100)	**1:1**	**3:2**	**2:3**	**4.5:5.5**	**1:1**

Table 1: Composition of buccal patch formulation.

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dispersing aqueous serratiopeptidase solution in clove oil (ratio 1:1 v/v) and Tween 80 mixture using Silverson homogenizer (Remi industries, India) at room temperature. This emulsion was added gradually in alcoholic solution of HPMC under continuous stirring. Then Eudragit L100 was slowly added to form homogenous mixture. This mixture was transferred in a petri dish and kept for drying to obtain buccal patch. The formulation was abbreviated as F5 (Table 1).

Characterization of buccal patch

Thickness: Thicknesses of the patches were measured using vernier caliper (Unique Enterprises, Mumbai) at different areas on the film and the average was calculated [8].

Patch weight: Three patches of each formulation (F1, F2, F3, F4 and F5) were taken and weighed individually on digital balance and results were analyzed for mean standard deviation.

Moisture content: The prepared patch formulations were weighed individually and kept in a desiccator containing calcium chloride at room temperature for 24 h. The patches were weighed again after fixed interval, until they showed constant weights. The percent moisture content was calculated by using following formula [2].

% Moisture content = Initial weight - Final weight / Final weight × 100

Folding endurance: Three patches of each formulation (1 × 1 cm^2) were cut by using stainless steel cutter. Folding endurance was estimated by frequently folding a small strip of patch at the same position till it breaks. The patch folded at the same place without breaking and will propose the value of folding endurance [9]. Results were analyzed for mean standard deviation.

Swelling index: The pre-weighed patch formulations of (1 × 1 cm^2) diameter were placed in 20 ml of phosphate buffer pH 6.8 at ambient temperature. After 1 h, the patches were removed and excess of water wiped with tissue paper and weighed. The swelling index can be determined by using the formula [10].

Swelling Index = Final weight - Initial weight / Initial weight × 100

Scanning electron microscopy (SEM): The size and shape of plain serratiopeptidase and surface morphology of buccal patch formulations were assessed by SEM (S-4800, Type II Hitachi, Japan) using the gold sputtering technique. The sample was placed in gold palladium coated stubs and photographs were obtained at proper resolution [11].

Entrapment efficiency: Buccal patch formulations (F1, F2, F3, F4 and F5) equivalent to 10 mg of serratiopeptidase and clove oil were dissolved in phosphate buffer pH 6.8. The contents of clove oil and serratiopeptidase were analyzed by UV-Vis spectrophotometer (UV1800, Shimadzu, Japan) at 280 nm and 291 nm, respectively. The encapsulation efficiencies of clove oil and serratiopeptidase were calculated by following formula.

% Drug entrapment efficiency = (Initial weight - Final weight / Initial weight) × 100

***Ex-vivo* mucoadhesive force:** The mucoadhesive strength of buccal patches was determined on goat buccal mucosa (obtained from local market) by measuring the force of detachment or the force of adhesion [12]. The goat buccal mucosa was fixed on the internal side of a beaker. The patch was wetted with 100 µL of phosphate buffer pH 6.8 and pasted to the rabbit buccal tissue by applying a light force with a fingertip for 1 min. The beaker was filled with 300 mL phosphate buffer pH 6.8 and kept at 37 ± 1°C. Then, the beaker was magnetically stirred at 50 rpm stirring rate to simulate the buccal cavity environment. The time required for detachment of these patches from the buccal mucosa was recorded. The experiment was performed in triplicate and the results were analyzed for mean and SD.

***In vitro* release studies of serratiopeptidase and clove oil:** *In vitro* release studies of patches were carried out by Franz-diffusion cells using dialysis membrane (Himedia, India). 15 ml of phosphate buffer pH 6.8 solutions was used as the receptor medium at 37°C for 50 rpm. The patches were placed in contact with the dialysis membrane. At scheduled intervals, 2 ml of aliquots were withdrawn for the analysis of clove oil and serratiopeptidase using UV-Vis spectrophotometer (UV 1800, Shimadzu, Japan) at 280 nm and 291 nm, respectively. The experiment was repeated for triplicate reading [13]. Aliquots were replaced with fresh buffer solution at the various intervals to maintain sink condition.

On the basis of results of encapsulation efficiency, folding endurance, mucoadhesion test and *in vitro* release studies of buccal patches, the formulation F5 was selected for further studies like *in vivo* drug release, ATR-FTIR, DSC and XRPD.

***In vivo* release studies of serratiopeptidase and clove oil:** Wistar rats were used for determination of *in vivo* release of clove oil and serratiopeptidase from formulation F5. Animals were housed in a room maintained at 25 ± 1.0°C for 12 h. They were allowed free access to food and water. Formulation F5 was applied in the buccal cavity of size (0.5 cm × 0.5 cm) bilaterally in light ether anesthetized animal. At scheduled time intervals 1, 2, 3, 4 and 24 h, the blood samples were collected in Eppendorf tubes by retero-orbital plexus method. The plasma was separated by centrifugation for 20 min at 3000 rpm and kept in refrigerator for 24 h. The release of serratiopeptidase and clove oil from formulation F5 was determined by UV-Vis spectrophotometer (UV1800, Shimadzu, Japan) at 280 and 291 nm, respectively [14].

Anti-inflammatory activity: Healthy Wistar rats weighing 120-150 g were used for anti-inflammatory activity of the formulation F5 by the carrageenan induced paw edema method. The animals were housed in a group of 6 in a single clean polypropylene cage. They were housed in an environmentally controlled room temperature 25 ± 1.0°C and relative humidity 50-60%. Under 12 h light dark cycles, the animals were fed on standard pelletized laboratory animal diet. The rats were fasted with free access to water for 12 h prior to the test. Paw edema was induced by 1% carrageenan solution in normal saline by injecting 0.1 ml (s.c.) into the right sub plantar region of the hind-paw. The control group received no treatment while formulation F5 was placed in buccal cavity of treatment group. The paw volume up to tibiotarsal articulation was measured at 0 h (V_0), 1, 3, 5 and 24 h (V_T) after carrageenan injection using a plethysmometer. Differences in paw volume between V_0 and V_T were taken as a measure of edema [15,16].

Analgesic study: The analgesic activity of the formulation F5 was evaluated by hot-plate technique [16]. Wistar rats weighing between 120-150 g were used for determination of analgesic activity. A solution of serratiopeptidase dose 5 mg/kg was prepared in normal saline water. **The patch was stuck in the upper oral mucosa**. The animals were placed on electrical heated surface of hot plate maintained at temperature 55°C to 56°C and latency time until either licking or jumping occurs was recorded at 0, 30, 60, 90 and 120 min.

ATR-FTIR: ATR-FTIR of plain serratiopeptidase, clove oil and formulation F5 were obtained by FTIR spectrophotometer (Agilent, USA) The wavelength was used in the range of 400-4000 cm^{-1} [17].

DSC: Thermal analyses of serratiopeptidase and formulation F5 were carried out using DSC (Shimadzu, Japan) with a scanning temperature 10°C/min [18].

X-ray powder diffraction (XRPD): Serratiopeptidase and formulation F5 were subjected to XRPD studies (Bruker, Germany). The samples were analyzed between 2θ angles of over 5-45°C.

Results and Discussion

Thickness

The thickness of the patches ranges between 0.41 ± 0.004 mm to 0.56 ± 0.005 mm. Each formulation has a uniform thickness. The thickness of patches was in the order of F4>F3>F2>F1 might be due to increase in Eudragit L100 concentration. Formulation F5 showed thickness 0.48 ± 0.006 mm was more due to entrapment of emulsion droplets in the patch.

Patch weight

The patch weight was in the order of F2>F3>F4>F1>F5 as shown in Table 2. Mass difference of the formulated patches was increased in F2 due to high concentration of hydrophilic polymer, HPMC.

Moisture content

Moisture content of the formulation was in the order of F2>F3>F4>F1>F5 due to the presence of hydrophilic nature of HPMC and hydrophobic nature of Eudragit L100. The high moisture content of the formulation F2 was perhaps associated with the major proportion of water retained in HPMC.

Folding endurance

Formulated buccal patches showed good folding endurance in order of F5>F1>F3>F2>F4 and no visible cracks. Formulations showed good film properties as shown in Table 2.

Swelling index

The percentage swelling index of different formulations was in the order of F2>F5>F1>F4>F3. The formulations F2 and F5 showed higher swelling index due to increase in wettability of higher HPMC concentration and the presence of matrix network structure to the movement of water molecules in w/o emulsion, respectively. The effect of Eudragit in swelling of the patches was negligible due to its hydrophobic nature (Table 2).

SEM

SEM studies showed the uniform distribution of serratiopeptidase and clove oil in formulations F1-F4; and their w/o emulsion throughout the patch formulation F5. The particle size of serratiopeptidase and emulsion droplets were 23 µm and 1 µm, respectively as shown in Figures 1 and 2. It was also observed homogenous, clear and regular surface of patches.

Entrapment efficiency

The % entrapment efficiencies of serratiopeptidase in formulated buccal patches were found in the order of F5>F4>F1>F3>F2 while clove oil was in the order of F5>F1>F4>F3>F2. The less values of standard deviation designated that serratiopeptidase and clove oil were homogeneously distributed throughout the formulated patches. This might be due to adhesive forces of drug and polymer was stronger than cohesive forces between drug molecules and polymer structures, which contributed to the successful encapsulation and stabilization of the drugs in the patch.

Formulation	Thickness (mm)	Patch weight (mg)	Moisture content (%)	Folding endurance	Swelling index (%)
F1	0.41 ± 0.004	0.623 ± 0.06	6.52 ± 0.07	13 ± 0.4	15.28 ± 1.7
F2	0.43 ± 0.008	0.652 ± 0.08	12.72 ± 0.09	9 ± 0.8	44.44 ± 1.4
F3	0.52 ± 0.007	0.646 ± 0.05	8.47 ± 0.07	10 ± 0.6	13.50 ± 1.1
F4	0.56 ± 0.005	0.629 ± 0.06	6.89 ± 0.07	8 ± 0.8	14.28 ± 1.2
F5	0.48 ± 0.006	0.615 ± 0.09	5.26 ± 0.06	15 ± 0.9	40.33 ± 1.8

Table 2: Physicochemical evaluation of patches.

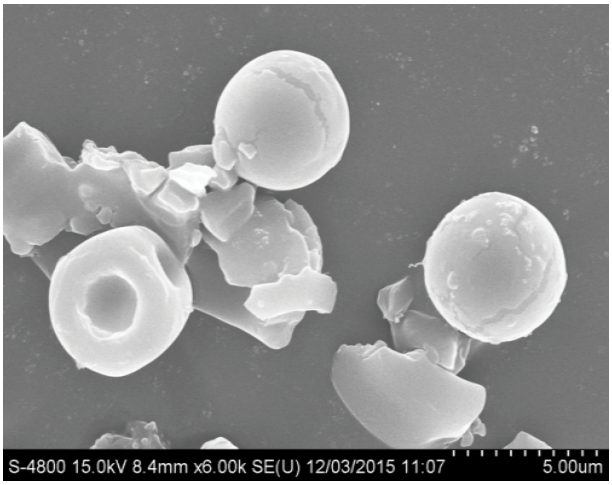

Figure 1: SEM of serratiopeptidase.

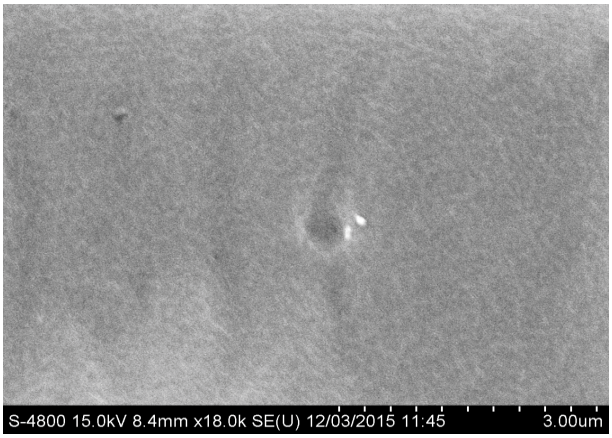

Figure 2: SEM of formulation F5.

Mucoadhesive strength

The mucoadhesion strength of formulations was in the range of 3.20 ± 0.12 to 9.80 ± 0.14 g as shown in Figures 3-5. It was observed that the longest adhesion time and the convenient bioadhesion strength reflect the different behavior of Eudragit which, in addition to its role as film-forming polymer, possesses non-neglectable mucoadhesive properties that enhanced mucoadhesion [13]. This study was sustained on the assumption that ionizable polymers exhibit the best mucoadhesive characteristics. Formulation F2 demonstrated low strength which can

be due to the high swellability of HPMC producing a non-adhesive mucilage layer near the polymer/mucosa interfacial surface and this sentence was supported by the result of the swelling index.

In vitro release studies of serratiopeptidase and clove oil

In vitro release profiles of serratiopeptidase and clove oil from formulated buccal patches were shown in Figures 6 and 7, respectively. Serratiopeptidase and clove oil released from formulation F5, showed highest release 74.32% and 80.65%, respectively. Serratiopeptidase and clove oil patches showed % release was in the order of F5>F2>F1>F4>F3 for 24 h in controlled manner. The formulation F5 ratio (1:1 w/w) of HPMC and Eudragit showed the highest percent release with an initial burst release for 4 h followed by controlled release for 24 h. As an inert nature of polymer, Eudragit, solvent penetration into the patch was the rate limiting factor for the release of the drug. At the start of the study, drug available at and near the surface of the patch dissolved quickly. When the dissolution process progresses, there was a greater resistance to the penetration of the solvent inside of the matrix film, due to the hydrophobicity of the Eudragit and the decreasing length of the solvent front. The drug immediately dissolved by dissolution medium and diffused from the interface between the patch surface and the surrounding media. Later slow down diffusion process led to controlled release of drug from mucoadhesive [14]. This outcome suggested that delivery of serratiopeptidase and clove oil as a controlled release formulation was possible with formulated patches with inter-polymer complexes of Eudragit and HPMC wherein emulsion droplets were uniformly distributed.

On the basis of results of encapsulation efficiency, folding endurance, mucoadhesion test and *in vitro* release studies of buccal patches, the formulation F5 was selected for further studies like *in vivo* release, ATR-FTIR, DSC and XRPD.

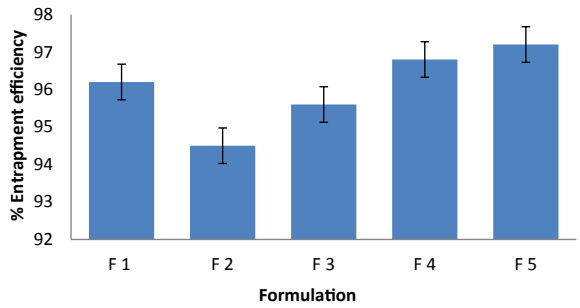

Figure 3: Entrapment efficiency of serratiopeptidase buccal patch formulations (F1-F5).

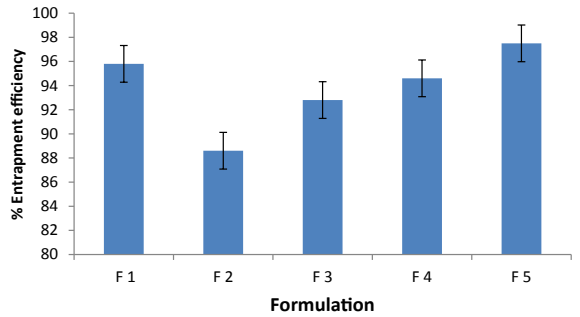

Figure 4: Entrapment efficiency of clove oil buccal patch formulations (F1-F5).

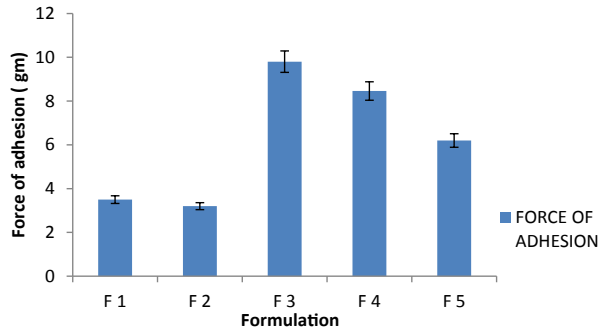

Figure 5: Mucoadhesive strength of formulations (F1-F5).

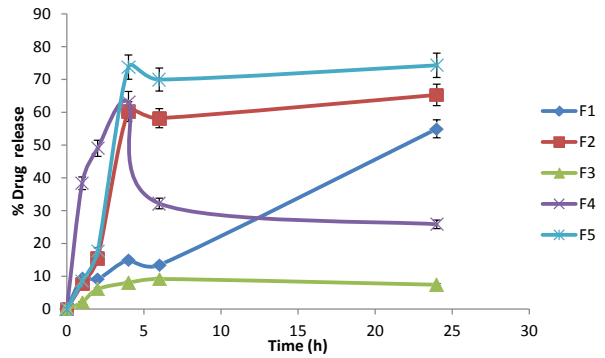

Figure 6: *In vitro* drug release profile of serratiopeptidase from formulations (F1-F5).

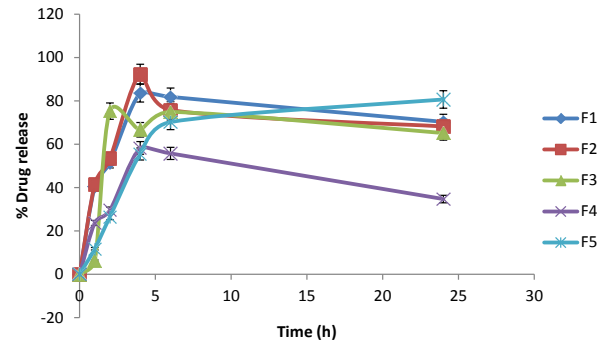

Figure 7: *In vitro* drug release profile of clove oil from formulations (F1-F5).

In vivo drug release study

In vivo release study showed that maximum drugs released was found in combinational effect serratiopeptidase and clove oil as compared to formulation F5. It indicates that increase in bioavailability of serratiopeptidase in emulsion formulation F5.

Analgesic activity

The results of the hot plate test are presented in Figures 7-9. All

drugs along with the formulation significantly ($P<0.05$) delayed response at selected time points after treatment. The formulation showed maximum effects when compared with serratiopeptidase and clove oil. This might be due to controlled release of serratiopeptidase and clove oil in animal treated with F5.

Anti-inflammatory effect

The increase in volume displaced by paw edema in control rats was observed. The serratiopeptidase and F5 showed significant reduction in volume displaced by paw edema in rats compared to control group (Figure 10).

FTIR

FTIR spectra of serratiopeptidase and clove oil and formulation F5 were shown in Figure 11. The characteristic peaks of serratiopeptidase were shown due to presence of aromatic NH_2 (3325 cm^{-1}), aliphatic NH (1032 cm^{-1}), aliphatic OH (1017 cm^{-1}) and aromatic C=C (1649 cm^{-1}). Clove oil spectrum exhibited peaks in the region of 3300-3600 cm^{-1}. The presence of -OH stretching and absorption peaks in the region of 1600-1680 cm^{-1} assigned to alkene CC and aromatic ring present. The spectrum of formulation F5 showed absorption bands on serratiopeptidase and clove oil, there is no change in the absorption bands due to emulsification. The FTIR spectra concluded that there was no chemical incompatibility between drugs and excipients used in the buccal patch formulation.

DSC

DSC thermograms of serratiopeptidase showed endothermic peak at 240°C matching to the melting point of the serratiopeptidase as shown in (Figure 12) while clove oil exhibited a broad exothermic peak at 162°C and endothermic peak at 282°C due to the thermal oxidative temperature and boiling point of eugenol, respectively. This indicated that the characteristic peaks of serratiopeptidase and clove oil appeared in the formulation F5 showing that there was no possible interaction between the drugs and the exicipients in the mucoadhesive buccal patch formulation.

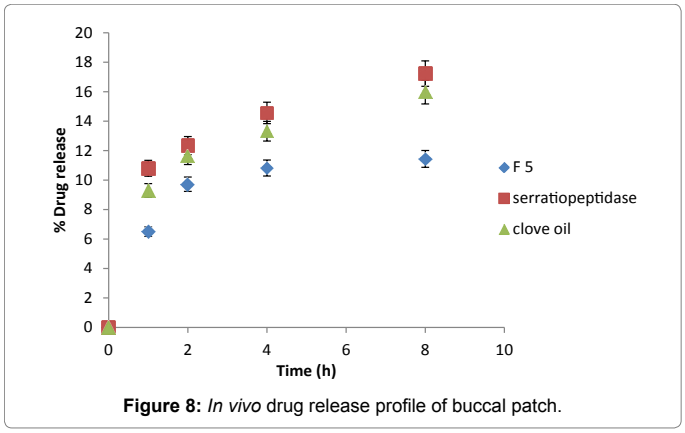

Figure 8: *In vivo* drug release profile of buccal patch.

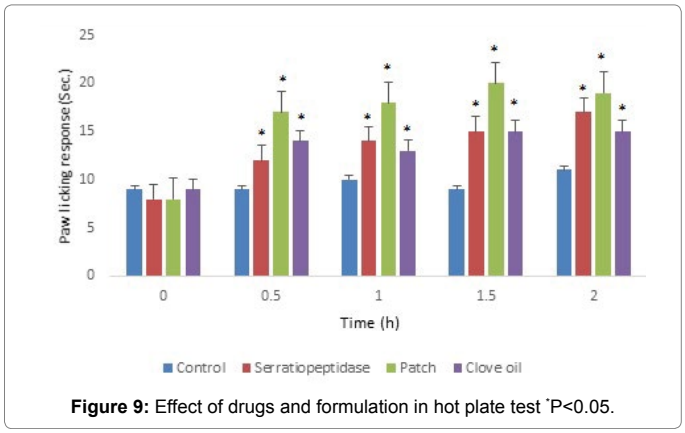

Figure 9: Effect of drugs and formulation in hot plate test *P<0.05.

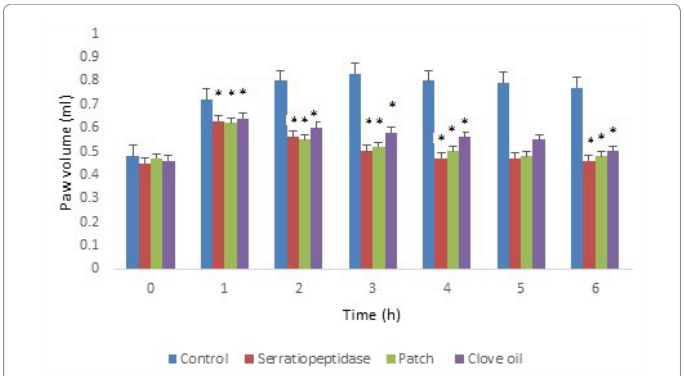

Figure 10: Anti-inflammatory effect of drugs and formulation F5 *P<0.05.

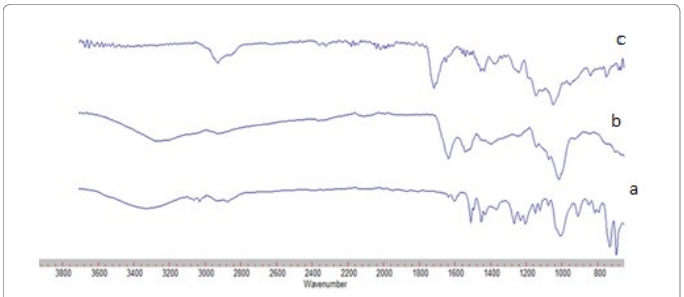

Figure 11: FTIR spectra of a) Clove oil b) Serratiopeptidase and c) Formulation F5.

XRPD

The comparison of plain serratiopeptidase and emulsified buccal patch formulation F5 showed a drastic change as shown in Figure 13. Formulation F5 showed reserve of amorphous nature of serratiopeptidase which was confirmed by this study. XRPD pattern of serratiopeptidase showed peaks at 11.2, 19.5 and 37.5 but this pattern was not observed in formulation F5.

Conclusion

The serratiopeptidase and clove oil buccal patch was prepared by emulsification method using HPMC and Eudragit L100 used in different ratio of 1:1, 3:2, 2:3, 4.5:5.5 and 1:1 exhibited significant results of bioadhesive, mucoadhesive properties, encapsulation efficiency and *in vitro* and *in vivo* release studies. The clove oil emulsified buccal patch of serratiopeptidase offered their combinational controlled release actions and is good alternative to avoid hepatic first pass metabolism.

References

1. Madhavi BR, Varanasi SNM, Prameela RA, Kumar GD (2013) Buccal Film Drug Delivery System-An Innovative and Emerging Technology. J Mol Pharm Org Process Res 1: 2-6.

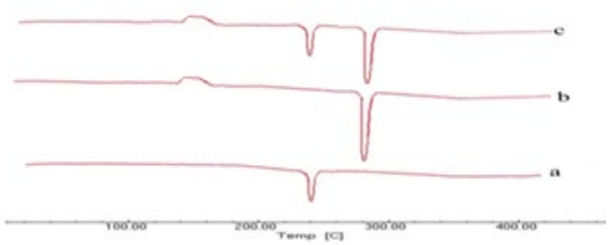

Figure 12: DSC of a) Serratiopeptidase b) Clove oil and c) Formulation F5.

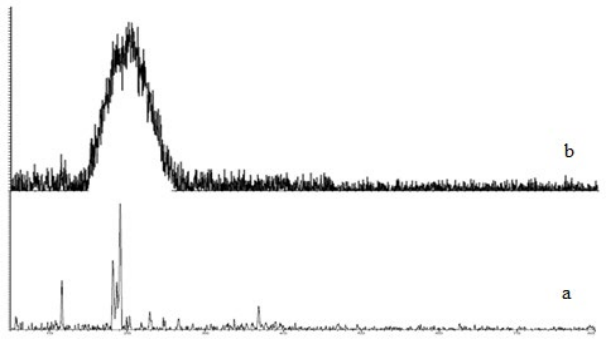

Figure 13: XRPD pattern of a) serratiopeptidase and b) formulation F5.

2. Reddy SN, Kumar BD, Kashyap NU, Vankata Sairam K, Ramya S (2012) Formulation and evaluation of pantaprazole buccal patches. Int J Pharma and Ind Res 2: 37-40.

3. Chaudhari SA, Mali AK (2013) Production, characterization and optimization of potent protease (serratiopeptidase) from *Serratia marcescence* E 15. Int Res J Pharm App Sci 3: 95-98.

4. Mohankumar A, Hari Krishna Raj R (2011) Production and characterization of serratiopeptidase enzyme from *Serratia marcescens*. Int J Bio: 3: 39-41.

5. Hosseini M, Kamkar MA, Rakhshandeh H (2011) Analgesic effect of clove essential oil in mice. AJP 1: 1-6.

6. Annigeri RG, Jadhav M, Juturu T (2015) Clinical evaluation of transmucosal mucoadhesive meloxicam patch in dental pain reduction: A preliminary study. Indian J Pain 29: 82-85.

7. Singhal P, Singh GJ, Sinha M, Saraf SA (2010) Formulation and evaluation of buccal patches of terbutaline sulphate. Int J Res Pharm Sci 1: 440-449.

8. Kumar SK, Nagabhushanam MV, Sambasivarao KSS, Bhikshapathi DVRN (2013) Formulation development and in vivo evaluation of zolmitriptan oral dissolving films. Int J Pharm Bio Sci 4: 638-654.

9. Khanna R, Agrawal SP, Ahuja A (1997) Preparation and evaluation of buccal film of clotrimazole for oral Candida infection. Ind J Pharm Sci 59: 299-305.

10. Perioli L, Ambrogi V, Angelici F, Ricci M, Giovagnoli S, et al. (2004) Development of mucoadhesive patches for buccal administration of ibuprofen. J Control Release 99: 73-82.

11. Shende PK, Trotta F, Gaud RS, Deshmukh K, Cavalli R, et al. (2012) Influence of different techniques on formulation and comparative characterization of inclusion complexes of ASA with ß-cyclodextrin and inclusion complexes of ASA with PMDA cross-linked ß-cyclodextrin nanosponges. J Incl Phenom Macrocycl Chem 74: 447-454.

12. Gupta A, Garg S, Khar RK (1992) Measurement of bioadhesion strength of mucoadhesive buccal tablet design of an in vitro assembly. Indian Drugs 30:152-155.

13. Shende P, Gaud R (2009) Formulation and comparative characterization of chitosan, gelatin, and chitosan-gelatin-coated liposomes of CPT-11-HCl. Drug Dev Ind Pharm 35: 612-618.

14. Li C, Koch RL, Raul VA, Bhatt PP, Johnston TP (1997) Absorption of thyrotropin-releasing hormone in rats using a mucoadhesive buccal patch. Drug development and industrial pharmacy 23: 239-246.

15. Bhandare AM, Kshirsagar AD, Vyawahare NS, Hadambar AA, Thorve VS (2010) Potential analgesic, anti-inflammatory and antioxidant activities of hydro-alcoholic extract of *Areca catechu* L. nut. Food Chem Toxicol 48: 3412-3417.

16. Shende PK, Bakal RL, Gaud RS, Batheja KN, Kawadiwale MS (2015) Modulation of serratiopeptidase transdermal patch by lipid-based transfersomes. J Adhes Sci Technol 29: 2622-2633.

17. Shende PK, Gaud RS, Jaiswal SB, Kochar NI (2007) Formulation and evaluation of alginate- gelatin beads for controlled release of glipizide. Indian Drugs 44: 945-948.

18. Shende P, Deshmukh K, Trotta F, Caldera F (2013) Novel cyclodextrin nanosponges for delivery of calcium in hyperphosphatemia. Int J Pharm 456: 95-100.

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