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RESEARCH PAPER

Formulation and Evaluation of Mucoadhesive Tablets Containing Eugenol for the Treatment of Periodontal Diseases

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ABSTRACT

Eugenol is the principle chemical constituent of clove oil and has been used to cure dental problems for ages. Eugenol is an integral part of the dentist's kit due to its analgesic, local anesthetic, anti-inflammatory, and antibacterial effects. It is used in the form of a paste or mixture as dental cement, filler, and restorative material. This study reports the development and evaluation of controlled-release mucoadhesive tablets for gingival application, containing eugenol, which are prepared by taking carbopol 934 P and Hydroxypropyl methylcellulose (HPMC) K4M in the ratio of 1:2, 1:1, and 2:1. Incorporation of eugenol (10 mg) in a mucoadhesive formulation provides controlled release for a period of 8 hours, which is advantageous over conventional use. In vitro mucoadhesion measured as detachment force in grams and the formulations show good correlation in vivo. The release study indicates that increase in carbopol increases the release rate of eugenol from the formulation whereas HPMC retards it. Increased in vitro bioadhesion is related to HPMC content of the formulation. The release kinetics of eugenol in vitro correlates with the in vivo results. This indicates the increased potential of eugenol as antibacterial, local analgesic, and anaesthetic treatment.

Key Words: Eugenol; Carbopol; HPMC; Mucoadhesion; Release pattern.

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INTRODUCTION

Clove oil is widely used by dentists to provide interim relief to patients. In the forms of ''Zinc oxide-Eugenol'' cement and medicated tooth powder, clove oil is used to treat in dental caries, spongy and bleeding gums, and gingivitis etc.[1] Eugenol, a phenolic compound, shows anti-inflammatory activity by inhibiting the enzyme cyclo-oxygenase-II,[2] analgesic activity due to selective binding at capsaicin receptor,[3] and antibacterial (1 mg/mL) activity against gram (+) ve and gram (−) ve microorganisms.[4] Eugenol inhibits macrophage function and thus modulates immune, inflammatory reactions in dental pulp and periapical tissue.[5–8] Eugenol also shows neuroprotective,[9] thromboxane and biosynthesis inhibitory,[4] anti-inflammatory, anticancer, anti-ischemic,[10] antihistaminic,[11] and antianaphylactic properties.[3]

Periodontal disease, a common health problem, involves a group of inflammatory conditions due to bacterial etiology affecting the supportive structures of teeth, gingiva, periodontal ligament, and alveolar bone. Treatment of periodontitis, an extension of inflammation into deeper tissues, with conventional root planning and scaling with orally administered antimicrobial agents results in dose-related undesirable side effects. Larger doses of the drug required for 5 to 7 days of therapy contribute to side effects. Oral hygiene systems such as mouth rinses are ineffective against periodontal pockets, which are receptive to treatment with local, sustained release, tetracycline-loaded hollow fibers and/or chlorhexidine slabs.[12] Mouth infections, such as gingivitis and stomatitis, are mostly caused by aerobic and anaerobic microbes and can be treated locally by antimicrobial and anti-inflammatory drugs, usually administered in buccal gel form or as mouthwashes. However, the disadvantage of these deliveries is that they are easily washed away by saliva, and the effective drug levels in the mouth are limited to a short period of time, necessitating repeated administration.

Interest in the development of mucoadhesive buccal dosage forms for systemic delivery and local targeting of drugs is increasing. Several such approaches for controlled drug delivery have been explored by Nagai[20] Previous studies have revealed that a range of putative mucoadhesive polymers in the form of gels and polymeric films create considerable technical problems. It has been proposed that the interaction between mucus and mucoadhesive polymers is a result of physical entanglement and secondary bonding, mainly hydrogen bonding and Van der Waals attractions. Physical properties such as rate of hydration and rheological properties of the polymeric formulations are likely to have a major impact on their bioadhesion and consequently their eventual duration of retention.

Analgesic efficacy and safety of an intraoral lidocaine patch for the treatment of toothache has been assessed by applying the system to the human gingiva.[13,14] Oral deliveries of the antimicrobial and anti-inflammatory drug tetracycline[12] for long term treatment of periodontal diseases has been reported.

At present, application of medicines for toothache are provided in the form of lotions, viscous liquids, or ointments, which are applied with a piece of cotton (eugenol). However, such a mode does not provide sustained action and also act on other parts of the oral cavity.[13] The potential side effects of administering systemic antibiotics and the inability of antiseptic mouthwashes to penetrate the periodontal pocket have fueled the interest in the sustained delivery of such agents within the pocket. However, these devices suffer a major disadvantage in that the polymer strip must be removed from the periodontal pocket after the complete release of agent, which might cause local mechanical irritation.

Treatment of gingivitis and periodontal diseases demands use of a combination of antibacterial, analgesic anti-inflammatory, and local anaesthetic agents in effective concentration at the site with extended residence time and fewer side effects. However, no single synthetic compound possesses these. So attention has been given to the natural product eugenol a suitable candidate that fulfills all the requirements. No such mention has been reported in the literature for eugenol in periodontal disorders. Formulations containing carbopol and Hydroxypropyl methylcellulose (HPMC) are safe and provide the desired adhesion.[15,16] In the present work the effect of carbopol and HPMC concentration on bioadhesive property and in vitro release of eugenol has been studied. The formulations were evaluated in healthy human volunteers for bioadhesion and release profile.

MATERIALS AND METHODS

Materials

Eugenol was purchased from E. Merck (Mumbai, Maharashtra, India) Ltd. Hydroxypropyl methylcellulose K4M (HPMC) and carbopol 934 P NF (CP) were supplied by Colorcon (Mumbai, Maharashtra, India) and B.F. Goodrich (Mumbai, Maharashtra, India)

respectively. Reagents and other chemicals of analytical grade were procured locally and used without purification. Double distilled water was used for diffusion and analytical studies.

Preliminary Studies

Thin Layer Chromatography of Eugenol

The purity of eugenol was confirmed by thin-layer chromatography (TLC). The samples were applied in the form of bands on a precoated silica gel 60 F_{254} (Merck, Mumbai, India) aluminum plate (Camag, Linomat IV, Switzerland). The solvent system of ethyl acetate and toluene (9.9:0.1) was used for development. Eugenol was detected by placing the plate in a chamber containing iodine vapor. The plate was visualized after spraying with vanillin-sulfuric acid reagent, and R_f values for standard and samples were recorded.

Viscosity Studies

The 0.5% w/v of polymeric dispersion of each polymer and that of the mixture were prepared separately by dispersing 500 mg in distilled water in a conical flask and volume was made up to 100 mL. This was kept for overnight stirring on a mechanical shaker. Resultant solutions were neutralized to pH 5.0 with 0.1 M sodium hydroxide solution. The viscosity of 0.5% w/v solutions of the individual polymers and mixtures of CP and HPMC in the ratios of 2:1, 1:1, and 1:2 were determined with the speed of 10 rpm at 25°C using the Brookfield programmable DV-II+ Viscometer with Wingather software.

Shear Stress Measurement

Shear stress measurement studies as described by Rao, Vani, and Rameshary were carried out on 3% w/v aqueous solutions of CP: HPMC and their mixtures in the ratios of 2:1, 1:1, and 1:2, respectively.[17] Two smooth polished glass blocks of $10 \times 10 \times 0.5$ cm were selected. One glass plate was fixed on a leveled table with an adhesive. To the upper block a thread was tied and passed through a pulley at a length of 12 cm. At the end of the thread a pan of 3 g weight was attached for the addition of weights. A drop of polymeric solution at a temperature of 25°C was kept at the center of the fixed block with a pipette, and the movable block was placed on it. A load of 100 g was applied such that the drop of polymer spread as a uniform film in between the two glass blocks. After keeping it for fixed time intervals of 5, 10, and 15 min, the applied load was removed and the weights were added into the pan just sufficient to pull the upper movable block or to make it slide down from the fixed base block. The sheer stress was expressed in gm/cm^2.

Preparation of Mucoadhesive Tablets

A bilayer tablets with a mucoadhesive layer and an impermeable cap layer were prepared. The adhesive layer consisted of 200 mg of physical mixture (PM) of CP and HPMC in the ratios mentioned in Table 1. In the case of drug-loaded formulations, 10 mg of eugenol was incorporated in the same layer. The backing membrane was composed of the physical mixture of polymers (in the same ratio as in adhesive layer) and magnesium stearate (Mg-st) in a 1:1 ratio. The tablets were prepared by compressing the adhesive layer in a die of 13 mm diameter under the pressure of 100 kg/cm^2 for 15 seconds using a hydraulic press (Spectra Lab, Mumbai, Maharashtra, India). The composition of backing layer was then placed on the above compact, and the two layers were compressed to a mucoadhesive tablet under the pressure of 200 kg/cm^2 for 30 s.

In Vitro Bioadhesion Testing

A modified balance method was used. The sheep intestine was cut into pieces and washed with Tyrode solution. A piece of intestine was tied to the support. The tablet was stuck to the lower side of the left-hand stainless steel pan. The weight of 5 g was placed in the left-hand pan, which lowered the pan along with the tablet over the mucosa. The balance was kept in this position for 3 min and then the 5 g weight was removed from the left-hand pan. The weights were added slowly

Table 1. Formula for preparation of the mucoadhesive dosage form.

Formulation code	Ratio of		
	Carbopol-934P:	HPMC-K4M:	Eugenol
A	1	1	–
AE	1	1	0.005
B	1	1	–
BE	1	1	0.005
C	2	2	–
CE	2	2	0.005

Note: Formulations and polymer mixtures in different ratios.

to the right-hand pan, until the tablet separated from the mucosal surface. This gave the bioadhesion strength of the tablet in grams.

In Vivo Bioadhesion

In vivo bioadhesion was performed on six healthy human male volunteers between 24 and 26 years old. The tablet was placed on the fingertip with the muco-adhesive surface facing upwards, and then applied to the upper gums so that the hydrophobic backing layer faced the cheek. Formulation was held in place for 30 seconds and allowed to remain. Where the formulation was displaced, residence time was recorded; in cases where the formulation did not fall off before 8 hr, the qualitative force required to remove it was recorded. The volunteers were also asked to notice any discomfort due the bioadhesive tablet during the study.

Water Uptake Studies

The tablet was placed in a beaker with distilled water, which was maintained at $37 \pm 1^{\circ}$ C using a cryostatic bath (Haake Phoenix C25P, Karlsruhe, Germany). The system was stirred at 100 rpm using a constant speed stirrer (Eurostar power control-visc, IKA Labortecnik, Staufel, Germany). After 5, 10, 15, 30, 45 min, 1, 2, 3, 6, 8 h, and 1-day time intervals the samples were removed, blotted, dried, and reweighed. The increase in weight was calculated and reported as percent water uptake.

Drug Content

The tablet was dissolved in methanol by sonication for 3 h.The solution was filtered through Whatman filter paper 41, and after sufficient dilutions with methanol, analyzed spectrophotometrically at 278 nm (JASCO V530, JASOO International Co., Ltd., Hachioji City, Tokyo, Japan). The eugenol-content was calculated from the standard curve obtained in methanol.

In Vitro Drug Release Studies

In vitro release of eugenol through a cellophane membrane (Dialysis tubing, MWCO 12500, Sigma Chemical Co., St. Louis, MO), was studied using Keshary–Chien type diffusion cell at $37 \pm 1^{\circ}$ C, and 22.0 mL of 2% w/v sodium lauryl sulphate solution (SLS) as a diffusion medium. One-mL samples were withdrawn each at 5-min to 8-h time intervals and replaced with fresh medium maintained at the same temperature. After sufficient dilution with 2% w/v SLS solution, samples were analyzed spectrophotometrically at 278 nm (JASCO-V500, Japan). The eugenol content was calculated from the standard curve obtained in 2% w/v SLS (R=0.999403, P=1.82, E-14, Y intercept= −0.37285, X variable=92.30912). The flux of eugenol was calculated considering the surface area of the tablet.

In Vivo Drug Release Studies

For in vivo drug release studies, six healthy human volunteers of either sex between 22 and 26 years old were selected. The study protocol was approved by the Drug Therapeutic Committee, and explained to the volunteers whose written consent was obtained. Volunteers were instructed not to take food or drink during the studies. The tablet was placed on the fingertip with the eugenol-containing mucoadhesive layer facing upwards and then stuck to the gums by holding it for 30 s. Tablets remained on the gums for different periods of time (0.5, 1, 2, 3, 4, 5, 6, and 8 h). The amount of remaining eugenol was estimated as mentioned for the in vitro study.

RESULTS

Eugenol is a phenolic chemical constituent found in various plants, mainly obtained from clove oil. It has been used to cure dental problems for ages. It is an integral part of a dentist's kit due to its analgesic, local

Table 2. Physicochemical properties of eugenol.

	Parameters			
	Boiling point	Specific gravity at 25°C	R_f value	λmax
Eugenol sample	253–255°C	1.0642–1.068	0.37	280 nm
Reported test sample	253–255°C	1.0654	0.37	278 nm

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Table 3. Comparison of viscosity of CP:HPMC combination.

Sr. no.	Composition of CP:HPMC	Viscosity at 10 rpm at 25°C in CP
1	2:1	3,743±14
2	1:1	5,759±23
3	1:2	43,719±51

anasthetic, anti-inflammatory, and antibacterial properties. The traditional method of keeping the clove oil soaked cotton swab between the teeth and cheek limits its efficiency due to short residence time. Hence it was incorporated in mucoadhesive polymer matrix and evaluated for the in vitro and in vivo bioadhesion and release performance.

Eugenol obtained from the market was standardized based on parameters such as boiling point, specific gravity, spectroscopic and chromatographly, and the results are compared with reported values (Table 2).[18] The same was used for the preparation of mucoadhesive tablets using CP, a bioadhesive polymer and HPMC, a bioadhesive and sustained-release polymer. The polymers used were evaluated for viscosity and shear stress measurement. Viscosity of three compositions of CP and HPMC in three ratios, 1:2, 1:1, and 2:1, are reported in Table 3. The order of increase in viscosity among polymer combinations was 2:1<1:1<1:2, which indicates that increase in viscosity is directly proportional to the proportion of HPMC. In vitro adhesion of these three combinations was measured in terms of shear stress; that is, the weight required to move the glass plates over each other. Results illustrate that shear stress increased with the increase in the proportion of HPMC in the combination, which is reported in Table 4.

The formulations of eugenol with CP and HPMC in three ratios, 1:2, 1:1, and 2:1, were prepared keeping the loading dose to 10 mg/tablet as a constant in the case of three drug loaded formulations. The tablets were evaluated for average diameter and thickness, weight variation, drug content, and content uniformity, which were found to be 13.8 (±0.030) mm, 1.63 (±0.030) mm, 0.250 (±0.001) g, 99.79% (±0.62), and 100.12%, (±0.53), respectively. The results reveal that prepared tablets comply with the Indian Pharmacopoeia (IP) limits.

Mucoadhesive tablet formulations without eugenol, A, B, and C, were evaluated for in vitro and in vivo bioadhesion. Bioadhesion of the formulations was measured in vitro as a detachment force measurement, or the force required to detach the formulation from the mucosal tissue. The reproducibility of the test system was initially examined using 10 tablets from the same batch. After each measurement, the tissue was replaced by fresh piece and finally detachment force was measured. The results in Table 5, were based on comparisons of mucoadhesive bond strengths of tablets of different batches. Formulation AE, with the highest proportion of CP among the three ratios, requires the most maximum force in grams to break the bond

Table 4. Shear stress measurement of polymers used in the mucoadhesive tablets.

		Weight required in grams				
		Trial				
Polymer(s)[a]	Contact time (min)	I	II	III	Average	SD
Carbopol	5	24.16	24.56	21.59	23.44	±1.60
	10	39.70	43.86	42.76	42.11	±2.16
	15	112	98.83	107.07	105.97	±6.65
HPMC	5	47.96	51.10	48.37	49.14	±1.71
	10	66.23	60.51	69.78	65.51	±4.68
	15	97.76	90.37	92.37	93.5	±3.82
CP:HPMC (2:1)	5	26.08	26.02	24.15	25.42	±1.10
	10	53.02	47.81	41.67	47.5	±5.68
	15	73.84	77.91	69.64	73.80	±4.14
CP:HPMC (1:1)	5	100.70	115.43	117.63	111.63	±1.21
	10	151.90	144.78	158.05	151.58	±6.64
	15	165.56	181.02	178.09	174.89	±8.21
CP:HPMC (1:2)	5	130.67	125.17	135.50	135.45	±5.16
	10	157.11	165.98	147.34	156.81	±9.32
	15	187.56	203.45	186.95	192.65	±9.36

[a]Polymer solutions of 3% w/v concentrations were prepared in water.

Table 5. Comparision between in vitro and in vivo adhesion.

Formulations	In vitro adhesion force in g average wt. (±SD)	In vivo adhesion performance in min.
CP:HPMC (2:1)	86.8±14.1	530±23
CP:HPMC (1:1)	45.1±3.6	475±16
CP:HPMC (1:2)	40.6±7.4	380±20

between the mucoadhesive polymer and the mucosal surface. The detachment force decreases to half with a 33.3% decrease in CP as indicated in formulation BE. Further reduction in the ratio of CP did not lowered the detachment force much, as there is no highly significant difference between the values.

In vivo bioadhesion studies showed that all three formulations adhered to buccal mucosa in 20–30 seconds and remained there in a swollen state for the longer period. Formulation AE remained at the site for more than 8 h, and the force required to detach the tablet was more. Medium bioadhesion was shown by the BE formulation, as it remained up to 8 h at the site but required less force for removal than AE. Whereas the CE systems detached after 6 h in two volunteers, the remaining four volunteers reported residential time of 7 h. These findings are in accordance with the second method carried out to measure in vitro bioadhesion forces. Good mucoadhesive properties (8 h) were observed in all the formulations, except for CE systems (6 h).

Water uptake tests are of great significance, as variation in water content causes a significant variation in the mechanical properties of formulations, especially those comprised of hygroscopic components. The capacity of the formulation to take up water is an important intrinsic parameter of the polymeric system in consideration of the release of the drug on the mucosal surface. Formulations A and AE, which contained higher proportions of CP, were found to absorb more water than the rest of the ratios (Fig. 1). The Water absorbing capacities of the systems decreased in the following order, A>AE>B>BE>C>CE, with decreasing CP and increasing HPMC concentrations.

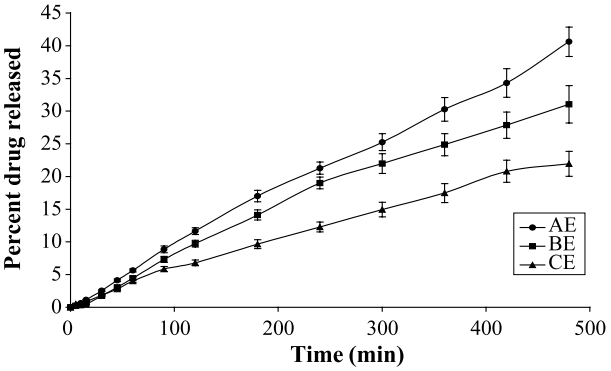

Figure 2. Comparison of in vitro release of eugenol from formulations (diffusion) (n = 3, $p < 0.05$).

In vitro release kinetics of different ratios was studied by diffusion study of eugenol from the semipermeable membrane using 2% SLS solution as diffusion medium. Results of the diffusion study (Figs. 2 and 3), show the percent of release and flux of eugenol in μg/cm^2/min per unit area and per unit time, respectively. Results showed that release of eugenol in 8 h from the formulation AE was higher, i.e., 40.61% followed by 31.06% from the formulation BE, while CE showed the lowest release of 21.93%. The flux of eugenol was in the same order as that of diffusion studies.

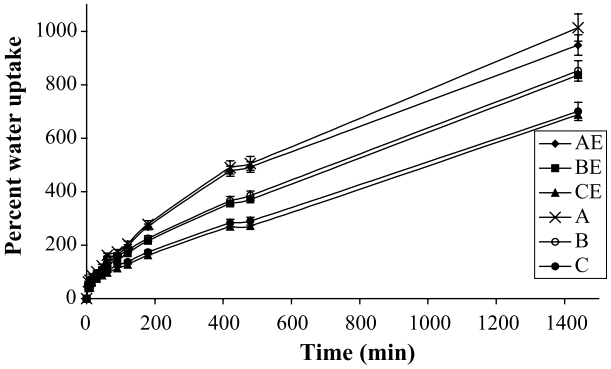

Figure 1. Comparison of percent water uptake (n = 3, $p < 0.05$).

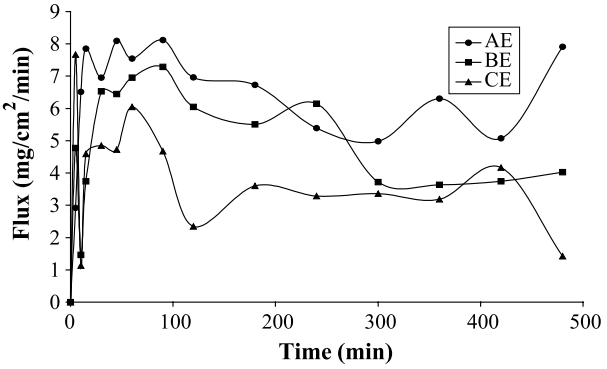

Figure 3. In vitro diffusion flux of eugenol from tablet.

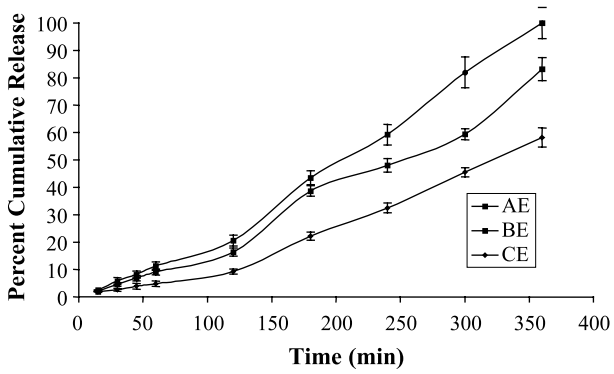

Figure 4. In vivo release of eugenol from tablets ($n = 3$, $p < 0.05$).

The in vivo release study showed the complete release of eugenol from formulation AE within 6 h, followed by BE having intermediate 82.3% release. The CE formulation showed 58.2%, the least release among all three combinations, as illustrated in Fig. 4. The order of release of eugenol is given as AE>BE>CE. Decrease in CP or increase in HPMC content of formulation decreased the rate of release.

DISCUSSION

In the case of shear stress measurement it was found that as the proportion of HPMC increased, the weight required to move the glass plates over each other increased. But in contrast to these results, detachment force measurement studies carried out on sheep intestinal mucosa showed that as the concentration of CP increased, the force required to detach the formulation increased significantly. These contradictory results could be explained by taking into consideration polymer properties. The use of HPMC, a highly viscous and adhesive polymer, resulted in higher shear stress under the former method. The wetting time is more for higher CP concentrations. The higher percentage of carboxylic acid groups gradually undergo hydrogen boding.[19] This particular aspect plays an important role in mucoadhesion. Thus in the latter method carbopol showed the higher detachment forces this is further supported by the results of in vivo bioadhesion study.

The tablet was applied to the upper gums with a fingertip. If the tablet is made of adhesive layer only, it first sticks to the finger after application. Therefore, the supporting layer, which is formed of PM and Mg-st and is not so sticky, plays a beneficial role in application. When the adhesive tablet is applied, it absorbs fluid and swells to form a gel-like layer, which covers the mucosal surface much longer than existing preparations such as ointments (less than 6 hours).[20]

Water uptake tests are of great significance as variation in water content causes a significant variation in mechanical properties of formulations especially when comprises of hygroscopic components. The capacity of the formulation to take up water is an important intrinsic parameter of the polymeric system in consideration to the release of the drug on the mucosal surface. Formulation A and AE, which contained higher proportion of CP were found to absorb more amount of water. Water absorbing capacity of system decreased in the following order A>AE>B>BE>C>CE; with decreasing concentration of CP.

In vitro release kinetics followed the zero order with an average release of 8.4, 6.8, and 4.7 μg/min in the case of formulations AE, BE, and CE respectively. This could be explained on the basis of the following hypothesis. Initially in dry state, eugenol in adsorbed form is distributed uniformly throughout the polymer matrix. After coming in contact with dissolution medium as gelation takes place, eugenol is dispersed in the form of very small droplets through a viscous gel matrix. This particular system could be termed as ''oil-in-hydrogel dispersion.'' The release of eugenol takes place from the surface of a swollen matrix by micellar solubilization with SLS. The movement of eugenol to the surface is governed by the viscosity of the external hydrogel phase. The viscosities determined in the preliminary study are in the order of 2:1<1:1<1:2 (carbopol:HPMC). Also, carbopol in its non-neutralized form does not gel completely, as it still remains in coiled form. As the proportion of CP increases, viscosity also decreases in the same order. Thus formulation AE, with a larger proportion of carbopol (2:1), because of its lower viscosity and incomplete gelation results in faster release of drug as compared to formulations BE and CE.

The flux was higher in the case of AE with higher proportion of CP, which might be because of faster hydration of polymer due to higher carboxyl group content and lower viscosity of gel phase. In contrast, the batch CE showed lower flux as the hydration time is more in the case of HPMC and the viscosity of gel phase is also higher. The formulation BE with equal proportions of both polymers shows higher initial flux due to faster hydration of CP. The subsequent decrease in the flux was observed due to slower diffusion of drug through the viscous HPMC gel region.

Release kinetics of eugenol in vivo followed the same trend as that of diffusion studies. The in vivo release rate of eugenol from the formulations was

doubled in vivo. This increased release can be attributed to the surrounding saliva medium, which hydrates the polymers and converts them to a gel. In diffusion studies only one surface of the tablet was exposed to the diffusion medium, which restricted the rate of hydration and therefore release of eugenol. The in vivo study shows the order of release of eugenol as AE>BE>CE. The decrease in CP content of the formulation decreased the rate of release, while HPMC showed reversal of results.

Better correlation was found between the results of the detachment force measurement method using sheep intestinal mucosa and the in vivo studies. The combination of CP and HPMC was found to be suitable for incorporation of eugenol in mucoadhesive dosage form. These two polymers act complementary to each other in that CP increases the bioadhesion and HPMC on the other hand helps in sustaining the release. Thus, the variation in their ratios could be manipulated as per the desired release profiles. Formulations containing higher proportions of CP showed faster release in both in vitro and in vivo. Thus, the study revealed that the mucoadhesive formulations showed good mucoadhesion properties with sustained release of eugenol for more than 8 hours. Clinical and antimicrobial studies against oral pathogens are in progress for determining the optimum formulation.

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