



RESEARCH ARTICLE

FORMULATION AND EVALUATION OF BUCCOADHESIVE GEL FOR THE TREATMENT OF ORAL SUBMUCOSAL FIBROSISPoonam Kumari^{*1}, Surender Singh Sourabh¹, Roshan Issarani¹

Department of Pharmaceutics, La choo Memorial College of Science and Tech, Pharmacy Wing, Sector-A, Shastri Nagar, Jodhpur, Rajasthan-342003, India.

Received 10 June 2014; Accepted 18 June 2014

ABSTRACT

Oral submucosal fibrosis (OSF) is a disease affecting the oral cavity and characterized by chronic, complex, irreversible, potentially malignant disorder (PMD) and crippling condition of oral mucosa, various delivery systems for administration & delivery of drugs to the oral cavity for treatment of OSF have been developed. The developed delivery systems mostly contain a single drug, while treatment of OSF is based on multiple drug approach. In the present work it is proposed to formulate and evaluate buccoadhesive gel of two drugs that is metronidazole and lycopene as topical antioxidant. In the present study five batches of metronidazole and lycopene gels were prepared using natural biodegradable polymer Carbopol 934p and sodium carboxy methyl cellulose. The influence of polymer type and polymers blend in varying ratio on the viscosity, buccoadhesive strength, spreadability, extrudability and drug release were evaluated. SCMC based gel showed fastest release in comparison with Carbopol based gel. Using polymer blend of SCMC with Carbopol resulted in a modification of both release and physical properties. The release of metronidazole was decreased with increasing amount of Carbopol and decreasing amount of SCMC. And increasing the viscosity of the gel formulations resulted in a retardation effect on the release of the drug. The study also showed that formulas containing Carbopol exhibited maximum viscosity with lower release rates and best buccoadhesion.

Key words: Oral submucosal fibrosis, metronidazole, lycopene, buccoadhesion**INTRODUCTION:**

The habit of chewing tobacco based products like Pan Masalas, Gutkha etc., contains many toxic chemicals which irritate the delicate skin of oral mucosa. This causes oral sub mucous fibrosis (OSMF) a pre-malignant fibrotic lesion of buccal region characterized by dense bands of collagen in the juxta-epithelia proceeded by inflammation and in later stages leads to oral cancer. In India 30-40% patients of oral inflammations are suffering with OSMF ⁽¹⁾. Metronidazole is bactericidal, front-line chemotherapeutic agent for treating infections caused by anaerobic bacteria such as *Prophylomonas gingivalis* because of the low minimum inhibitory concentration (MIC) required. MIC for susceptible anaerobic bacteria generally ranges from 0.1 to 8µg/ml. Such a drug has to be formulated as buccoadhesive delivery system to be delivered via transmucosal route ⁽²⁾.

Research shows that inflammation in the oral tissue is associated with OSF and periodontitis. The real culprit with inflammation is oxidative stress, a disturbance in the balance of oxidants and antioxidants. Although infections

are major trigger for inflammation and oxidative stress, there are numerous other causes, such as poor diet, alcohol consumption and nicotine use or chemical pollutants. Antibacterials control the micro-organisms that contribute to periodontitis, OSF and other infections, but they do not necessarily address the free radical and oxidative stress that accompany inflammation.

The categories of drugs used for the treatment OSF include antibacterials, anti-inflammatory, analgesic, local anesthetic, antiseptic. These agents are applied topically over the affected surface and topical therapy is supplemented with systemic addition of antioxidants. However preliminary observation has showed that antioxidants when applied topically along with OSF therapeutic agents result in clinical improvement of the condition dramatically. Therefore an antibacterial along with an antioxidant shall be combined in one dosage form such as buccoadhesive gel for application on the affected area in oral cavity ⁽³⁻⁷⁾.

Bioadhesive dosage forms have been used to target local disorders at the mucosal surface to reduce the overall

dosage required and to minimize the side effects that may be caused by the systemic administration of the drugs. Bioadhesive formulations use polymers as the adhesive component. These polymers are often water soluble and when used in a dry form, they attract water from the mucosal surface and this water transfer leads to a strong interaction. These polymers also form viscous layers when hydrated with water, which increases the retention time over the mucosal surfaces and leads to adhesive interactions. An attempt has been made, in the present work, to develop buccoadhesive gels of Metronidazole and lycopene by conventional method, using a blend of natural polymers such as Carbopol and sodium carboxy methyl cellulose. The objectives of the study were to investigate the performance of natural polymers and effect on the release characteristics of the Metronidazole (antibacterial) gels⁽⁸⁾.

MATERIALS AND METHOD:

Metronidazole and lycopene were procured from Uttaranchal Biotech Ltd. Rudrapur, U.S. Nagar (D.K.) as a gift sample. Carbopol 934p, sodium carboxy methyl cellulose, sodium metabisulphate, mannitol and other chemical and solvents were of analytical grade/IP/equivalent grade and procured from laboratory.

Preparation of buccoadhesive gel:

1- **Preparation of single polymer gel:** Carbopol or sodium carboxymethyl cellulose powder was sprinkled on preheated deionized (DI) water (40°C), with continuous stirring at 400 rpm, after 15 min speed was increased upto 900 rpm to prepare homogenous mixture of carbopol (phase 1). SMBS and mannitol were weighed and dissolved separately in minimum quantity of DI water, both solutions were mixed well (phase 2). Accurately weighed metronidazole, was dissolved in water by aid of small amount of ethanol as cosolvent and lycopene was dissolved in ethanol. Both were mixed well (phase 3). All phases were mixed and magnetically stirred at 700 rpm for 15 min, to obtain homogenous mixture. 0.1N NaOH/0.1N HCl was used to adjust pH to 6.6.

2- **Preparation of combination polymer gel:** Different gel formulations were prepared with various ratios of Carbopol to sodium carboxymethyl cellulose of 3:1, 2:2 and 1:3 by the same method mentioned previously. Different formulations of metronidazole and lycopene buccoadhesive gel are given in Table-1⁽⁹⁾.

Surface pH:

5 gm of the buccoadhesive gel was weighed in a 100ml beaker. 45ml of water was added to disperse the buccoadhesive gel. The pH of suspension was determined using digital pH meter.

Viscosity:

Viscosity of buccoadhesive gel of metronidazole and lycopene was determined at 25°C using Brookfield viscometer. The sample was sheared with spindle No-95 at 50 and 100rpm⁽¹⁰⁾.

Drug content:

Drug content was determined by taking 1gm sample of gel and analytically assayed to calculate the drug present in the sample using UV-Visible spectrophotometer at λ_{max} 320nm for metronidazole and 401.5 nm for lycopene.

Extrudability:

The formulation was filled in a clean, lacquered aluminum collapsible tube, extrudability was then determined by measuring the amount of gel extruded through the tip when a constant load of 1 Kg. was placed on the pan which was collected and weighed. The percentage of gel extruded was calculated using the formula:

$$\text{Extrudability (\%)} = W_E / W_C \times 100$$

Where

W_E = weight of extruded buccoadhesive gel

W_C = total weight of buccoadhesive gel in collapsible tube

Spreadability:

Spreadability was measured by apparatus, which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. A rectangular ground glass slide was fixed on this block. An excess of gel (about 2gm) under study was placed on this ground plate. The gel was then sandwiched between this plate and another glass plate having the dimensions of the fixed ground plate and provided with the hook. A 1Kg weight was placed on the top of the two plates for 5 minutes to expel air and to provide a uniform film of the gel between the plates. Excess of the gel was scrapped off from the edges. The top plate was subjected to pull of 150 gms, with the help of string attached to the hook and the time (in seconds) required by the top plate to cover a distance of 10cm is noted. A shorter interval indicates better spreadability. The spreadability can be calculated using the formula⁽¹¹⁾:

$$S = m \times l / t$$

Where -

S = Spreadability (gm.cm/sec)

m = weight tied to the upper slide (gm)

l = length of the glass slide (cm)

t = time (sec)

Adhesion strength:

The buccoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from buccal mucosal tissue using modified physical balance (Fig 1) method. A section of buccal mucosa was cut from the goat buccal cavity and

instantly fixed with the mucosal side out, on to glass slide using thread. The area of each exposed mucosal membrane was 2 cm². The slide with buccal tissue was stored at 37⁰C for 10 minutes. Another slide with buccal tissue was connected to the left side of two arm balance and the stored slide was fixed on height adjustable-pan. To the exposed tissue on the slide, a constant amount of 0.1 gm gel was applied. The height of the slide was adjusted so that the gel could adhere to the mucosal tissue of both slide. A force of 100gm was applied for 2 minute to ensure intimate contact between the tissue (Fig 2) and the sample. After removal of preload force, water was added slowly to previously weighed beaker placed on the right hand pan until slide get detach. The bioadhesive force expressed as the detachment stress in dyne/cm², was determined from the minimal weight that detach the tissue from the surface of each formulation using the following equation-

$$\text{Detachment stress (dyne/cm}^2\text{)} = \text{m.g /A}$$

Where-

m- the weight added to the balance

g- acceleration due to gravity taken as 980 cm/sec²

A-Area of tissue exposed ⁽¹²⁾

In-vitro drug release:

The release studies were conducted using Franz diffusion cells using egg membrane and dialysis membrane. Egg membrane was obtained by immersing egg in 10% HCl in a beaker. It was kept until the calcium shell was dissolved completely. Egg yolk was pump out by puncturing the membrane and washed with DI water. Both egg & dialysis membranes were soaked over the night in phosphate buffer solution. Dialysis membrane and egg membrane (surface area= 3.14 cm²) was fitted into the place between the chambers of cells. The receptor phase (15ml) composed of phosphate buffer solution (pH 6.6) and temperature was maintained at 37⁰C. The formulations were placed on the donor side. The receptor phase was stirred at 250rpm during the study. A pre-determined amount of gel (1 gm contained 1mg metronidazole and 8 mg lycopene) was mounted on the donor side of Franz cell. 1ml from the receptor phase was withdrawn and the same volume was replaced by fresh solution. The sample was diluted upto 10 ml by using receptor medium. Samples were assayed spectrophotometrically for amount of metronidazole and lycopene released. Each test was carried out in triplicate and the mean of three observations was reported separately for metronidazole through egg membrane and dialysis membrane and lycopene through egg membrane and dialysis membrane. Considering zero-order, constant

drug release for 4 hr, a theoretical release profile was computed which is also presented in Table-3 ⁽⁹⁾.

Release kinetics:

Different models for release kinetics were applied to the *in-vitro* release data. Among all the models the best fit model having highest correlation coefficient was selected to describe the sustained character of formulation. To analyze the mechanism of drug release from the buccoadhesive gel the release data were fitted into following models (e.g. zero order, first order, Higuchi, Hixon-crowell and Korsmeyer-Peppas model) which are presented Table 4. For each of the models the dependent parameters i.e. cumulative percentage drug released v/s time, log %drug released v/s time, percentage drug released v/s square root of time and cube root %drug unreleased v/s time and fraction release v/s time were used to calculated correlation coefficient and suggest a model that best fits the release kinetics is shown in Table-4 ⁽¹³⁾.

Antimicrobial activity:

Antimicrobial activity was determined by agar diffusion test employing cup plate technique. The drug was allowed to diffuse through a solid agar medium. The standard minimum inhibitory concentration (MIC 8 µg/ml) of control and developed formulation containing metronidazole were prepared. A total of 60 ml of nutrient agar media was prepared and sterilized at 120⁰C temperature 15 lb/sq-inch pressure for 15 minutes in an autoclave. Sterilized nutrient agar solution was poured into the petri plate contain each 20 ml & left at room temperature, this was done in an aseptic condition. 0.5ml of microorganism suspension (*Staphylococcus aureus* (MTCC-96)) was spread on agar plate with the help of spreader. After solidification of the media, sterile solution of metronidazole (standard solution lower concentration (50ppm), S₁ and higher concentration (100ppm), S₂) and the developed formulation diluted suitable with sterile distilled water (test solution (100ppm), T) were poured into the cup of sterile nutrient agar Petri plate. After allowing diffusion of the solutions at room temperature for 30 min then incubated at 37⁰C for 24 hr. After 24 hr of incubation the result of antimicrobial activity of the optimized gel formulation (F₅) against the *Staphylococcus aureus* indicates satisfactory Zone of Inhibition (ZOI) ⁽¹⁴⁾.

RESULT & DISCUSSION:

Surface pH:

The surface pH of gel was in the range of 6.4 ± 0.3-6.8 ± 0.4. This is the buccal pH (5-7.5pH) and may be suitable for oral application without any discomfort and irritation.

Thus, it may be assumed that all the formulations are applicable for oral mucosal treatment (Table-1).

Viscosity:

The viscosity of gel formulations were in the range of 3160 ± 94 to 14466 ± 18 at 50 rpm and 2118 ± 35 to 13240 ± 87 at 100 rpm (Table-2). Effect of rpm on viscosity of the gel is shown in Figure-3. Shear thinning phenomenon, an advantageous property of buccal gel, was observed (Figure-3) the viscosity of gel was found with increase in the rpm. However viscosity is indirectly proportional to rate of shear ($F = \eta G$), which is directly proportional to change in velocity or rpm ($G = dv/dr$), so with increase in rpm increase in rate of shear, which leads to thinning of gel and that ultimately decreases the viscosity of the gel to small extend.

There was significant difference in viscosity among the gel formulations containing different polymers/polymer blends and their concentration (F1-F5) at 50 rpm ($p < 0.05$, $F = 12957.99$, $F_{crit} = 3.478$, $df = 4$ and 10 , single factor ANOVA) and at 100rpm ($p < 0.05$, $F = 13788.34$, $F_{crit} = 3.478$, $df = 4$ and 10 , single factor ANOVA).

It may be concluded that polymer types and their blends affect the viscosity of the formulations. The apparent viscosity values were used as a measure of gel consistency. These values appeared to be markedly different; revealing variability in net work structure. Carbopol based gels showed higher viscosity values indicating higher consistency which may be due to its cross-linked structure and molecular weight reflecting this rheological behavior.

Drug content:

Percent drug content in formulation was found to be range $81.41 \pm 0.69\%$ to $99.42 \pm 1.05\%$ (metronidazole) and $82.58 \pm 0.10\%$ to $98.52 \pm 2.07\%$ (lycopene) with a small variation indicating uniform drug distribution in the buccoadhesive gel (Table-2).

Extrudability:

The packing of gels have gained a considerable importance in delivery of desired quantity of gel from jar or extrusion of gel from collapsible tubes. In the present study extrudability of gel formulations was determined based upon the quantity of % gel extruded from tube on application of certain load. More the quantity of gel extruded, better the extrudability. The extrudability of formulated gel was in the range of $3.5 \pm 0.1\%$ to $7.6 \pm 0.2\%$ and of the marketed preparation was $6.5 \pm 0.2\%$ which is shown in Table-2. There was significant difference in extrudability among the gel formulations containing different polymers/polymer blends and their concentration (F₁-F₅) and marketed formulation (mkt)

($p < 0.05$, $F = 224.857$, $F_{crit} = 3.106$, $df = 5$ and 12 single factor ANOVA).

It may be concluded that polymer types and their blends affect the extrudability of the formulations. The order of increasing % extrudability of formulations were $F_2 > F_5 > F_{mkt} > F_4 > F_3 > F_1$ respectively.

Spreadability:

Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to the affected parts. The therapeutic efficiency of a formulation also depends upon its spreading coefficient, higher the spreading coefficient values better the spreadability. Spreadability of formulated gel was in the range of 8.57 ± 0.39 to 46.88 ± 1.42 gm.cm/sec (Table-2). There was significant difference in spreadability coefficient among the gel formulations containing different polymers/polymer blends and their concentration (F₁-F₅ & F_{mkt}) ($p < 0.05$, $F = 178.068$, $F_{crit} = 3.108$, $df = 5$ and 12 single factor ANOVA).

It may be concluded that polymer types and their blends affects the spreadability of the formulations. The order of increasing spreadability of formulations were $F_2 > F_5 > F_{mkt} > F_4 > F_3 > F_1$ respectively.

Adhesive force:

The buccoadhesive properties of prepared gel formulations were evaluated of the detachment force required to overcome the adhesive bond between each formulation and the buccal mucosa. The polymers employed in these formulations have been described as bioadhesive and, therefore, it would be anticipated that the formulation would display good mucoadhesive properties. It also was noted that factors such as the molecular weight of polymer, the type and degree of cross-linking agent, molecular architecture and the polymer amount in the gel influenced the mucoadhesive performance. The adhesion force of formulated gel was in the range of 2517.81 ± 49.27 to 8705.67 ± 74.37 dyne/cm² (Table-2). There was significant differences in mucoadhesive property among the gel formulations containing different polymers/polymer blends and their concentration (F₁-F₅) ($p < 0.05$, $F = 855.804$, $F_{crit} = 3.478$, $df = 4$ and 10 , single factor ANOVA).

It may be concluded that polymer types and their blends affects the buccoadhesion of the formulations. Mucoadhesive force in term of detachment stress (Table-2) indicated that the mucoadhesive forces for Carbopol based formulations were much more than other formulations which may be attributed to the high viscosity of Carbopol based gel. Carbopol also has a very high percentage (58-68) of carboxylic group in its chemical structure that gradually undergo hydrogen

bonding with sugar residue in the oligosaccharide chain in the mucous membrane resulting in the formation of strengthened network between polymer and mucus.

In addition may also adopt more favorable macromolecule confirmation with the accessibility of its functional group for hydrogen bonding, while other polymers only undergo superficial buccoadhesion. Buccoadhesion of SCMC blend based gels was affected by polymer molecular weight and also viscosity of formulation. On the other hand the charge of the polymer tended to affect the buccoadhesive force, where nonionic polymer appears to undergo a smaller degree of adhesion compared to anionic polymer. This explains why the F₁ formulation, anionic polymer based gel, had buccoadhesive force higher than F₂ formulation, nonionic polymer based gel.

In-vitro release:

In the all formulations (F₁-F₅) at the end of 240 min, percent metronidazole release was 39.64%, 85.53%, 43.27%, 55.20%, 81.64% and 93.92% (F_{mkt}) across egg membrane and 34.86%, 83.13%, 42.57%, 54.83% and 79.58% across dialysis membrane percent lycopene release was 35.86%, 73.61%, 40.07%, 52.77% and 69.94% across egg membrane and 33.98%, 69.73%, 39.82%, 51.21% and 68.27% across dialysis membrane (Table-3). There was significant differences in release profile among the gel formulations containing different polymers/polymer blends for metronidazole through egg membrane and dialysis membrane (F₁-F₅, F_{mkt}) (p<0.05, F=152.053, F_{crit}=3.478, df=4 and 10, single factor ANOVA), (F₁-F₅) (p<0.05, F=254.671, F_{crit}=3.478, df=4 and 10, single factor ANOVA), respectively Figure-4,5. There was significant differences in release profile among the gel formulations containing different polymers/polymer blends for lycopene through egg membrane and dialysis membrane (F₁-F₅) (p<0.05, F=139.036, F_{crit}=3.478, df=4 and 10, single factor ANOVA), (p<0.05, F=133.272, F_{crit}=3.478, df=4 and 10, single factor ANOVA), respectively Figure-6,7.

Effect of polymer:

Gels with particular polymer were prepared to study the effect of polymer type on the release profile. Figure-8 showed the release profile of metronidazole and lycopene from formula F₁ and F₂, and release of metronidazole from F_{mkt}. Being an anionic and water soluble, SCMC (4%) based gel (F₂) released more than 81% of metronidazole within 3 hrs, and approximately 30% of drug released within 30 minutes. This formula showed burst release due to rapid dissolution of the gelling polymer in pH of the dissolution medium, water soluble base (1%) based gel (F_{mkt}) released more than

93% within 4 hr, this marketed preparation showed fast release due to rapid dissolution of the gelling polymer which amount is 1%. Carbopol gel showed integrity beyond 4 hrs and did not dissolve completely even after 4 hrs. More retardant effect was obtained with carbopol this may be attributed to the highest viscosity of this gel than other gel preparations.

Effect of polymer combination:

To obtain adequate of the drug, it is thought to prepare formulas containing mixture of SCMC (fast drug release polymer) and carbopol (slow drug release polymer). Figure-9 show the release profile metronidazole and lycopene from gel with combination polymers. The release rate constant for carbopol: SCMC gels decreased significantly with decreasing amount of SCMC and increasing amount of carbopol, this could be described as the corresponding reduction in the number and dimension of the channel by increasing viscosities of the formulations. Gel containing carbopol: SCMC showed lower rate of release which produce water swollen gel that may substantially reduce the penetration of the dissolution medium into the gel and as the result the drug release.

Release kinetics:

From the above results it was found that the drug release was best fitted Korsmeyer-Peppas model since it showed highest value of 'r' for formulation F₁, F₃ and F₄. The 'n' value for batch is greater than 0.5, hence, it is clear that the formulation is showing non-fickinian and anomalous diffusion (appendix Table 3). Also the release of drug from F₁ formulation was best fitted to zero order kinetics, there by meaning that release is independent of drug concentration. Drug release from F₂ was best fit in to Higuchi model; it means that drug release from gel was diffusion controlled. While drug release from F₅ was best fit in to Hixon Crowell model, there by meaning that release was dissolution controlled. It means that polymer layer dissolved and released the drug, then next layer dissolve and release drug.

Antimicrobial efficacy:

Antimicrobial efficacy study was performed on F₅ formulation using Gram+ve *Staphylococcus aureus* organism. The Zone of Inhibition of F₅ buccoadhesive gel found to be 17.7±1.15 mm. The result of antimicrobial activity is shown in Table-5. Diameter of ZOI is shown in Figure-10.

The study indicated metronidazole approximately retained its antimicrobial activity when formulated as buccoadhesive gel system against selected *Staphylococcus aureus*.

Table 1: formulation of metronidazole and lycopene buccoadhesive gel (%w/w)

Ingredients (%w/w)	Mtz	L	CP	SCMC	M	SMBS	W	Surface pH*
Batch code								
F ₁	0.1	0.8	4.0		2	0.02	q.s	6.6 ± 0.2
F ₂	0.1	0.8		4.0	2	0.02	q.s	6.4 ± 0.3
F ₃	0.1	0.8	3.0	1.0	2	0.02	q.s	6.5 ± 0.4
F ₄	0.1	0.8	2.0	2.0	2	0.02	q.s	6.7 ± 0.4
F ₅	0.1	0.8	1.0	3.0	2	0.02	q.s	6.8 ± 0.4

#pH adjusted to 6.6 using 0.1N sodium hydroxide (NaOH)/0.1N HCl. *Data represents mean ± SD of triplicate determination

Mtz= Metronidazole, L= Lycopene, CP= Carbopol 934p, SCMC= Sodium carboxy methyl cellulose, M= Mannitol, SMBS= Sodium meta bisulphite, W= Water

Table 2: Results of evaluation of prepared buccoadhesive gel of metronidazole and lycopene.

Batch code	Viscosity (cps)*		Drug content (%)*		Extrudability	Spreadability	Adhesion force
	50rpm	100rpm	Mtz	lyco			
F ₁	14466 ±	13240 ±	81.41 ± 0.69	82.58±0.1	3.5 ± 0.2	8.57 ± 0.39	8705.67 ±
F ₂	3160 ± 94	2118 ± 35	99.42 ± 1.05	98.52±2.0	7.6 ± 0.2	46.88 ± 1.42	2517.81 ±
F ₃	13140 ±	11534 ±	89.43 ± 1.20	89.70±1.8	4.2 ± 0.1	10.86 ± 0.94	6726.97 ±
F ₄	6756 ± 88	4314 ± 69	90.56 ± 1.09	89.56±1.0	5.3 ± 0.3	14.42 ± 1.03	5810.07 ±
F ₅	5554 ± 82	4162 ± 83	98.58 ± 1.05	97.69±1.5	6.8 ± 0.2	30.43 ± 1.69	5492.43 ±
F _{mkt}	-	-	-	-	6.5 ± 0.2	29.45 ± 1.32	-

*Data represents mean ± SD of triplicate determination

Table 3: Results of *in-vitro* release of metronidazole and lycopene at 240 min from buccoadhesive gel of metronidazole and lycopene

Drug (Memb)	Formulation code					
	F ₁	F ₂	F ₃	F ₄	F ₅	F _{mkt}
Mtz (EM)	39.64±2.4	85.53±3.28	43.27±3.17	55.20±3.2	81.64±2.86	93.92±2.2
Mtz (DM)	34.86±2.6	83.13±2.37	42.57±2.48	54.83±1.8	79.58±2.35	
Lyco (EM)	35.86±2.6	73.61±2.79	40.07±2.58	52.77±2.3	69.94±2.18	
Lyco (DM)	33.98±2.1	69.73±2.35	39.82±2.42	51.21±2.5	68.27±2.66	

Table 4: Model fitting of *in-vitro* release data

Formulation Code/Drug/Me		ZOK	FOK	HM	HCM	KPM			Best fit model	
		R	r	r	r	r	k	n		
F ₁	M	M _E	0.997	0.859	0.967	0.996	0.997	0.004	0.8	ZOK, KP
		M _D	0.997	0.886	0.963	0.997	0.996	0.002	0.8	ZOK, HC
	L	M _E	0.996	0.881	0.962	0.995	0.996	0.002	0.9	ZOK, KP
		M _D	0.995	0.899	0.949	0.993	0.995	0.001	1.0	ZOK, KP
F ₂	M	M _E	0.920	0.849	0.988	0.971	0.982	0.004	0.6	HM
		M _D	0.979	0.669	0.986	0.965	0.982	0.002	0.6	HM
	L	M _E	0.912	0.672	0.982	0.949	0.978	0.002	0.6	HM
		M _D	0.912	0.681	0.982	0.952	0.957	0.001	0.6	HM
	M	M _E	0.974	0.819	0.980	0.983	0.988	0.042	0.9	KPM
		M _D	0.979	0.827	0.980	0.987	0.989	0.038	0.9	KPM

F ₃	L	M _E	0.980	0.845	0.975	0.988	0.989	0.033	0.9	KPM
		M _D	0.986	0.848	0.976	0.992	0.994	0.024	0.9	KPM
		M _D	0.981	0.791	0.982	0.995	0.993	0.006	0.9	HCM
F ₄	M	M _E	0.971	0.793	0.981	0.984	0.985	0.004	0.8	KPM
		M _D	0.974	0.802	0.980	0.985	0.987	0.003	0.9	KPM
	L	M _E	0.983	0.814	0.980	0.992	0.993	0.002	0.9	KPM
		M _D	0.984	0.822	0.979	0.992	0.991	0.002	0.9	HCM
F ₅	M	M _E	0.990	0.783	0.981	0.997	0.996	0.009	0.8	HCM
		M _D	0.990	0.789	0.981	0.998	0.996	0.008	0.8	HCM
	L	M _E	0.981	0.786	0.984	0.996	0.993	0.007	0.8	HCM
		M _D	0.981	0.791	0.982	0.995	0.993	0.006	0.9	HCM

Table 5: Data for Zone of Inhibition of optimized formulation gel of metronidazole

Microorganism	Concentration of solution	ZOI Mean± SD (mm)*
<i>S.aureus</i>	S ₁ (50ppm)	12.3±0.58
<i>S.aureus</i>	S ₂ (100ppm)	19.3±0.58
<i>S.aureus</i>	T (100ppm)	17.7±1.15



Figure 1:

Figure1: Photograph of modified physical balance,

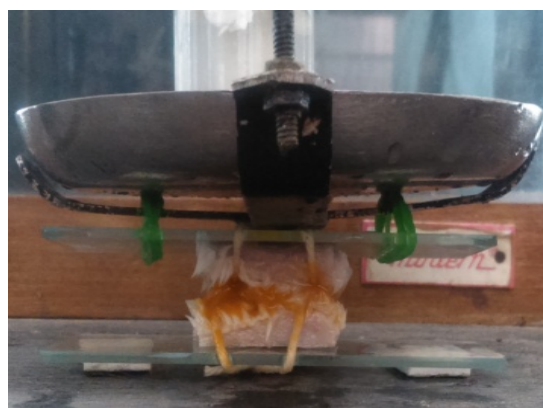


Figure 2:

Figure 2: Intimate contacts between the tissue

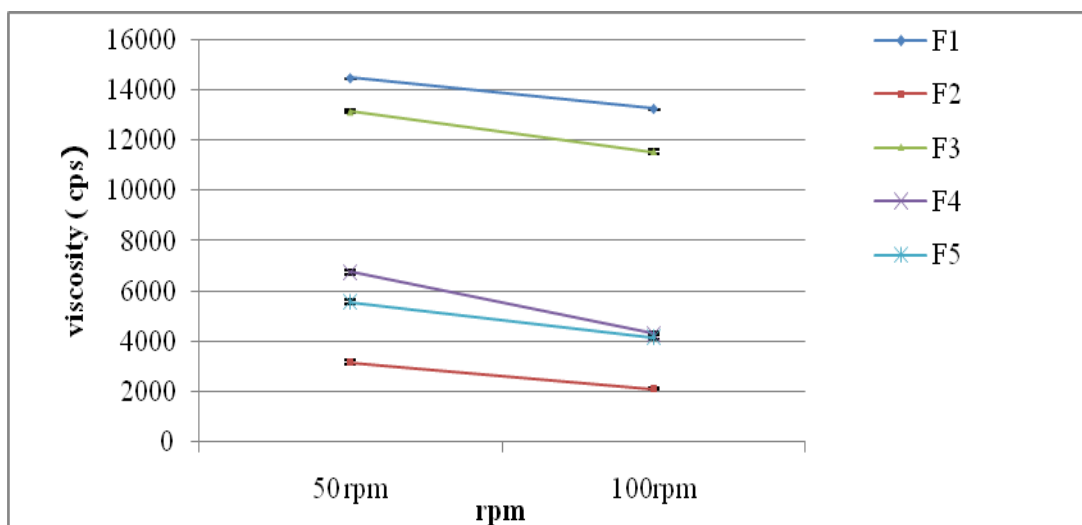


Figure 3: Effect of rpm on viscosity of buccoadhesive gel containing metronidazole and lycopene

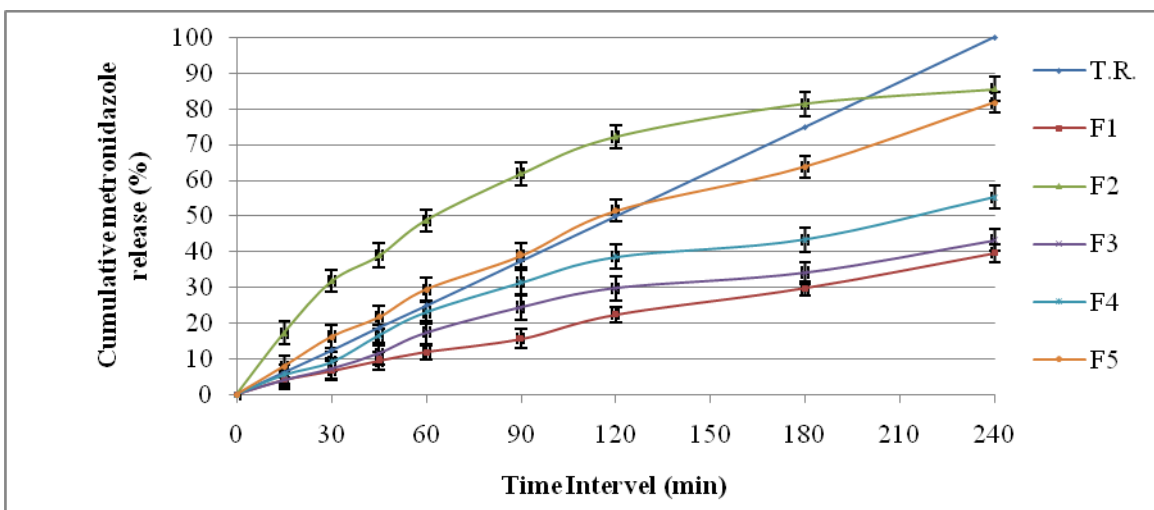


Figure 4: *In-vitro* release profile of metronidazole through egg membrane

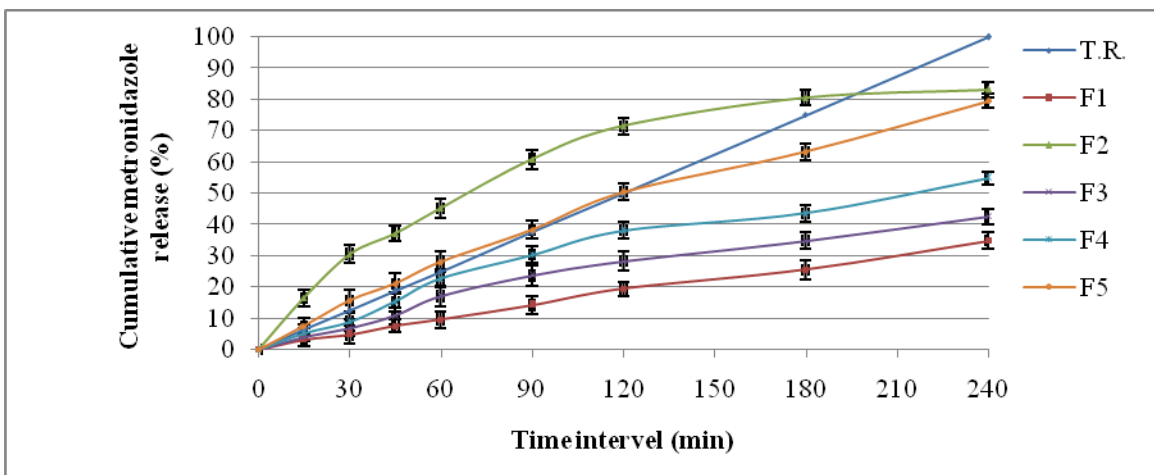


Figure 5: *In-vitro* release profile of metronidazole through dialysis membrane

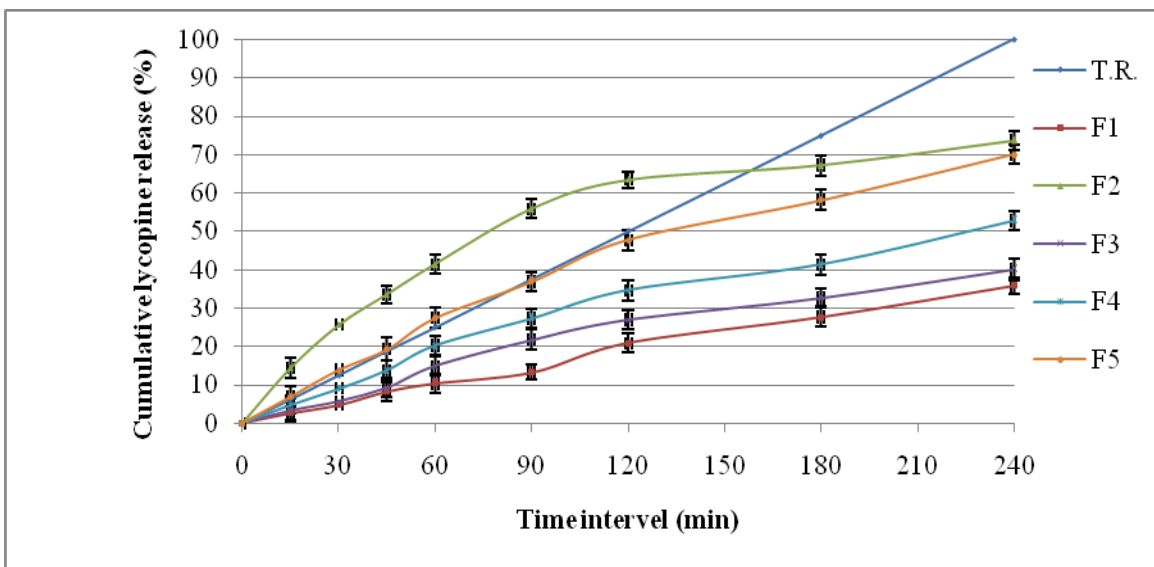


Figure 6: *In-vitro* release profile of lycopene through egg membrane

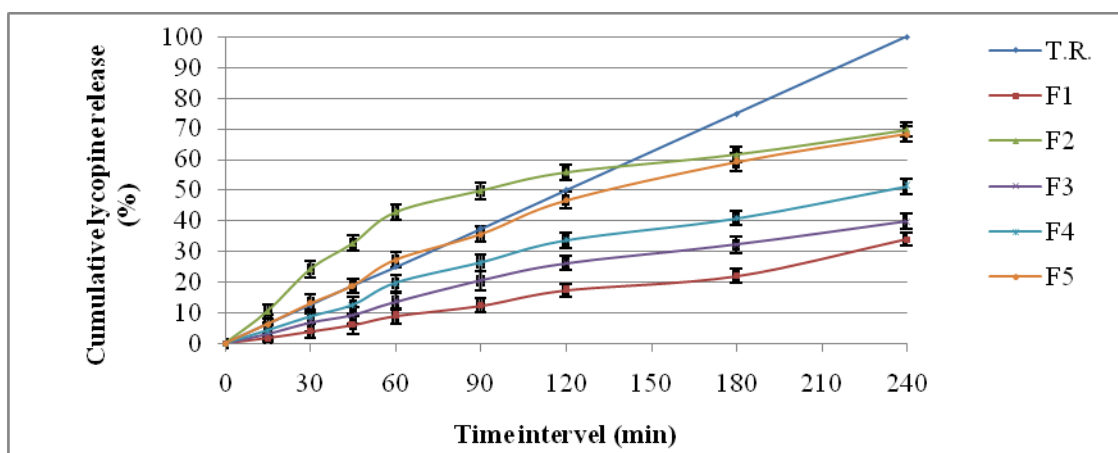


Figure 7: *In-vitro* release profile of lycopene through dialysis membrane

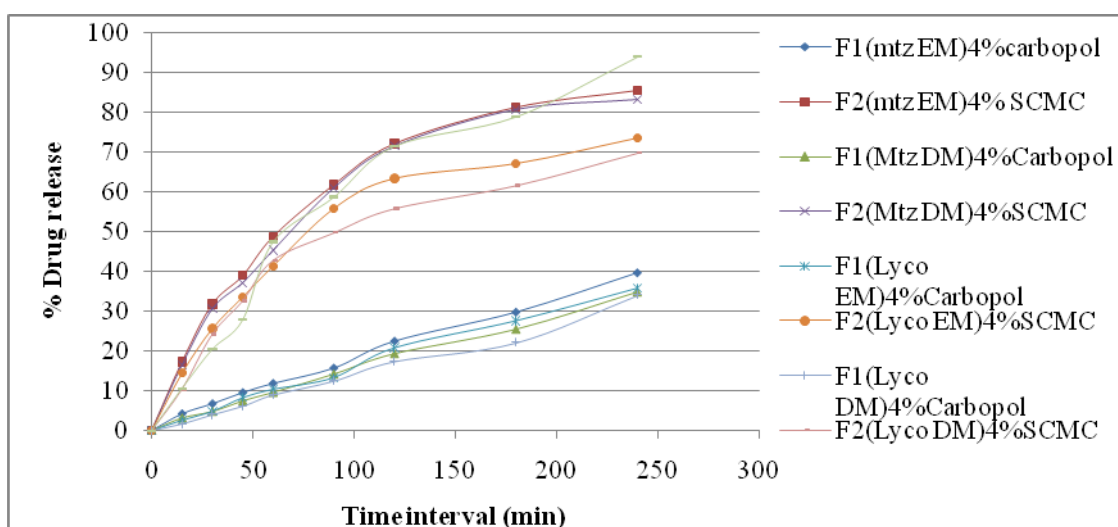


Figure 8: Effect of polymer type on metronidazole and lycopene release from buccoadhesive gel formulation through egg and dialysis membrane

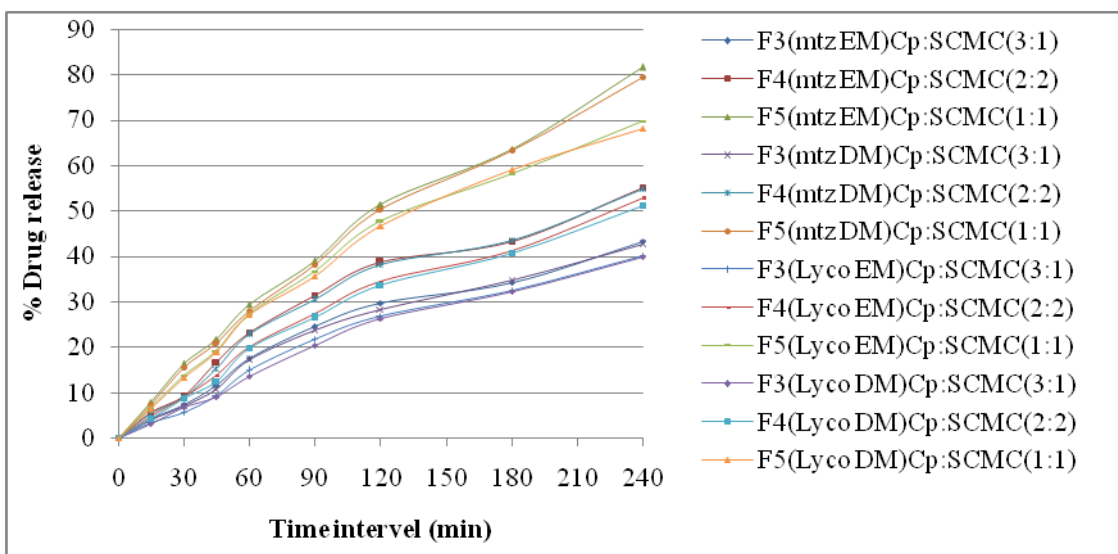


Figure 9: Effect of polymer combination on metronidazole and lycopene release from (Carbopol: SCMC) buccoadhesive gel through egg and dialysis membrane

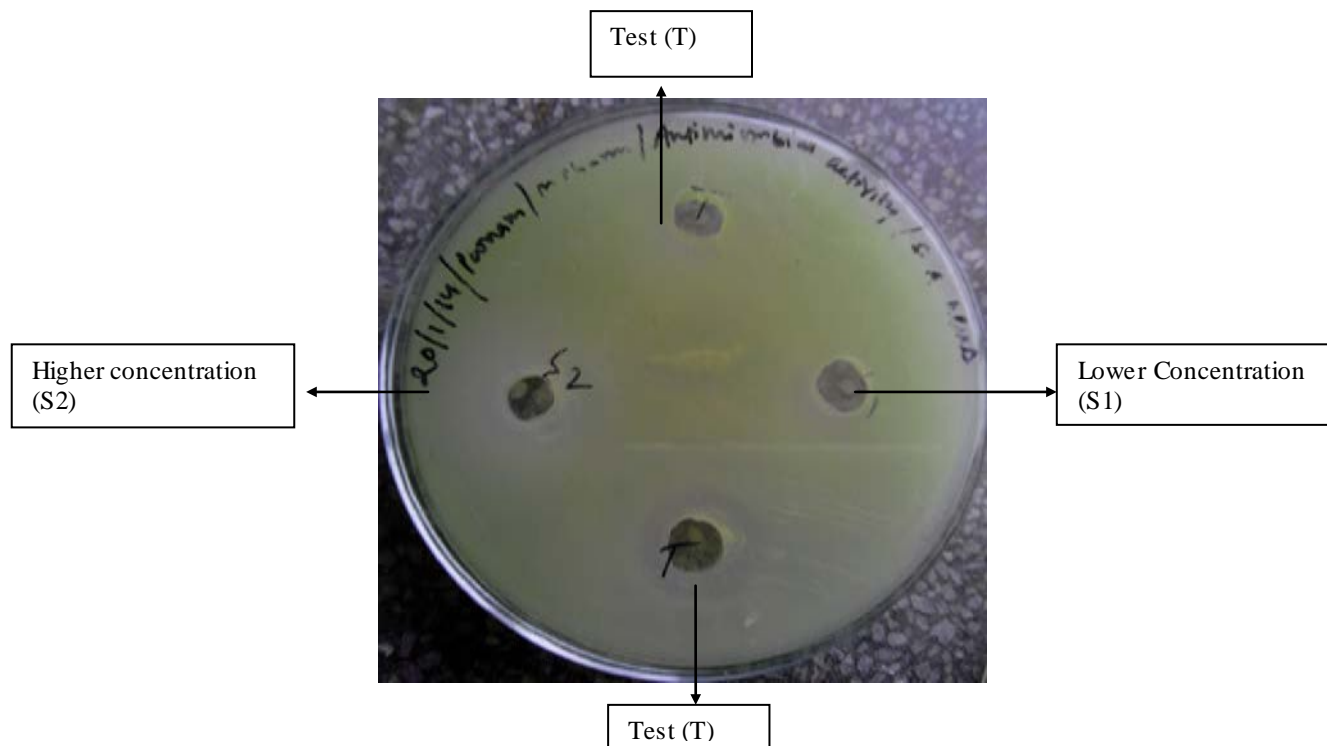


Figure 10: Zone of Inhibition (mm) of formulation F₅

CONCLUSION:

The release rate of metronidazole and lycopene from the prepared buccoadhesive gel as well as the other evaluation parameters were influenced by the type of polymer (Carbopol or SCMC) and combination of polymers (Carbopol and SCMC).

Formulated formulation F₁ with 4% Carbopol showed higher buccoadhesion but release profile was not similar to the theoretical release profile. Formulation F₂ with 4% SCMC showed maximum release within 4 hr but it showed burst release due to rapid dissolution of the gelling agent in pH of dissolution medium, and also showed lowest buccoadhesion, spreadability, viscosity and extrudability. Formulation F₃-F₅ with combination of Carbopol and SCMC (3:1, 2:2, 1:3) release rate constant for Carbopol: SCMC gels decreased significantly with decreasing amount of SCMC and increasing amount of Carbopol, this could be described as the corresponding reduction in the number and dimension of the channel by increasing viscosities of the formulations. Gel containing Carbopol: SCMC showed lower rate of release which produced water swollen gel that may substantially reduce the penetration of the dissolution medium into the gel and as the result the drug release, Formulation F₅ with high concentration of SCMC (3%) and low concentration of Carbopol (1%) showed similar release profile to the theoretical release profile. Also spreadability and extrudability of gel F₅ was comparability to the marketed

product (REXIDINE[®]-M), so F₅ selected as optimized formulation. In conclusion 1% Carbopol: 3% SCMC can be use as vehicle for buccoadhesive drug delivery system to the oral cavity because of their good buccoadhesion, spreadability, extrudability and release profile reported. The developed gel formulation could be a promising local alternative treatment of OSF and could be subjected further *in-vivo* study in rats by inducing OSF, and there after treating with optimized preparation, skin irritation test and stability study will be performs.

REFERENCE:

1. Vijaybhaskar D., et al, Histopathological effect of mucoadhesive herbal gel on tobacco carcinogenicity, International Journal of Pharmaceutical & Biological Archives 2012;3(2):394-399.
2. Ramadan E., et al, Formulation and evaluation of metronidazole bioadhesive matrices for treatment of periodontitis, Bull Pharmaceutical Science, Assiut University 2010;33(1):79-94.
3. Battino M, et al. The antioxidant capacity of saliva. Journal of Clinical Periodontology 2002; 29:189-194.
4. Miricescu D, et al. The antioxidant potential of saliva: Clinical significance in oral diseases. Therapeutics, Pharmacology and Clinical Toxicology 2011; 15 2:1-5.
5. Gupta A, et al. Lipid peroxidation and antioxidant status in head and neck squamous cell carcinoma

- patients. *Oxidative Medicine and Cellular Longevity*, April-June 2009.
6. Hershkovich O, et al. Age-related changes in salivary antioxidant profile: Possible implications for oral cancer. *The Journals of Gerontology* 2007; 62A 4:361-366.
 7. San Miguel SM, et al. Bioactive antioxidant mixtures promote proliferation and migration on human oral fibroblasts. *Archives of Oral Biology* 2011; doi:10.1015/jarchoralbil.2011.01.001.
 8. Yellanki K.S., et al, Formulation, characterization and evaluation of metronidazole gel for local treatment of periodontitis, *International Journal of Pharma and Bio-Sciences* 2010;1(2):1-9.
 9. Sulayman H. T., Some variables affecting formulation of tinidazole mucoadhesive oral gel, *Asian Journal of Pharmaceutical Sciences* 2011;10(2):165-179.
 10. Suresh K. P., et al, Ciprofloxacin loaded glycerylmono oleate periodontal gels formulation and *In-vitro* evaluation, *International Journal of Comprehensive Pharmacy* 2012;11(5):1-4.
 11. Purushotham Rao K., et al, Formulation of topical oral gel for the treatment of oral sub mucosal fibrosis (OSMF) 2011;3(1):103-112.
 12. Jelvehgaria M., et al, Mucoadhesive and drug release properties of benzocaine gel, *Iranian Journal of Pharmaceutical sciences*, autumn 2006;2(4):185-194.
 13. Pornsak S., et al, Swelling, erosion and release behavior of alginate-based matrix tablet, *European Journal of Pharmaceutics and Biopharmaceuticas* 2007;66:435-450.
 14. Sonjoy M., et al, Formulation and Evaluation of an *in-situ* gel forming ophthalmic formulation of moxifloxacin hydrochloride 2012;2(2):78-82.