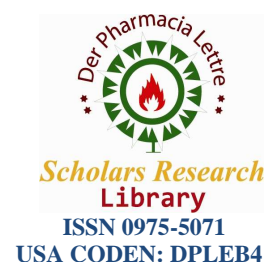




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Development and evaluation of mucoadhesive films of Acyclovir in oral environment- along with model study on Chlorhexidine

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ABSTRACT

Oral hygiene is important issue related with preventing oral/ systemic infections. In the present work attempt are made to develop and evaluate of the mucoadhesive films of with mouth freshening effect. Work consisted of product development of mucoadhesive films using drug acyclovir and chlorhexidine gluconate. The films were formulated, exposed to peppermint oil to produce mouth freshening effect. Evaluations of film formulations were performed for thickness, folding endurance, percent swelling and mechanical properties, mucoadhesive strength, invitro residence time, and invitro drug release. The optimized formulations were compared with the marketed product. The three months stability studies were carried for the optimized film formulations.

Keywords: Acyclovir; Chlorhexidine; Mucoadhesive films; *Invitro* drug release; Tensile strength; Mucoadhesive strength.

INTRODUCTION

Oral hygiene is important issue related with preventing oral/ systemic infections. The oral mucosa has many properties which make it an attractive site for drug delivery but also provides several challenges for researchers investigating novel delivery techniques to overcome. Many different formulations including sprays, tablets, mouthwashes, gels, pastes and patches are presently used for delivery into and/or across the oral mucosa. The buccal route of administration has a number of advantages including bypassing the gastrointestinal tract and the hepatic first pass effect. [1]

Mucoadhesive films are retentive dosage forms and release drug directly into a biological substrate. Furthermore, films have improved patient compliance due to their small size and reduced thickness, compared for example to lozenges and tablets. [2] Common oral viral infections cause primary herpetic gingivostomatitis, or oral herpes. In some hosts, it becomes latent and may periodically recur as a common cold sore. [3] Specific oral bacterial species have been implicated in oral diseases such as caries and periodontitis and in several systemic diseases, such as bacterial endocarditis, aspiration pneumonia, osteomyelitis in children, preterm low birth weight, and cardiovascular disease. [4] Acyclovir is antiviral can be used in the local viral infections and chlorhexidine are the antibacterial/ antiplaque agent which can be preventive measures in the oral bacterial infection and to maintain daily oral hygiene.

MATERIALS AND METHODS

2.1. Materials

Acyclovir and Chlorhexidine gluconate (Otto lab, Mumbai) was used as a model drug, HPMC K15 (Colorcon Asia Pvt. Ltd Goa), Sodium alginate and Gelatin (Research lab fine chemicals, Mumbai) were selected as a natural mucoadhesive polymer, the sodium alginate also act as the sustained release adjuvant. The polyethylene glycol-400

is used as the plasticizer in the film formulations. The peppermint oil (Research lab fine chemicals, Mumbai) was used to give the mouth freshening effect to the films. Distilled water was used as the preparation of casting solvent.

2.3. Preparation of mucoadhesive films of acyclovir

The polymeric solution of the 500mg HPMC K15 as a film forming polymer [5], sodium alginate and gelatin was prepared according to the concentrations given in the table (2). PEG-400 was taken as the 30% w/w of total quantity of the polymer concentration. As that of the drug is soluble in the water, the formulation was made to get the thin and clear film. The loading of the drug was optimized at the 0.33mg/cm² of the film formulation. At this concentration it was observed that no any precipitation of the drug on the surface of film. Above this concentration the drug is get precipitated from at the surface of the film.

The polymeric solution was prepared in the 50ml of the distilled water by constant. Stirring in another beaker 50ml of distilled water the drug us dissolved and that of slowly added in the polymeric solution for the uniform distribution of the drug in the casting solvent. The resultant solution was obtained was of 100ml quantity. Drug was added in such a way that the final formulation as getting a clear thin and film.

This solution was allowed to stir for the net 6hrs. The casting solution containing a drug was poured in the mould and kept at the room temperature overnight for evaporation of casting solvent. The dried films was carefully removed from the mould and wrapped in the aluminium foil for stored for further practical treatment and evaluations. Evaluation of acyclovir film for the required parameters was performed.

2.4. Preparation of mucoadhesive films of Chlorhexidine

Chlorhexidine gluconate solution was added drop wise in formulation with continuous stirring in the same blank polymeric solution prepared for the formulation of films of acyclovir. PEG-400 was taken as the 30% w/w of total quantity of the polymer concentration. As that of the films are only for the oral hygiene, the drug was loaded in a concentration such that the final concentration of film contains Chlorhexidine gluconate 1mg/cm²

Each film of prepared formulations of Acyclovir and Chlorhexidine gluconate are then exposed with the peppermint oil and then again evaluated for the drug release.

2.5 Evaluation of films of acyclovir for required parameter-

Films are evaluated for the following parameters:

1. Thickness:-

Similarly, three films of each formulation were taken and the film thickness was measured using Micrometer Screw Gauge (Aerospace-0-150 Digital Caliper) at three different places and the mean value was calculated.

2. Surface pH of Films:-

The film to be tested was placed in Petri dish and moistened with 0.5ml of distilled water and kept of 30s. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibration for 1 min. The average of three reading was taken for each formulation. [6]

3. Folding Endurance:-

Three films of each formulation of size (2×2 cm) were cut by using sharp blade. Folding Endurance was determined by repeatedly folding a small strip of film at the same place till it broke. The number of times, the film could be folded at the same place without breaking gave the value of folding endurance.

4. Percent Swelling:-

After determination of the original film weight and diameter, the samples were allowed to swell on the surface of agar plate kept in an incubator maintained at 37 °C. Increase in the weight and diameter of the patches ($n = 3$) was determined at preset time intervals (1–5 h). The percent swelling, %S, was calculated using the following equation:

$$\%S = \frac{x_t - x_0}{x_0} \times 100$$

Where, X_t is the weight or diameter of the swollen patch after time t , and X_0 is the original patch weight or diameter at zero time. [7]

5. *In vitro* Residence Time:-

The *in vitro* residence time was determined using USP disintegration apparatus. The disintegration medium was 800 ml of pH 6.8 phosphate buffer maintained at $37 \pm 2^\circ\text{C}$. The segments of porcine buccal mucosa, each of 3 cm length, were glued to the surface of a glass slab, which was then vertically attached to the apparatus. Three mucoadhesive films of each formulation were hydrated on one surface using pH 6.8 PB and the hydrated surface was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down. The film was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time required for complete erosion or detachment of the film from the mucosal surface was recorded (n=3). [1]

6. *In vitro* Dissolution:-

In vitro drug release study was carried out by using the USP type-1 dissolution apparatus using. One Film of each formulation was fixed to the central shaft using a cyanoacrylate adhesive. The dissolution medium was used 250 ml of phosphate buffer pH 6.8. The rotation speed was 50 rpm at 37°C . The drug release was analyzed spectrophotometrically at 252nm for acyclovir formulation and at 254nm for chlorhexidine formulation. One film was placed into each vessel. [8, 9]

7. Mechanical Properties:-

The mucoadhesive films should be mechanically strong. An ideal buccal film should be flexible, elastic, soft yet adequately strong to withstand breakage due to stress from mouth activities. Two mechanical properties namely tensile strength and percent elongation were determined for the evaluation of film. Tensile strength is the maximum stress applied to which the film specimen breaks and can be calculated from the applied load at rupture and percent elongation of film is increase in the length of the film when it gets breaks at its maximum stress. The mechanical properties of the film were determined by using previously designed and calibrated apparatus for determination of tensile strength and percent elongation. [10] The films of size 20x40 mm dimension was taken and that was fixed in the fixed jaw and movable jaw, stress is applied to the film movable jaw and force at which the film gets break is further calculated as the tensile strength using formula. The maximum increase in the length of the film during applying a load to the film was measured as the percent elongation of the film at that of the break point

$$\text{Tensile strength (kgmm}^{-2}\text{)} = \frac{\text{force at Break (kg)}}{\text{Initial cross sectional area of the sample(mm}^2\text{)}}$$

$$\text{Elongation at break (\%mm}^{-2}\text{)} = \frac{\text{Increase in Length(mm)}}{\text{Original Length}} \times \frac{100}{\text{Cross sectional area(mm}^2\text{)}}$$

8. Mucoadhesive Strength:-

Mucoadhesive strength of the film formulation was determined by using the previously calibrated assembly for the determination of mucoadhesive strength of the film. The porcine buccal mucosal membrane was used for determination of mucoadhesive strength. [11] The fresh porcine mucosal membrane was purchased from the local slaughter house and then it was washed using the isotonic phosphate buffer pH 6.8. The piece of fresh membrane was glued to a support (glass block) with the Cyanoacrylate adhesive. The glass block was then lowered into the container, which was then filled with isotonic buffer pH 6.8 kept at $37 \pm 1^\circ\text{C}$, such that the buffer just reaches the surface of mucosal membrane, and keeps it moist. This was then kept below the left hand side of the assembly. The test film was glued with the same adhesive to the rubber block hanging on the left hand side of assembly. The rubber block lowered along with the film over the mucosa with the weight 5g. The attachment of the film to the mucosal membrane was kept in this position for 3 minutes and then slowly water was added to the container on the right hand side by using the burette. The force of detachment of the two surfaces was obtained. Weight of the water was measured. Then the mucoadhesive strength of the film was obtained using the following formula. Three films were tested on each mucosal membrane. After each measurement the tissues were thoroughly and gently washed with the phosphate buffer (pH 6.8) and left for 5 minutes before the next experiment. Three reading was taken for the each.

RESULTS AND DISCUSSION

3.1. Evaluation of the mucoadhesive films of acyclovir

Films are evaluated for the physicochemical properties and mucoadhesive properties (Table 1 and 2). Figure 2 and 3 are graphs of the percent cumulative drug release and time. The release study was performed using USP type II dissolution apparatus. The release study was performed for each formulation before and after exposure to the peppermint oil. It was observed that the formulation F2 gives the longer release i.e. upto 44 minutes. There is no any

significant was difference observed in the release of the drug in the film formulation before and after exposure to the peppermint oil.

3.2. Evaluation of Chlorhexidine gluconate loaded films

Films are evaluated for the physicochemical properties and mucoadhesive properties (Table 3 and 4). After loading of the drug Chlorhexidine gluconate in the same formulation of the blank films the films, it was observed that there is no any significant difference observed in the evaluation parameters of the film formulation in the folding endurance, mechanical properties, swelling index, surface pH, *invitro* residence time mucoadhesive strength of the film formulation.

Figure 5 and 6 are graph of the percent cumulative drug release and time. The release study was performed using USP type II dissolution apparatus. The release study was performed for each formulation before and after exposure to the peppermint oil. It was observed that the formulation F2 gives the longer release i.e. upto 46 minutes. There is no any significant was difference observed in the release of the drug in the film formulation before and after exposure to the peppermint oil.

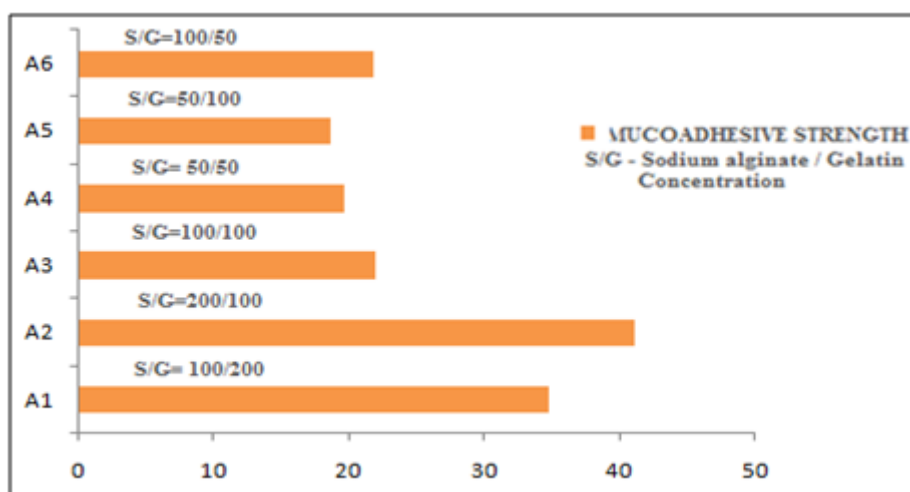


Figure 1 Graph of Mucoadhesive strength of acyclovir loaded film

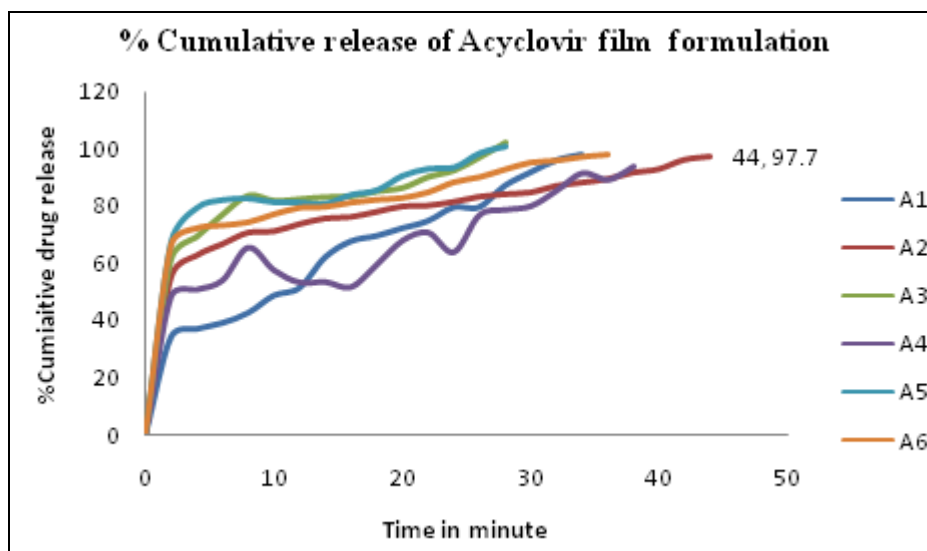


Figure no.-2 Graph of % Cumulative Release of drug from Acyclovir Loaded Films Formulations

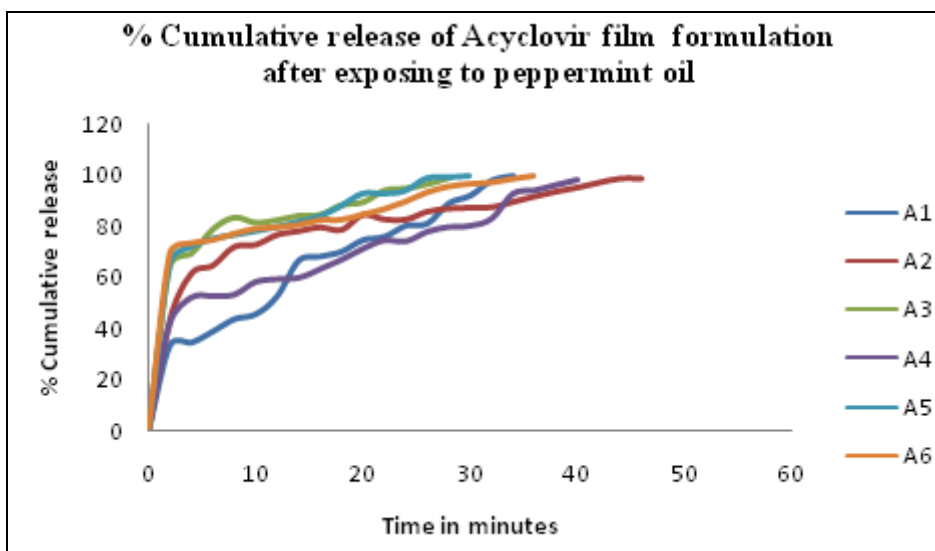


Figure 3 Graph of % Cumulative Release of drug from Acyclovir Loaded Films Formulations after Exposure to Peppermint Oil

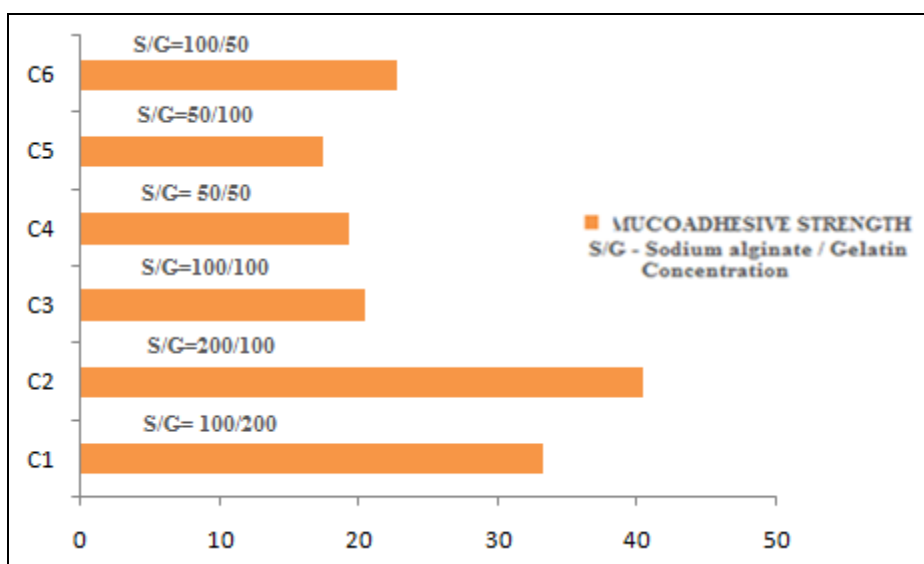


Figure -4 Graph of Mucoadhesive strength of chlorhexidine gluconate loaded films

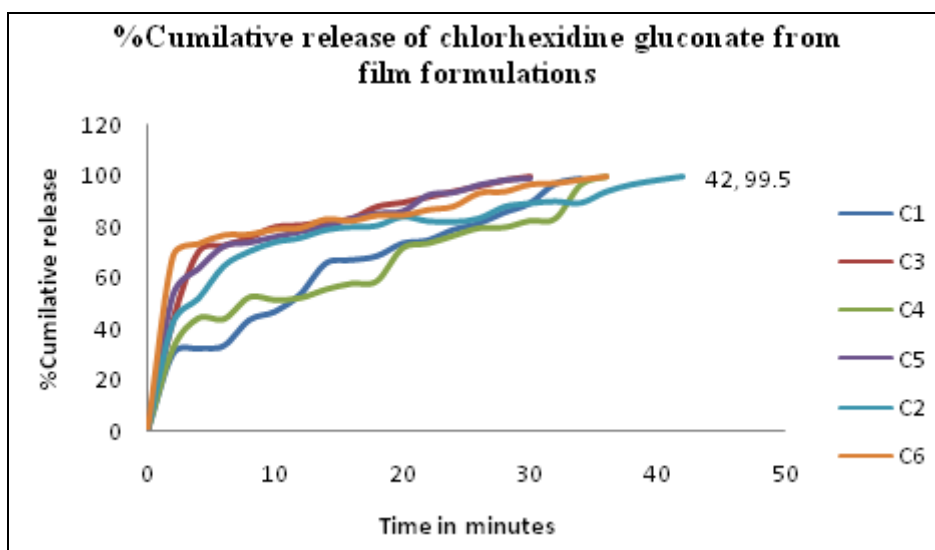


Figure-5 Graph of % Cumulative Release of Drug from Chlorhexidine Gluconate Loaded Films Formulations

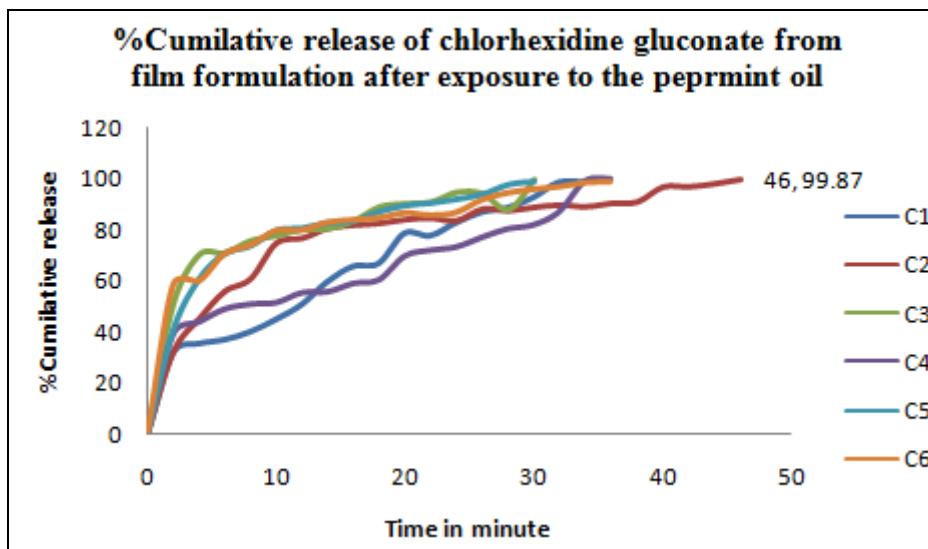


Figure -6 Graph of % Cumulative Release of Drug from Chlorhexidine Glucoante Loaded Films Formulations after Exposure with Peppermint Oil.

Table 1 Evaluation of acyclovir loaded films for physical mechanical and *invitro* residence time

Formula	Thickness	T.S.	P. E.	F.E.	Percent swelling	Surface pH	<i>In Vitro</i> residence Time (min)
A1	0.087 ±0.33	3.31±0.45	99.99 ±0.21	174.34±0.32	82.47 ±0.43	6.4 ±0.32	34
A2	0.081 ±0.44	2.9±0.54	118.94 ±0.98	128.54±0.98	84.54 ±0.75	6.8 ±0.12	52
A3	0.077 ±0.27	2.59 ±0.22	219.94 ±0.32	227.54±0.64	89.65 ±0.64	7.8 ±0.91	28
A4	0.068 ±0.64	3.25 ±0.12	76.90 ±0.74	128.23±0.88	85.540 ±0.83	7.1 ±0.32	26
A5	0.098 ±0.46	3.12 ±0.90	115.78 ±1.32	135.65±0.54	92.43 ±1.38	6.6 ±0.43	30
A6	0.089 ±0.47	4.11 ±1.32	98.43±0.33	171.65±0.82	81.54 ±0.93	7.0 ±0.54	17

$n=3$, ±SD; T. S. - Tensile strength (kg/mm²), P.E. - Percent Elongation, F.E. - Folding Endurance

Table- 2 Evaluation of the acyclovir loaded films for mucoadhesive strength

Formula	Conc. of sodium alginate/ Gelatin (g)	Mucoadhesive Strength (g)
A1	100/200	34.73±0.32
A2	200/100	41.12±0.93
A3	100/100	21.83±0.52
A4	50/50	19.65±0.42
A5	50/100	18.54±0.71
A6	100/50	21.76±0.89

$n=3$, ±SD

Table- 3 Evaluation of Chlorhexidine Gluconate Loaded Films for Physical, Mechanical and *Invitro* Residence Time

Formula	Thickness	T.S.	P. E.	F.E.	Percent swelling	Surface pH	<i>InVitro</i> residence Time(min)
C1	0.097 ±0.85	2.72 ±0.12	128.94 ±0.74	172.94 ±0.23	70.47 ±0.34	7.0 ±0.43	36
C2	0.085 ±0.93	2.92 ±0.73	123.53 ±0.83	127.33 ±0.46	81.99 ±0.29	7.3 ±0.84	47
C3	0.089 ±0.12	1.80 ±0.98	117.6 ±0.73	289.92 ±0.85	88.85 ±0.12	7.1 ±0.83	29
C4	0.073 ±0.43	3.19 ±0.45	107.6 ±0.83	183.94 ±0.23	82.02 ±0.65	6.8 ±0.23	31
C5	0.082 ±0.43	2.6 ±0.54	64.12 ±0.23	154.09 ±0.74	95.43 ±0.55	6.9 ±0.88	34
C6	0.095 ±0.84	2.8 ±0.23	126.78 ±0.12	161.12 ±0.74	81.34 ±0.83	7.1 ±0.85	19

$n=3$, ±SD; T. S. - Tensile strength (kg/mm²), P.E. - Percent Elongation, F.E. - Folding Endurance

Table- 4 Evaluation of Chlorhexidine Gluconate Loaded Films for Mucoadhesive Strength

Formula	Conc. of Sodium Alginate/ Gelatin (g)	Mucoadhesive Strength (g)
C1	100/200	33.12±0.64
C2	200/100	40.32±0.22
C3	100/100	20.43±0.43
C4	50/50	19.23±0.54
C5	50/100	17.32±0.50
C6	100/50	23.12±0.43

$n=3, \pm SD$

CONCLUSION

The films were exposed to the peppermint oil vapours for producing the mouth freshening effect to the films. These films were subjected for evaluated for the *invitro* drug release before and after exposing to the peppermint oil. It was found that there is no significant effect on the drug release profile of the formulation before and after exposing to the peppermint oil. Formulation A2 gives the longer release up to the 44 and 46 minutes before and after exposure to the peppermint oil respectively and C2 gives the longer release up to the 42 and 46 minutes before and after exposure to the peppermint oil respectively.

The formulations A2 and C2 were found to be having better mucoadhesive properties, *invitro* residence and having the longer release of drug from the film formulation. This is due to the presence of higher ratio of the sodium alginate and gelatin as that of sodium alginate may be acting as the sustain release adjuvant with the of presence of HPMC K15.

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