FORMULATION OF A WATER SOLUBLE MUCOADHESIVE FILM OF LYCOPENE FOR TREATMENT OF LEUKOPLAKIA

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ABSTRACT

The new formulation of mucoadhesive film for the treatment of leukoplakia (pre stage of oral cancer) was developed by using lycopene as a model drug, so that higher concentration is achieved in buccal cavity. As the film was intended for local effect, no drug release was performed. Solvent casting method was selected for film preparation. Lycopene is completely water insoluble, while other excipients are completely water soluble, so uniform film formation is a major challenge. Viscosity of vehicle, thickness of the film, tensile strength, bending strength, film swelling and erosion properties, and *ex vivo* mucoadhesion time and force were the criteria to optimize the film formation using propylene glycol as plasticizer.

Keywords: Leukoplakia, lycopene, tensile strength, film swelling property, erosion of film, Mucoadhesive films

INTRODUCTION:

Leukoplakia is a condition in which thickened, white patches form on gums, on the inside of cheeks and sometimes on tongue. These patches can't easily be scraped off. Tobacco, either smoked or chewed, is the main culprit, but irritation can also come from other sources, such as long-term alcohol use. People with compromised immune systems sometimes develop an unusual form of the disorder called hairy leukoplakia. In general, leukoplakia isn't painful, but the patches may be sensitive when you touch them or eat spicy foods. A small percentage of leukoplakic patches show early signs of cancer, and many cancers of the mouth occur next to areas of leukoplakia.[1]

The usual treatment for leukoplakia is to remove the source of the irritation. For most people, stopping smoking or alcohol consumption clears the condition. When this isn't effective or if the lesions show early signs of cancer, your dentist may choose to remove leukoplakic patches using a scalpel, a laser or an extremely cold probe that freezes and destroys cancer cells (cryoprobe). Researchers have investigated the effects of retinoids (derivatives of Vitamin A) that are used to treat leukoplakia. Beta carotene, an antioxidant that's converted to vitamin A in your body, also may completely or partially reduce leukoplakic patches. Lycopene- a potent anti-oxidant has shown promising results in the treatment of leukoplakia.[2]

However, the success of the treatment is dependent of the pharmaceutical dosage form used for its administration in the oral cavity, since it is important to maintain the drug concentration higher than the minimal inhibitory concentration in the salivary fluid, over an extended period of time. Therefore, a mucoadhesive sustained release formulation could be advantageous compared to commonly used conventional pharmaceutical dosage forms, which usually have short residence times at the site of administration. This problem may be resolved using bioadhesive dosage forms, which can improve intraoral administration and reduce the dosage frequency as they are able to produce a sustained release of the drug while remaining adhered to the mucosa surface. Among novel mucoadhesive drug delivery systems, tablets and films are the most prominent. Buccal films offer advantages over adhesive tablets in terms of flexibility and comfort. Moreover, buccal films are also suitable for protecting wound surfaces, which is important when the affection produces ulcerative lesions. The insolubility in water and light sensitivity of lycopene must be considered as a major feature for the formation of a water soluble film. Considering all these parameters, we have plan two methods for solubilization of lycopene. i.e. using surfactant to solubilize lycopene and using a vehicle in which all the excipients and drug is soluble.[3]

MATERIALS AND METHODS:

1. Materials

Lycopene (Gift from BASF Corporation, USA), Povidone K30, Glycerine, Isopropyl alcohol (SD Fine Chemicals Ltd, India), PEG 400 (Laffons Petrochemicals Limited), Carbopol 934 (Acrypol 934,Corel Pharma Chem, India), Propylene Glycol (Suvidhinath laboratory and Sulab's Lab Reagents, India). Other chemicals were of analytical grade and purchased from Loba Chemicals and Qualigens Fine Chemicals, India.

2. Formation of the lycopene loaded water soluble Mucoadhesive film

As lycopene is water insoluble, to make it solubilize in formulation, two methods were selected. The selection of plasticizer should be based on the tensile strength, but here the selection is done on the ease to remove from Teflon coated petri plate. Thus on the basis of ease to remove film from the petri plate, propylene glycol (PG) - 0.25% was selected as a plasticizer.

2.1 Using surfactants

Surfactants selected for the studies are polaxomer, sodium lauryl sulfate, ascorbyl palmitate, docusate sodium, polysorbate 80 and α- tocopherol.

The formation of water soluble Mucoadhesive film by using surfactants is given below:

Dissolve 0.122gm of carbopol 934 in 6 ml of water by heating and stirring on magnetic stirrer. To it add described quantity of surfactant as per given in table 1 and dissolve it with the help of magnetic stirrer. Lycopene is added to the above vehicle and kept for stirring. Now add 0.5 gm PVP K30 and 0.55 gm HPMC K15 and solubilize it with glass rod. Add 12.5 ml of IPA and 0.25% propylene glycol to it. Now the vehicle is kept for degassing and then is spread on Teflon coated petri plate and dried at 60°C for 2 hrs.[4]

Table 1 shows the formulation along with the percentage of surfactant and solubility of lycopene.

Table-1: Formation of film by using surfactants:

PVP:HPMC	Surfactant	Observation
1:1	Poloxamer(0.5%)	Drug remains insoluble
1:1	Poloxamer(1.0%)	Drug remains insoluble
1:1	Poloxamer(1.5%)	Drug remains insoluble
1:1	SLS (0.5%)	Drug remains insoluble
1:1	SLS (1.0%)	Drug remains insoluble
1:1	SLS (2.0%)	Drug remains insoluble
1:1	SLS (3.0%)	Drug remains insoluble
1:1	Ascorbyl Palmitate (0.05%) and Alpha tocopherol (0.025%)	Drug remains insoluble
1:1	Ascorbyl Palmitate (0.05%) and Alpha tocopherol (0.05%)	Drug remains insoluble
1:1	Polysorbate 80 (1.0%)	Drug remains insoluble
1:1	Docusate Sodium (0.5%)	Drug remains insoluble

2.2 Using a Vehicle in which all excipients and drug is soluble

Lycopene provided was 10%, and thus it contains excipients which were causing problem in formation of film. To overcome this, lycopene was first of all taken in water and properly homogenized by cyclomixer. Then the vehicle was kept in centrifuge for 30 min at 25˚C and 25000 RPM. The supernatant was removed and the drug was solubilized by properly homogenizing by cyclomixer.

The drug was first of all solubilized in chloroform and then the vehicle was chosen in which all the excipients having hydrophilic nature are soluble and the drug in chloroform becomes miscible. After making a miscible system, the vehicle is kept for degassing. Then the system was spread on Teflon coated petri plate and kept in dryer at 60˚C for 2 hrs.

A. Take 4 ml water and solubilize the described quantity of carbopol 934 (if given in formula) by heating on a magnetic stirrer for about 90 min. The stirring speed should be optimum, so that no vortex is formed (to prevent air bubble formation). Add described quantity of HPMC E15 and solubilize it with a glass rod. Add 6 ml of iso propyl alcohol (IPA). Stir it on magnetic stirrer for 15 min. Add described quantity of the PVP K30 and solubilize it.

B. Take the desired dose of lycopene and solubilize it in 5 ml of chloroform.

Now add B part to A and mix properly. Add described quantity of propylene glycol and mix well.

The vehicle was then degassed to remove all the air bubble. After degassing, the vehicle is spread on a Teflon coated petri plate and kept for drying at 60˚C for 2 hrs.[5]

Table 2 shows the formulation of film by drug-polymer miscible vehicle system along with time required to dissolve in mouth.

3. Characterization of water soluble mucoadhesive film

The optimized Mucoadhesive water soluble films were subjected to further characterization:

3.1 **Viscosity of the vehicle[6]:** This test is mainly done to check the spreadability on the flat surface. From the viscosity, the vehicle is proper or not can be checked. Viscosity was measured by dial gauge at 50˚C, RT of 29.9˚C, RPM 20, Dial-8, Off vam 10.7% viscometer.

3.2 **Thickness of film[7]:** The thickness of film is important for the brittleness of film. Thickness is depended on the area of the petri plate to be spread. The thickness of each film was measured at five different locations (center and four corners) using a micrometer screw gauge and a mean value of five locations was used as a film thickness. Generally it should be less than 200 μm.

3.3 **Tensile strength[8,9]:** The polymer film was cut into a narrow strip with a width of 10 mm and 30mm in length. The film was placed between the higher and the lower grip of a Chatillon Digital Force Gauge (a model Instron 1121) mounted on a test stand, aligning the long axis of the specimen and the grip with an imaginary line by joining the points of attachment of the grips to the machine. The two grips were kept at a distance of 10mm in a same plane, and the hand wheel attached to the lower gripwas rotated gradually until the film ruptured. The load at the moment of rupture was recorded and tensile strength was calculated using the following equation:

Tensile strength (σ) =force or load (F)/MA

Where F is the maximum load in Newton and MA is the minimum cross-sectional area of the film specimen in square millimeter.

3.4 **Film swelling properties and erosion characteristics**[6,10,11]: They were evaluated by determining the percentage of Hydration and Matrix Erosion or Dissolution (DS). Each film was divided in portions of 4 cm^2 (2 cm×2 cm) and cut, weighed (W1) and immersed in simulated saliva fluid at pH 6.75 for predetermined periods of time (10, 20, 30, 40, 50, 60, 70, 80, 90 min). After immersion, the films were wiped off from the excess surface water using filter paper and weighed (W2). The swollen films were dried at 60˚C for 24 h and kept in the desiccator over 48 h and after drying the weighting was repeated (W3). This experiment was performed in triplicate (n=3).

Percentage of Hydration and Matrix Erosion (DS) were calculated by using the following expressions:

% of Hydration= (W2-W1)/W2×100

DS= (W1-W3)/W1×100

Graphs are plotted on Graph 1 and 2 for swelling and erosion studies respectively.

3.5 ***Ex vivo* mucoadhesion time**[12]**:** The *ex-vivo* mucoadhesion time was performed (n = 3) after application of the films on freshly cut goat buccal mucosa. The goat buccal tissues were fixed on the internal side of a beaker with cyanoacrylate glue. Each film was divided in portions of 4 cm^2 and cut, a side of each film was wetted with 0.1 ml of simulated saliva fluid and was pasted to the goat buccal tissue by applying a light force with the finger tip for 20 s. The beaker was filled with 800 ml of the simulated saliva fluid and was kept at 37˚C. After 2 min, a 150 rpm stirring rate was applied to simulate the buccal cavity environment and film adhesion was monitored during 8 h.

3.6 ***Ex-vivo* mucoadhesion force**[12]**:** *Ex-vivo* adhesion strength was assessed by a simple modification in the weighing balance using goat buccal mucosa. For mucoadhesive measurements, films were cut in portions of 4 cm^2 and pasted on a support, connected to the one part of weighing balance with cyanoacrylate glue and the balanced with a preload. A piece of goat buccal mucosa was glued on a support and kept in a vessel. The free side of the film was wetted with 0.1 ml of simulated saliva fluid and pasted to the goat buccal tissue by applying a light force with the finger tip for 20 s. The vessel was filled with simulated saliva fluid at 37˚C and the measurement was started after 2 min. The maximum adhesive force is the average of three measurements (n = 3).

Table-2: Formation of film by using a vehicle in which all excipients and drug is soluble

B No.	Drug in chloroform	Excipients Ratio				Observation (using PP petriplate)	Mouth Dissolution time (min.)
		PVP K30	CP 934	HPMC E15	Variable Excipient		
1	50 mg/5 ml	1	-	-	1.8 (HPC EF)	Film is difficult to remove from petriplate	40
2	50 mg/5 ml	1	-	1	1 (Na CMC)	Film is formed (precipitate of Na CMC seen in less amount)	40
3	50 mg/5 ml	1	-	1	2.1 (Na CMC)	Film is formed	70
4	50 mg/5 ml	1	-	1.2	-	Film is formed	45
5	50 mg/5 ml	1	0.07	0.8	-	Film is formed	40
6	50 mg/5 ml	1	0.09	1	-	Film is formed	55
7	50 mg/5 ml	1	0.07	1.6	-	Film is formed	65
8	50 mg/5 ml	1	0.09	1.6	-	Film is formed	70
9	50 mg/5 ml	1	0.07	1.1	-	Film is formed	90
10	50 mg/5 ml	1	0.09	1.1	-	Film is formed	90
11	50 mg/5 ml	1	0.07	1	0.4 (Na alginate)	Film is formed	60
12	50 mg/5 ml	1	0.07	-	2 (Na alginate)	Film is formed	65

RESULTS AND DISCUSSION:

On the basis of time required to dissolve in mouth, batch 9 was selected as optimized. The result for the mucoadhesive film for batch 9 is given in table 3.

Rheological property of gel for film formation - Only analysis of the optimized formulation was done which may contribute to understanding the structure of the film, the collected data could also permit evaluation of the behavior of the gels as mucoadhesive pharmaceutical dosage forms. It is widely known that this kind of system has been studied as a potential dosage form in local and transmucosal drug delivery systems. The analyzed results can give information about film structures. Nevertheless,

further studies will need to be carried out regarding mucoadhesive gel formulations. Result is given in table 3.

Table-3: Results of characterization of water soluble Mucoadhesive film

Test	Water soluble Mucoadhesive film Batch 9.
Viscosity	210±15 cps
Thickness	80±10 µm
Assay	97±4 %
Tensile strength	302±65 gm/80 µm thickness
Ex vivo mucoadhesion time	70±5 min
Ex vivo mucoadhesion force	20±2 gm/ cm^2
Dissolution time in mouth	90±5 min.

Each data point represents the mean±S.D. of three replicates.

Film swelling and erosion property:

The effect of composition on the swelling index of the film is shown in Fig.1. The film was not dissolved nor eroded, indicating that the cohesiveness of the polymers is sufficient to guarantee the stability of the system. The film was rapidly swelled within 40 min and thereafter gradually reached a plateau. Since Carbopol is used as a cross-linking agent, it is expected that it can retain more water and higher swelling degree as its concentration increases.

The erosion test of the mucoadhesive film was conducted to evaluate the resistance force of the films in simulated saliva fluid (SSF). The fast erosion of the films in SSF may pose the problems, such as unexpected burst release of drug and short residence time on the buccal mucosa. The reaming percentage of the film expressed as a function of time is shown in Fig. 2.

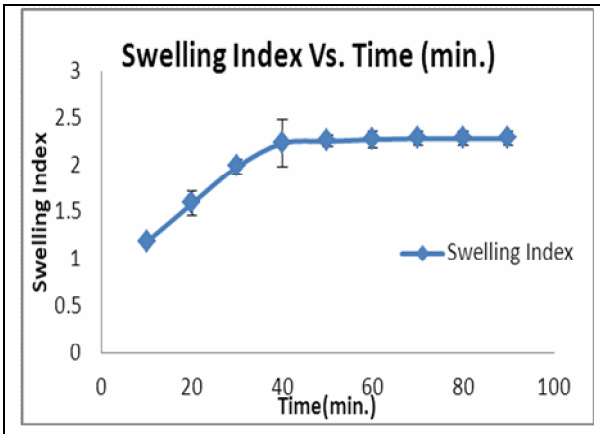

Figure 1: Swelling index of polymeric films. A film of 2 cm×2 cm in size was cut and immersed in SSF (pH 6.2). Each data point represents the mean±S.D. of three replicates.

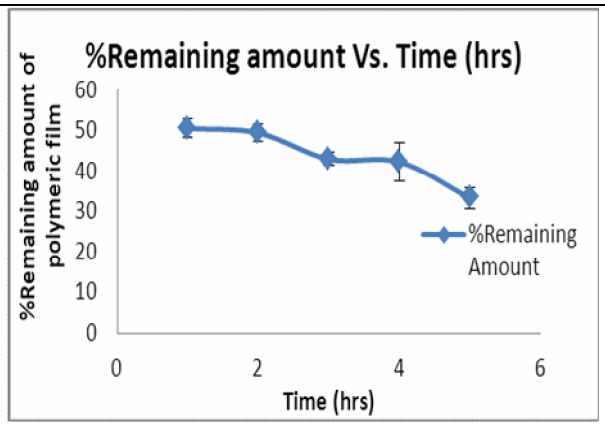

Figure 2: Erosion degree of polymeric films. A film of 2 cm×2 cm in size was cut and immersed in SSF (pH 6.2). Each data point represents the mean±S.D. of three replicates.

The film was eroded quickly between 2 to 5 h and then gradually increased before reaching a plateau. At 5 h, the film made of C:H:P = 0.07:1:1.1 was eroded up to 67%. Though higher concentrations of Carbopol showed greater swelling capability, the erosion rate of the film decreased as Carbopol content in the film increased.

***Ex-vivo* mucoadhesion time:**

The *ex-vivo* mucoadhesion time is important for mucoadhesion property of film. If the mucoadhesion time is more, than the drug absorption will be more through that site. After the detachment of the film, the drug will be absorbed in the GIT. Thus for the drugs which are having high first pass metabolism, drugs degraded in GIT and drugs which are causing GI irritation, the mucoadhesion time should be more. The mucoadhesion time of the optimized formula was 70 min. Result is given in table 3.

***Ex vivo* mucoadhesion force:**

The *ex vivo* mucoadhesion force is important when the person is chewing something or is drinking water. At this time his tongue can detach the film, and thus the pharmacological action will not be observed as desired. Thus as the mucoadhesion force is more, it's difficult to detach from the mucosa. The *ex vivo* mucoadhesion force of optimized formulation was found to be 20 gm/cm^2. Result is given in table 3.

CONCLUSION:

The main advantage of this formulation is that it contains a lower drug dose, sufficient for therapeutic effect as it is located directly on the site of the patch, if compared to traditional systemic therapies. Moreover, this buccal film is very tolerable and comfortable because it is non-irritant and may be preferred over adhesive tablet in terms of elasticity, flexibility and capability to protect the wounded or inflamed surfaces. The film is having high mucoadhesion force, and thus difficult to remove from site by tongue. The time required to dissolve is also high compare to other formulations and thus, the concentration of lycopene can be achieved in higher amount. The film will also have advantage as it doesn't require water for intake.

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