Current Trends in Biotechnology and Pharmacy
Vol. 17(2) 796 - 807, April 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2023.2.18

Development and Optimization of Curcumin and Lycopene Mucoadhesive Buccal Patches Using Response Surface Methodology

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Abstract

Buccal patches are the ideal mode of delivery for the systemic release of drugs because they give enhanced bioavailability by bypassing hepatic first-pass metabolism and allowing through the jugular vein, direct access to the circulatory system. The present research work is on the development and optimization of a mucoadhesive buccal patch of curcumin and lycopene for embattled release in oral cancer therapy. Using polymers like ethocel and methocel, buccal patches were produced by the solvent-casting method. Buccal patches were evaluated by disintegration time, folding endurance, % drug release, surface pH study, swelling study, mucoadhesive strength and stability studies. The developed buccal patches were evaluated for cytotoxicity in KB cell line using MTT assay. Folding endurance of formulation was found to be 167.66. The disintegration time of buccal patch was found to be 3.34 minutes. Thus, it can be said that the oral cancer treatment using the buccal patch formulation that has been created may be innovative.

Keywords: Buccal patches, Curcumin, Lycopene, Oral cancer, MTT assay.

Introduction

The improvement of controlled medication deliverance systems, either through, the oral mucosa using mucoadhesive polymers has gotten a lot of importance in current years. For local or systemic distribution, the buccal tablets delivery routes are becoming increasingly frequent. (1) The oral cavity, in scrupulous, seems to provide the unique profit of improved availability, a precise yet comparatively penetrable epithelial barrier for delivery of drugs, unidirectional drug instability, fast and simple exclusion of the pharmaceutical formulations leading suggestion, efficient drug permeability, and evade of liver function first-pass metabolism, all of which contribute to enhanced bioavailability and patient compliance.(2) The buccal route has been utilized for both local drug delivery and systemic drug delivery in a variety of dosage form forms, including sticky gels, capsules, films, patches, pastes, mouthwashes, and sprays.(4) Localized aphthous ulcers, gingivitis, periodontal diseases, and xerostomia are all treated using mucoadhesive dosage forms. Due to their outstanding flexibility and simplicity of usage, mucoadhesive tablets appear to be the most popular dose form for buccal administration of medication. They can be used on the cheek, gums, lips, palate, and other parts of the oral cavity.(4)

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Oral cancer was among the most recurrent and invasive cancers, accounting for 5% of all cancer deaths globally. Oral cancer is treated with radiotherapy, chemotherapy, and surgical excision, all of which have serious side effects for patients. These unfavourable treatment side effects are caused by the therapeutic drugs' nonspecific activity.(5) Chemotherapy's side effects have been decreased through developments in drug delivery technologies during the last four decades. In addition, the advancement of nanotechnology in anticancer therapy has aided in the development of new diagnostic and treatment procedures. The most desirable treatment for oral cancer is targeted therapy, which focuses on particular site delivery and therefore to reduces side effects and systemic toxicity. The solubility, stability, and bioavailability of therapeutics supplied by nano delivery systems made of polymers were improved, accumulating even inside tumor cells.(6) Oral strips was designed to be applied locally to the tongue or buccal cavity. When compared to traditional measures, mucoadhesive buccal patches provided advantages.(7) Buccal patches provide the advantage of increased residence duration and drug release owing to the narrow holding time of oral gels in the mouth cavity.(8)

One of the most widely utilized methodologies in this design and optimization of targeted drug release is response surface methodology (RSM). The methodology is based on ideology of design of experiments, includes the exploit several forms of trial designs, the development of polynomial numerical equations and the mapping of retort more than experimental province in order to resolve the best formulation (s). The process needs very petite experimentation and time, and it has proven to be significantly more effective and cost-efficient than traditional dosage form formulation procedures. Numerous RSM design types are available for the formulations' quantitative optimization, including the central composite design, 3×3 factorial design, and Box-Behnken design, etc.(9,10)

Polymers are frequently used in contemporary pharmaceuticals technology, and they have been crucial to the advancement of medication delivery. Polymers are used as carriers in targeted therapy, allowing for synchronized drug distribution while also lowering medication bitterness.(11) The nature of ethyl cellulose and methylcellulose is hydrophobic. It's a free-flowing white to light powder that's frequently employed in the production of controlled drug release. Ethyl cellulose and methyl cellulose are suitable to be used in tablets, oral capsule, ophthalmic or vaginal formulations, and topical therapies since they have few side effects.(12) A number of natural chemicals built-in the daily diet (or should be present) have been demonstrated to have chemo preventive properties. (13) Numerous biological processes are carried out by polyphenols, including carotenoids (lycopene, carotenoid epigallocatechin-3-gallate, resveratrol, ellagic acid, quercetin, and curcumin), minerals (selenium, zinc), C, D, and E vitamins. Cell growth inhibition, autophagy activation, growth factor decreases, signalling systems that control angiogenesis or tumour growth as well as the suppression of inflammation are only a few of the molecular mechanisms that are involved, have been proven to prevent cancer beginning and progression.(14-18) Aim of this work is development and characterization of buccal patches containing curcumin and lycopene encumbered in ethocel and methocel polymer.

The goal of this study was to characterize buccal patches in assessing their efficacy as targeted drug delivery for oral cancer therapy. A Central mergeddesign was used as computer-aided optimization technique. The quantity of the release retardant served as one of the study's independent variables, polymer-ethocel (X1) and propylene glycol (X2) and carbomer(X3). The dependent variables study was the disintegration time (Y1) and folding endurance (Y2) and % drug release (Y3).

Materials and Methods

Materials and reagents

Curcumin and lycopene were procured from Sigma Aldrich, India. Ethocel and methocel were purchased from TCI chemicals, India. Carbopol and Propylene Glycol were procured from SRL chemicals, India. Millipore water was used in this work. The remaining substances were all of analytical grade.

Development of drug loaded buccal patch

A petri dish was used to make a series of buccal patches using the solvent casting process. The patches were made up of different percentages of ethocel and methocel, which were dissolved in ethanol and then mixed with Curcumin for 30 minutes. Ethanol: water (1:1) Lycopene solution was added to the prior solution and agitated continuously for additional 30 min. The carbopol solution was made by boiling Millipore water with continual stirring before adding it to the polymeric solution. With intermittent shaking, the determined amount of propylene glycol was included to the solution. The casting solvent was therefore reduced to 30ml and agitated for 24 hours to ensure complete dissolution, before being placed in a vacuum desiccator to eliminate any remaining air bubbles. The rate of evaporation was controlled and prevents patch blistering. Then the solution was cast in a glass petri dish covered with an inverted glass funnel. At room temperature, the solvent was allowed to fade away for 24 hours. The dry patch was isolated, sliced into 2x2 cm square portions. It was covered in aluminium foil and kept in a desiccator.(19-21)

Preparation of buccal patch using experimental design

Utilizing central composite design, the formulation was improved. The selected factors were Ethocel (0.83-1.67gm, factor A), Propylene glycol (4.16-5.84 ml, factor B) and Carbomer 0.66-1.34 gm, factor C). The response studied was Disintegration time, foldi8ng endurance and %drug release. The buccal patch was prepared using central composite design (Design expert software version 12.0). The design expert programme displayed the complete 20 runs. The studied response and variables were shown in Table 1.

Table 1: Central composite arrangement for factors and responses

STD	Run	Space type	Factor A: Ethocel (gm)	Factor B :Propylene glycol (ml)	Factor C: Carbomor (gm)	Response 1 Disintegtaion time (Sec)	Response 2 Folding Endurance	Response 3 Drug Release (%)
15	5	Center	1.25	5	1	3.3	206	32.7891
18	8	Center	1.25	5	1	3.3	206	32.7891
19	9	Center	1.25	5	1	3.3	206	32.7891
20	11	Center	1.25	5	1	3.3	206	32.7891
17	13	Center	1.25	5	1	3.3	206	32.7891
16	15	Center	1.25	5	1	3.3	206	32.7891
10	3	Axial	1.956	5	1	6.45	215	41.0472
14	4	Axial	1.25	5	1.57	4	214	43.1234
9	12	Axial	0.543	5	1	6.45	212	42.0240
13	16	Axial	1.25	5	0.428	3	205	33.4518
12	18	Axial	1.25	6.412	1	2.3	202	49.4505
11	20	Axial	1.25	3.587	1	2.6	198	45.123
7	1	Factorial	0.83	5.84	1.34	4.0	209	52.8442

4	2	Factorial	1.67	5.84	0.66	3.0	204	42.8248
5	6	Factorial	0.83	4.16	1.34	3.3	207	92.9218
1	7	Factorial	0.83	4.16	0.66	2.45	203	40.0776
6	10	Factorial	1.67	4.16	1.34	3	208	53.1674
2	14	Factorial	1.67	4.16	0.66	2	200	39.4123
8	17	Factorial	1.67	5.84	1.34	5.1	211	43.9561
3	19	Factorial	0.83	5.84	0.66	2.15	201	18.2612

Table 2: Statistical parameters obtained from ANOVA

Responses	F-value	P-Value	Adjusted R^2	Adequate precision
Disintegration time (DT)	5.11	0.0089	0.9908	8.9078
Folding Endurance	16.02	0.0001	0.8768	14.8771
Percentage Drug Release	4.98	0.0097	0.9534	9.5887

Evaluation of prepared buccal patches

Study of drug–polymer interactions

Fourier Transform Infrared Spectroscopy

Nicolet 520P FT-IR spectrometer wavelength in the range of 4000-500 cm^{-1} was used for the study of drug and polymer interaction. KBr pellet method was used for sample preparation.

Differential scanning Calorimetry analysis

Differential scanning Calorimetry (DSC) analysis was used to analyze the Drug-polymer interactions. DSC is the one of the thermal analytical techniques. Perkin Elmer Differential Scanning Calorimeter (DSC 6000 – Perkin-Elmer) was used to analyze drug and polymer samples at a heating tempo of 10ºC / min under air environment and an air was flushed at a flow rate of 5 mL/ min.

Folding endurance and thickness

It was done to demonstrate the effectiveness of the softener and assess the tensile of the patch made with various polymers. Folding endurance is nothing but the number of folds needed to break any polymer patch. The exact folding endurance was tested manually by repeated folding of a tiny (2 x 2 cm) section of the film in same spot until it was broken. The folding endurance of patch was determined by how many times it was folded in the same position without breaking or cracking the patch. The same process was repeated for all 3 formulations.

In vitro disintegration time

The interval at which a film disintegrates when it comes into touch with water or a buffer is known as the disintegrating time. A buccal patch of 2 x 2 cm was placed inside the disintegration device, which was kept at 37±0.5°C and filled with pH 7.4 buffer. The time it took for the patch to disintegrate was recorded.

Determination of in vitro drug release

Buccal patch preparations were characterized in vitro using Franz diffusion cells. This is a reliable technique for estimating drug transport from topical formulations through the skin. With a synthetic cellophane membrane and 30.0 ml of phosphate-buffered saline (pH 7.4) injected into the receptors section of the diffusion cell, studies on in vitro drug release were done. A patch of 2 x 2 cm was cut and stitched on. The

membranes in the client compartment received it. The cellophane membrane was then evenly widened. The assembly was continuously kept at 50 rpm at 37.0 2.0°C. In order to keep the receptor phase volume at 30 ml, samples (1.0 ml aliquots) then were set aside at proper times (0, 1, 2, 3, 4, 5, 6, 7, 8, and 10 hr) and supplied with the medium. The patches that contained curcumin and lycopene underwent spectrophotometric analysis at wavelengths of 427 nm, 440 nm, and 375 nm, respectively.

Weight variation

For weight uniformity, all prepared patches were individually weighed. The weight was determined using a weighing balance for analysis (Shimadzu AX 200, Kyoto, Japan). Individual weights were compared to the state median.

Uniformity of drug content

The patches were trampled in a mortar and pestle with water/acetone solvent to check for drug content homogeneity (1.5cm1.5cm dimension) and water or alcohol as a patch solvent containing curcumin and lyopene. The content was then filtered by using 0.45 m syringe. Then the solutions were diluted with simulated saliva. Samples were analyzed at a maximum wavelength of 426 and 260.6 nm (Shimadzu, Japan, UV 1800 spectrophotometer) by using solvent plain. Using the calibration curve, the drug substance was calculated.

Surface pH determination study

pH at the surface of the patches were measured at intervals of 1, 2, 3, 4, and 5 hours by inserting them into distilled water in 10 ml glass tubes. Close to the surface with digital pH meters tip allowing the patch to settle. Then it was equilibrating for 1 minute before the recording begins and this determination was carried out in three times.

Swelling study

On petri dishes, 25 mL of artificial saliva was kept at room temperature. The patch starting weight (W1) was measured. The films with moisture on their surface were gently removed with filter paper after 60 minutes. The swollen patches were weighted (W2), and the formula below was used to estimate the swelling percent.

$$\text{Swelling index} = (W2-W1)/W1\times100$$

Mucoadhesive study of buccal patches

PBS (pH 6.6) was used as the moistening agent, and the modeling substrates were recently excised pig buccal mucosa that was purchased from a slaughterhouse. For a total of 10 minutes, a horizontally positioned crushed buccal patch was “sandwiched” between two or three layers of clipped model tissue substrates. A constant mass of 50 gm was then placed on top. The bio adhesive strength was evaluated using an Extech 475040 Force Gauge Meter in terms of the force needed to dislodge the patch from the animal buccal mucosa (Extech Instruments Corp). When the patches were removed from porcine buccal mucosa, upward tension was congested and the force reading was obtained.

Morphology of buccal patches

Morphology of the developed patches were analyzed using scanning electron microscopy (SEM; JEOL JMS-6390 apparatus). In order to ensure the electron beams strong conductivity across the samples, carbon coatings were applied to them. As the electron beam traverses the sample, signals in raster images that contain information about the sample’s surface topography and other characteristics are produced.

Stability studies

For the preparation optimized patch, stability studies were conducted over 180 days. Patch was preserved in the incubator, that was maintained at 37 ± 0.05°C and 75 ± 5 RH, for stability studies. The prepared patch medication content and physical appearance were exam-

ined after a 30-days interval. The procedure specified in the section was followed to determine the drug content.

Cytotoxicity assay

Optimized buccal patch 2x2cm was prepared, sterilized by ultraviolet radiation and then incubated with 1ml of DMEM medium at 37°C with 5% CO_2 for 24hrs. Sample solution, from these serial two-fold dilutions (6.25 – 100 µg) was prepared. From NCCS, a KB cell line was purchased. In a medium (DMEM) enriched with 10% inactivated Fetal Bovine Serum (FBS), 100 IU/mL penicillin, and 100 g/mL streptomycin, stock cells were cultivated until confluent. The growing environment consisted of a humidified atmosphere with 5% CO_2.

Using appropriate media containing 10% FBS, the monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL.

About 100 µL of diluted cell suspension (1 x 10^5 cells/well) was added to each 96 well microtiter plate. A partial monolayer was formed after 24 hours. The monolayer was rinsed with medium once after the excess was flicked off. The partial monolayer in the microtitre plate received 100 L of test samples at various concentrations. The plate was subsequently incubated for 24 h at 37 °C in a 5 % CO_2 environment. (22,23)

Results and Discussion

Drug polymer incompatibility study

The FT-IR and DSC studies were used to determine drug excipient compatibility.

FT-IR

The FT-IR spectrum analysis of the drug and polymer mixture was used to identify any physical or chemical modifications of the drug's properties. Curcumin's FTIR spectra were found to be in the following ranges: -OH group: 3382.67 cm^{-1}; aromatic CH group: 2969.72 cm^{-1}; C=C and C=O group: 1510.60 cm^{-1}; and C-O-C stretching: 1205.44 cm^{-1}. Lycopene's FTIR spectra were found to be in a range between 282.959 cm^{-1} and 2005.58 cm^{-1} for the CH group and 1512 cm^{-1} for the C=C group. When comparing the spectra of the created films with the original peak of the drug and polymers, it was found that there was no substantial change, indicating that there was no drug-polymer interaction. The IR chromatogram was shown in Figure 2. The final formulation FTIR spectra shown many peaks indicating that the chemical structure of the drug was conserved with efficient loading into the formulation. Curcumin and lycopene had no chemical interaction with the physical mixtures of polymers (ethocel, methocel, carbomer, propylene glycol) utilised in this buccal formulation study.

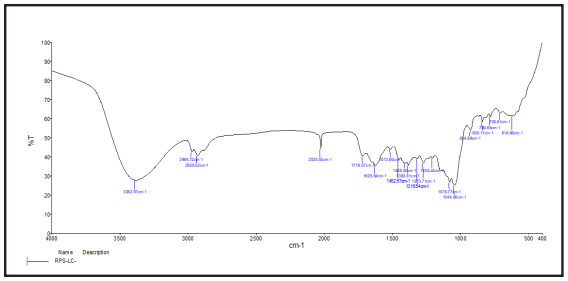

Fig. 2: IR spectrum for Physical mixtures

DSC

DSC thermogram of curcumin, lycopene and polymer was studied. It was found that curcumin shown endothermic peak at 184.81°C, Lycopene shown endothermic peak at 153.11°C, ethocel shown exothermic peak at 348.98°C, methocel shown exothermic peak at 306.22°C, Carbomer showed endothermic peak at 246.70°C. Physical mixture pure drug and polymers showed endothermic peak at 184.81°C, 323.5°C, 153.11°C, 348.98°C, 306.22°C and 246.70°C. This suggests that there were no interactions between drug and polymer as well as excipients used in buccal patch formulations. The DSC thermogram was shown in Figure 3.

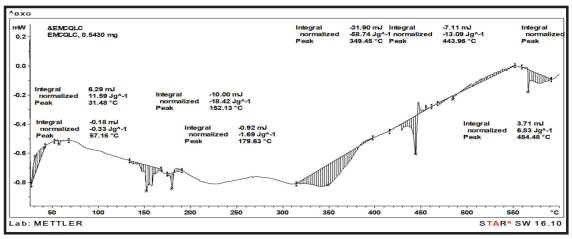

Fig. 3: DSC Thermogram for formulated buccal patch

Evaluation

In this research work, buccal patch of curcumin and lycopene were prepared with polymer combination of ethocel and methocel by using solvent casting technique. A total 20 number formulation were prepared using a central composite design (Table 1). Flat yellow non-transparent patches were obtained and cut into 1.5 cm squares on either side.

Statistical optimization

Disintegration time, folding endurance and % drug release obtained by the various levels of 3 independent variables, ethocel - Polymer, propylene glycol - plasticizer and carbomer – Film thickening agent, were subjected to multiple regressions to yield the final equation.

Disintegration time (DT)

$Y = +3.33 + 0.0940 * A + 0.2193 * B + 0.5478*C+0.3375*AB+0.0500*AC+0.625*BC + 0.8833 * A^2 - 0.5221 * B^2 - 0.5109*C^2$(+0.3375*AB) + Sign of coefficients indicates positive effects of polymer and plasticizer concentration on DT. The model is significant, according to the F-value of 5.11 percent. An F-value this large could only happen to owe to noise 0.89 %. P-values under 0.0500 indicate that the model terms are important.

Folding endurance

$Y=+206.08+0.58918A+1.01*B+3.09*C+0.8750*AB+0.3750*AC+0.3750*BC+2.15*A^2-2.63*B^2+0.7325*C^2$(+0.8750*AB) + Sign of coefficients indicates positive effects of polymer and plasticizer concentration on folding endurance. The model is suggested to be significant by the F-value of 16.02 percent. Only 0.01 percent of the time is it possible for noise to cause an F-value this large. The modelling terms are considered significant when the P-value is less than 0.0500.

Percentage drug release

$Y = +32.63-2.03*A-4.42*B+8.68*C+7.01*AB-9.07*AC-3.89*BC+4.26*A^2+6.15*B^2 +2.97*C^2$(-9.07*AC) negative sign of coefficients indicates negative effects of polymer and thickening agent concentration on percentage drug release. The F-value of 4.98 % suggests that the model is significant. An F-value this large might be caused by noise only 0.97 percent of the time. Model terms that have P-values less 0.0500 are considered significant.

The irrelevant terms (P> 0.05) were eliminated from the models to use the backward elimination method to produce an easy-to-understand and practical model. Although in statistical modelling the adjusted R2, which takes the number of linear regression variables into account, is commonly chosen, R2 always drops when a linear regression variable is removed. The experimental data demonstrated a satisfactory fit to second order polynomial equations because the corrected R2 values were well within the allowed bounds of R2 0.90. All of the reduced models had p values below 0.05, indicating that they were all significant models. The signal to noise ratio is measured by the value of appropriate precision. A ratio higher than 4 is desirable. The ratio was found to be in the range from 8.90-14.87which indicated on adequate signal. Hence the model was significant.

Response surface plot

It is highly helpful to investigate the interaction effects of these factors on the response using 3D surface plots for all response variables. Figure 1 shows 3D response surface plot. From this design optimized formulation was found. The optimized conditions of ethocel 1.67 gm, propylene glycol 5.84 ml and carbom-

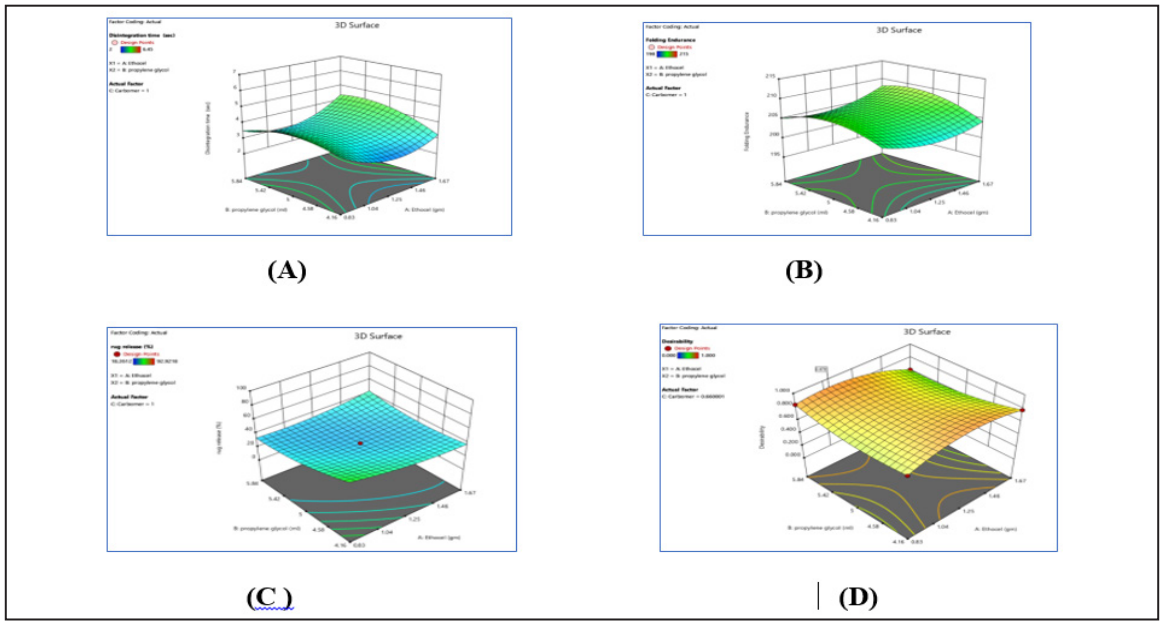

Fig. 1: 3D response plot for (A) Disintegration Time (B) Folding Endurance (C) Percentage Drug Release (D) Desirability function

er 1.34gm used. According to the later value of D, the projected reaction values were DT = 3.34sec, Folding endurance = 167.66, and percent drug release 73.05 %.

Disintegration time (DT):

Decrease the value of DT with the increase the con of plasticizer. At high drug polymer ratio the DT is more. The higher DT is recorded at low con of plasticizer and high drug polymer ratio. But excessive amount of polymer increases the film becomes brittle. Both factors would be considered while controlling the DT. Polymer and Plasticizer at elevated concentration would generate patch which fulfil a prerequisite for rapid disintegration.

Folding endurance

The plasticizer is in charge of giving the film flexibility, while the film forming (Polymer) ensures enough strength. Therefore, a buccal patch with desirable prominence would result from a suitable combination of both of these elements. The findings showed that polymers and plasticizers have a beneficial effect on folding endurance. The poly nomial equation suggested the concentration of plasticizer has a hopeful influence of the folding endurance. Because plasticizers relax linear polymeric chains, presumably by creating hydrogen bonds that increase flexibility, they are considered to be the key contributing element for folding endurance.

% Drug release

Decreases in the value of the percent drug release with increases in the amount of the film thickening agent and rises in the percent drug release with increases in formulation's polymer content. The highest percentage drug release recorded at low drug polymer ratio and high thickening agent concentration. When con of polymer increases then drug was decreases since drug remains inside the matrix of polymer.

The desirability function of the Derringer D is the geometric mean, weighted average, or the average of the different popularity functions. Desirability has uses D accepts values between 0 and 1. Weight values can range from 0.1 to 10, with weights below 1 denoting less relevance

and weights above 1 denoting higher importance for the aim. The table listed the requirements for each response's optimization.

It was evident from the preceding figure that a particular set of coordinates produced a high Desirability value (D= 0.879) were Ethocel 1.06 gm, Propylene glycol 5.84ml and carbomer 0.66gm used. The predicted response values corresponding to the later value of D were DT= 2 sec, Folding endurance = 198, % drug release 18.26%. By processing the experimental results under ideal circumstances, the model's prediction effectiveness was verified. Within 1-6 %, it was discovered that the observed and experimental differences were in good agreement.

Physical evaluation of buccal patches

Thickness and folding endurance

The thickness of the patchwork film increases with the weight of the polymer, vice versa. Weight of the batches' films, 260.56 mg. Patch film thickness: As the polymer concentration rises, so does the patch film thickness. The formulation's patch thickness ranges between 0.18- and 0.22-mm. Low standard deviation scores indicate that the movie is physically equal. The folding endurance gauges a film's resistance to rupturing. After manually measuring the folding resistance, it was discovered that the film's folding resistance increases along with the polymer concentration. The film's folding endurance was discovered to be 167.66.

The films' folding endurance values were discovered to be quite favorable, and as a result, they displayed good mechanical and physical properties. The film's surface pH was discovered to be between 6.06 and 6.07 for buccal patch formulations. Table 3 presented the outcomes. All of the films' surfaces had pH values that fell within the range of salivary pH. The pH of the surfaces of all formulations showed no discernible variation. Since the observed surface pH values of all of the formulations were found to be somewhat neutral, they should be quite comfortable and less likely to irritate the buccal mucosa.

The mucoadhesive strength of buccal patches found to be 6.01 g. The disintegration time of drug loaded buccal patches was found to be 3.34minutes. The Patches disintegration time was within the limit. Hence it was easily dissolved in saliva.

Table 3: Physical evaluation of optimized drug-loaded buccal patches

Evaluation parameter	Result
Disintegration time (min)	3.34
Folding endurance	167.66
Weight (mg)	260.56
Thickness (mm)	0.18
surface pH	6.06
Mucoadhesive strength (g)	6.01
Content uniformity (%)	97.95769

SEM analysis of buccal patches

Figure 4 depicts the cross split of buccal patch compositions seen below a scanning electron microscope. The composition's cross-section inside the patch showed a consistent, non-porous structure. Non-homogeneous texture is visible at greater magnifications was clearly obvious. On evaporation, it is reasonable to suppose that the dissolved curcumin may be affected by the type of solvent used. Micron-sized aggregates have formed as a result of the precipitation. Sizes and are spread throughout the polymer solution. Any there was no texture non-uniformity found suggesting that the medicine has been distributed properly throughout the matrix of polymer.

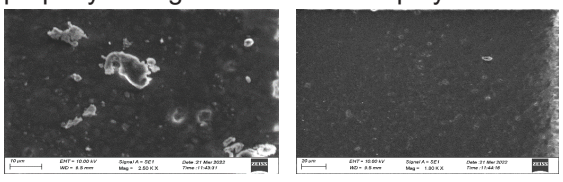

Fig. 4: Scanning electronic microscope image for optimized buccal patch

in vitro drug release study

In-vitro release of curcumin and lycopene encumbered optimized buccal patches was performed in pH 6.8 phosphate buffer. The results were shown in Figure 5 and Table 4. The *invitro* drug release profiles of buccal patches, which is containing ethocel, methocel, propylene glycol and carbomer polymers in the ratio of 1:1,1:2, 2:1, 1:3 and 3:1. The most significant factor disturbing the velocity of drug liberate from the buccal tablets with the drug and polymers ratio. In all the formulations the drug release was ranging from 73.5% to 95.6%.

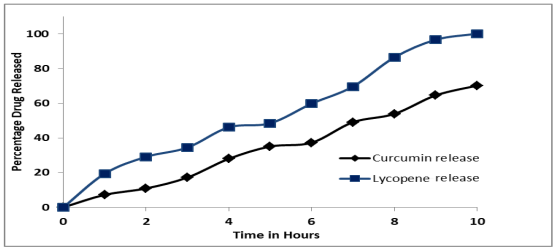

Fig. 5: *In vitro* drug release study of buccal patch

Table 4: Evaluation of the drug-loaded optimized buccal patch's in-vitro drug release

Time (Hours)	Curcumin release	Lycopene release
0	0	0
1	7.2	19.4
2	10.9	29.1
3	17.2	34.5
4	28	46.2
5	35.1	48.5
6	37.3	59.8
7	49.1	69.7
8	53.9	86.5
9	64.6	96.7
10	70.2	100.2

Surface pH

The created patch's surface pH was carefully calibrated to account for potential irritability during in-vivo tests.

Stability studies

The optimized buccal patch samples were taken after 30 (1 month), 60 (2 months), 90 (3 months) days. For 90 days at 40°C±5°C and 75% RH, the tensile strength, drug content, and percentage of drug release of the sample (optimized) were evaluated. Reports were displayed in Table 5. Stability studies for the formulations of patches were conducted for 90 days. The prepared patches displayed maximum stability with no noticeable physiochemical alterations after defined intervals of 30 days

Table 5: Stability study report of drug-loaded optimized buccal patches

Evaluation parameter	After 30 days	After 60 days	After 90 days
Colour and appearance	No change	No change	No change
% drug content (Curcumin)	96.7±1.66	95.3±1.42	93.2±0.98
% drug content (Lycopene)	97.59±1.98	95.22±1.25	94.3±1.87
% drug release (Curcumin)	70.2±2.69	68.6±2.01	67.4±1.22
% drug release (Lycopene)	100.2±2.55	99.8±1.46	95.1±1.37

Cytotoxicity study (MTT assay)

MTT assay used to measure the cell viability. Cell viability of prepared buccal patches was tested in KB cell lines at various concentrations (Figures 6 and 7). At varying concentrations of 12.5, 25, 50, 100 and 200µg/ml, the prepared buccal patches showed cell viability was reduced in a dose dependent method with a significant difference between the control and test groups. When the drug concentration was increased from 12.5 to 200µg/ml, cell diffusion was improved. The IC50 value of the test sam-

ples for the production of curcumin and lyopene loaded buccal patches was found 2.888.

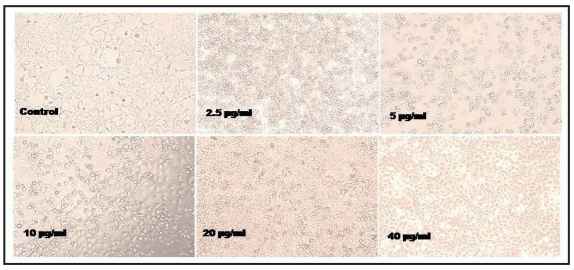

Fig. 6: Cytotoxicity study for different concentration

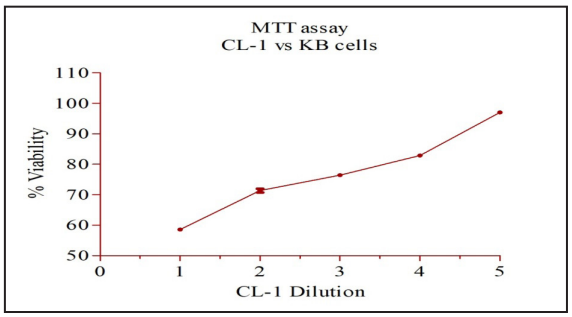

Fig. 7: Cell viability (%) of drug loaded buccal patch formulation

Conclusion

According to the study's findings, in-vitro drug release pattern of the patches were sufficient and good enough ensuring good bio-availabilty of the drugs. The optimized formulation CL5 had good mucoadhesion, were irritation-free, and released the medication entirely by a diffusion process and this study also recognized the relevance of using curcumin and lycopene combination for formulating buccal patch. As a result, the perspectives and approaches of buccal mucoadhesive patches can be considered as a unique treatment for oral cancer.

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