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FORMULATION AND INVITRO EVALUATION OF MUCOADHESIVE BUCCAL PATCHES OF CURCUMIN, LYCOPENE, AND QUERCETIN

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Abstract

The goal of this study was to develop a mucoadhesive buccal patch containing curcumin, lycopene, and quercetin for targeted administration in oral cancer. Buccal patches were made using ethocel and methocel polymers and the solvent casting process. Buccal patches were evaluated by disintegration time, folding endurance, % drug release, surface pH study, swelling study, mucoadhesive strength and stability studies. The developed patches were evaluated for cytotoxicity in KB cell line using MTT assay. Folding endurance of formulation was found to be 217.33. Formulation disintegration time was observed to be 6.51 minutes. As a result, it may be concluded that perhaps the developed buccal patch formulation could be a novel treatment for oral cancer.

KEYWORDS: Buccal patches, curcumin, lycopene, quercetin, oral cancer, MTT assay.

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INTRODUCTION

Oral cancer was among the most frequent 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.[1] 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. Therapeutics administered using nano delivery systems comprised of polymers have demonstrate to improved bioavailability, stability, solubility, and accumulation inside the tumor cell.[2]

In recent decades, there has been an increase in a lot more in developing controlled drug release delivery methods, either through the oral mucosa or through mucoadhesive polymers. Buccal mucoadhesive drug delivery systems are becoming more popular for locally or systemically distribution.[3]The oral cavity, in particular, seems to provide the unique benefits of increased availability, a rigorous yet comparatively penetrable epithelial barrier for delivery of drugs, All of these factors lead to better bioavailability and patient compliance: progressive drug flux, elimination of pharmaceutical formulations, enhanced drug permeation, and bypass of first-pass hepatic metabolism.[4] In a variety of dosage form types, including adhesive gels, capsules, film, buccal patches, ointments, mouthwash and sprays, the buccal route has been utilized for both topical and systemic drug delivery. Localized aphthous ulceration, periodontitis, and xerostomia are treated with mucoadhesive dosage forms. Due to their excellent flexibility and simplicity of application, mucoadhesive tablets appear to be the most extensively utilized route of administration for buccal drug distribution. They can be used on the lips, teeth gums, cheeks, and palate, among other mouthparts. [6]

Oral strips were designed to be applied locally to the tongue or buccal cavity. When compared to traditional procedures, mucoadhesive buccal patches provided advantages.[7] Buccal patches have the advantage of increased residence time and medicine release due to the limited duration of buccal patches in the mouth cavity.[8]

Polymers are widely used in current drug delivery applications, and they have played an essential role in drug delivery progression. In targeted therapy, polymers are utilized as carriers, allowing for controlled drug distribution while also reducing medicine bitterness.[9]The nature of ethylcellulose and methylcellulose is hydrophobic. It's a free-flowing white to light powder that's commonly employed in the controlled drug release delivery system manner. Ethyl cellulose and methyl cellulose are safe to be used in oral solid preparation like tablets and capsules, vaginal and ophthalmic preparation, and local therapies since they have few side effects.[10]A number of natural chemicals included in the daily diet (or should be present) have been demonstrated to have chemopreventive properties. Polyphenols (e.g., curcumin, resveratrol, quercetin, epigallocatechin gallate), carotenoids (carotene, lycopene), vitamins (D, E, C), and minerals (zinc) all play a role in biology. Several molecular processes have been shown to deter or reverse cancer stage initiation and cancer cell progression, including cell growth inhibition, apoptosis/autophagy activation, growth factor reduction, and signal transduction pathways controlling tumor growth or angiogenesis, and inflammation inhibition.[11-16]

The Response Surface approach (RSM) is one of the most commonly used methodologies in the design optimization techquies of targeted medication delivery system. The method, which is based on the design of experiments principles, comprises the use of various experimental designs, the production of polynomials mathematical formulas, as well as the mappings of the responses over the experimental region to select the optimal formulations. The process needs very little experimentation and time, and it have proven to be significantly more effective and cost-efficient than traditional dosage form formulation procedures. Central Composite Design(CCD), 3 factorial design techniques, and Box–Behnken design are only a few of the

RSM designs available for quantitative formulation optimization techniques.[17, 18]The aim of this work is development and characterization of buccal patches containing curcumin, quercetin and lyopene loaded in ethocel and methocel polymer. The purpose of this research was to characterize buccal patches in assessing their efficacy as targeted drug delivery for oral cancer therapy. As a computer-aided optimization approach, a Central Composite Design (CCD) was used. The amount of release resistant polymer-ethocel(X1) and propylene glycol (X2), as well as carbomer, were the independent variables in this investigation (X3). The disintegration time (Y1), folding endurance (Y2), and percent drug release were the dependent variables studied (Y3).

MATERIALS AND METHODS

Materials and Reagents

Curcumin, Quercetin and Lycoene 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 research study. All of the other chemical reagents and chemicals was a great analytical grade.

METHODS

Preparation 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 and Quercetin for 30 minutes. Ethanol: water (1:1) Lycopene solution was added to this solution and agitated for additional 30 minutes. The carbopol solution was made by boiling Millipore water with continual stirring before adding it to the polymeric solution. The determined amount of propylene glycol was added to the polymeric solution with intermittent shaking. The casting solution 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. After that, the mixture was placed into a Petri dish with an inverted glass funnel on top. The solution was allowed to evaporate for 24 hours at room temperature. The dry region was cut into 2x2 cm squares and isolated. Before even being placed in a desiccator, it was covered in aluminum foil.[19-21]

Preparation of Buccal Patch using Experimental Design

The formulation was optimized by using central composite design. 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). Disintegration time, folding endurance, and percent drug release were all investigated. A central composite design was used to optimize the formulation of the buccal patch. The design-expert software presented a total of 20 runs. Table 1 shows the response and variables that were studied.

Evaluation of prepared Buccal patches

Drug and polymer interaction study methods

a. FTIR (Fourier Transform Infrared Spectroscopy) analysis

Nicolet 520P FT-IR spectrometer wavelength in the range between of 4000 cm^{-1} - 500 cm^{-1} was used for the evaluate of drug and polymer interaction. The samples were prepared by using KBr pellet method

b. DSC (Differential Scanning Calorimetry) analysis

Drug-polymer interactions study was analysed by using DSC. DSCis the one of the thermal analytical technique.Perkin Elmer Differential Scanning Calorimeter (DSC 6000 – PerkinElmer) was used to analyse drug and polymer samples under an air environment, with a heating rate of 10^0 C/min at a rate of 5 ml/min, air was flushed.

In vitro disintegration time

The time it takes for patches to disintegrate when it reacts with water or a buffer is known as disintegrating time. A buccal patch of size 2×2 cm was placed inside the disintegration apparatus filled with pH 7.4 buffer maintained at 37±0.5°C and the time taken for the buccal patch to disintegrate were noted down.

Folding endurance and thickness

This test was performed to measure the toughness of the patch made from various polymers and to check the efficacy of the plasticizer. Folding endurance is the degree of folds necessary to break a polymeric patch. Mechanical folding endurance was measured by folding a tiny strip of film (2 cm) in the same position until it broke. The couple of times the patch could be folded in the same spot before breaking or cracking were used to measure the value of folding endurance. Three patches of each type were used in the experiment.

***In vitro*drug release study**

The Franz diffusing cell was used to characterize buccal patch formulations in vitro. This is an effective method for estimating drug transport across the skin from topical preparations. In vitro, drug release studies were conducted using a synthetic cellophane membrane and 30.0 ml of phosphate buffer (pH 7.4) inside the diffusion cell's donor compartment. A patch of 2 × 2 cm size was cut and attached. It was administered to the donor compartment's membrane. Then it was evenly dispersed throughout the cellophane membrane. The assembly was kept at a constant room temperature of 37.0±2.0°C at a rotational speed of 50 rpm. At appropriate time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 hr), samples (1.0 ml aliquots) were withdrawn and replaced with a quantity of buffer solution to preserve the receptor compartment volume at 30 ml. The curcumin, lycopene and quercetin contains patches were analyzed spectrophotometrically at 427nm, 440nm and 375nm respectively.

Weight variation

All prepared patches were individually weighed to ensure weight homogeneity. An analytical weighing balance was used to determine the weight (Shimadzu AX 200, Kyoto, Japan). The weights of individuals were compared to the state median.

Uniformity of Drug content

To evaluate for drug content homogeneity, the patches were mashed in a mortar and pestle using water and acetone solvent (1.5cm dimension). Alcohol/water is used solvent for Patches containing curcumin, quercetin and lycopene. The content was then filtered by using 0.45m syringe. Then the solutions were diluted with simulated saliva. The samples were analysed at a maximium wavelength of 426 to 260 nm (Shimadzu, Japan, UV 1800 spectrophotometer) by using solvent alcohol and water blank. The drug content of the patches was calculated from the calibration curve.

Surface pH of buccal patches

The pH at the surface of the patches was measured after 1, 2, 3, 4, and 5 hours in glass tubes containing 20 ml of distilled water. 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. The above determination was carried out in three times.

Swelling study of buccal patches

On Petri dishes, around 20 ml of fake saliva was stored at ambient temperature. The initial patch weight (W1) was determined. After 60 minutes, the films with humidity on their surface were carefully removed with filter paper. After the swelling patches were weighed, the swelling percentage was computed using the formula below (W2).

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

Mucoadhesive study of buccal patches

The model substrate was detected and removes porcine buccal mucosa obtained from a slaughterhouse, and the moistening fluid was PBS (pH 6.6). A compression buccal patch was horizontally inserted or "sand wedged" within two or three layers of clipped tissue substrates for a total 10 minutes, with a constant mass of 50 grams of a total of 10 minutes. Mucoadhesive strength was measure using an Extech 475040 Force Gauge Meter in terms of the force required to remove the patches from the animal mucosal membrane (Extech Instruments Corp). When the buccal patches was withdrawn from the porcine mucous membrane and the intensity reading was taken, the upward tension stopped.

Morphology character of Buccal patches (SEM)

SEM was used to examine the morphology of the produced patches (SEM; JEOL JMS-6390 apparatus). The samples were coated with carbon to improve electron beam conductivity; the beam passing through the sample generates a signal in a raster which contains information on the surface morphology topographical composition and other features.

Stability studies

For the preparation optimized patch, stability studies were conducted over 180-days. The patch was maintained for stability tests, and the incubation was kept at 36±0.5 □C and 75±5% relative humidity. The prepared patch medication content and physical appearances were examined after a 30-days interval. The procedure specified in the section was followed to determine the drug content.

Cytotoxicity Assay

Optimized buccal patch 2x2cmwas 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 this serial two fold dilutions (6.25 – 100 µg) were prepared. NCCS provided the KB cell line. Stock cultures were incubated until confluent in DMEM media consists of supplemented with 10% inactivated Fetal Bovine Serum, penicillin (100 IU/mL), and streptomycin (100 g/mL) in a moisturised environment of 5% of CO_2.

Using an appropriate medium containing 10% FBS, the monolayer culturing was inoculated and the cell density was regulated to 1.0 x 105 cells/mL. Each 96-well microtiter plate received 100 l of diluted cell suspension (1 x 105 cells/well). After 24 hours, a partial monolayer had formed. The monolayer was cleaned once with medium after the precipitate was flicked off. In microtiter plates, 100 L of various concentrations of test substances were applied to the partial monolayer. The plate was then incubated at 37°C for 24 h in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well. The samples were incubated at 37°C for 4 hours in a 5% CO2 environment. The supernatant was discarded, and 100 mL of DMSO was added in its place. To dissolve the produced formazan, the plate has been gently shaken. A microplate reader was used to measure the absorbance at a wavelengths of 570 nm.

The percentage of viability was calculated using the following formula, % viability = Sample abs/Control abs x 100.[23]

RESULTS

In the present research work, buccal patch of curcurmin, quercetin and lycopene were prepared with polymer combination of ethocel and methocel by using solvent casting techniques. 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. The buccal patches were shown in figure -1

Figure 1 Buccal Patch

Table 1 Central composite design for buccal patch formulation

Space type	Factor A Ethocel (gm)	Factor B Propylene Glycol	Factor C Methocel (gm)	Response 1 DT (min)	Response 2 Folding Endurance	Response 3 Drug Release (%)
Centre	1.25	5	1	6.45	208	85.500
Centre	1.25	5	1	6.45	208	85.500
Centre	1.25	5	1	6.45	208	85.500
Centre	1.25	5	1	6.45	208	85.500
Centre	1.25	5	1	6.45	208	85.500

Centre	1.25	5	1	6.45	208	85.500
Axial	1.95	5	1	6.3	216	84.406
Axial	1.25	5	1.57	6	215	84.967
Axial	1.25	5	0.48	5.3	204	98.329
Axial	1.25	3.58	1	5.1	200	32.196
Axial	1.25	6.41	1	6	218	30.746
Axial	0.54	5	1	6.5	219	80.490
Factorial	1.67	4.16	1.34	6	207	31.616
Factorial	0.83	4.16	1.34	7	220	42.928
Factorial	0.83	5.84	0.66	8	208	48.727
Factorial	0.83	4.16	0.66	6.3	219	79.185
Factorial	1.67	5.84	0.66	6.3	212	152.135
Factorial	1.67	5.84	1.34	6	212	59.316
Factorial	1.67	4.16	0.66	6.3	218	83.826
Factorial	0.83	5.84	1.34	6.3	218	77.330

Optimization of independent variables

Disintegration time, Folding endurance and % drug release obtained by the various levels of three independent variables. Ethocel, Propylene glycol and carbomer were used as Polymer, plasticizer, Film thickening agent. These agents were subjected to multiple regressions to yield a final equation.

Disintegration time (DT)

$$Y = +6.32+0.0979*A+0.0376*B+ 0.1155*C+0.$$

$$-0.2246*B^2-0.1892*C^2$$

(+0.2250*AB) + sign of coefficients indicates positive effects of polymer and plasticizer concentration on DTT his model's F-value of 11.87 percent indicates that it was important. There is barely a 0.05 percent probability that an F – value this high will occur due to noise. There were P-values compared with fewer than 0.0500 found. It stated that model terms are important. All response variables have a 3D surface plot, which is highly significant for studying the interplay impact of different factors on the response. 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. Patches with high concentrations of polymer and plasticizer would meet the conditions for rapid dissolving.

Folding Endurance

$$Y=+208.10-1.54*A+1.19*B+1.35*C+2.50*AB-1.75*AC+1.50*BC+3.73*A^2+0.7204*B^2-0.8971*C^2$$

(+2.50*AB) positive sign of coefficients indicates positive effects of polymer and plasticizer concentration on folding endurance. The model's F-value of 13.69 percent indicates that it was significant. Due to noise, there will only be a 2.07% probability that an F – value this high will occur. The film former (polymer) ensures sufficient strength, while the plasticizer gives the film its flexibility. As a result, a balanced combination of each of these elements would result in a buccal patch of acceptable quality. The findings revealed that polymers and plasticizers have a good impact on folding endurance. The poly nominal equation suggested the concentration of plasticizer has a promising influence of the folding endurance. Because plasticizer is a major contributor to folding endurance, it allows the linear polymeric chain to relax, presumably by establishing hydrogen bonds, resulting in increased flexibility and, as a result, higher folding endurance numbers.

Percentage Drug Release

$$Y = +85.36+6.25*A+7.14*B-12.83*C-11.51*A^2$$
$$+3*10C^2$$

(+17.17*AC) positive sign of coefficients indicates positive effects of polymer and thickening agent concentration on percentage drug release. The model F-value of 4.46 % represent that it is significant. Due to noise, there will only be a 1.43 % probability that an F – value this high will occur. Tends to increase in the value percent drug release with an increase in the amount of thickening agent and decreases in percent drug release with an increase mostly in polymer concentration of the formulation. The highest percentage drug release recorded at low drug polymer ratio and high thickening agent concentration. When the concentration of a polymer increases, the drug diminishes because the drug remains within the polymer matrix. The response surface plots for three responses and desirability plots were shown in figure 2.

The desirability function of Derringer The geometric mean weighed or not, of the various desirability functions is defined as D. Desirability functions D have a range of values ranging from 0 to 1. Weights can range from approximately to 10; weighs less than 1 indicate that the objective is less important, while weights larger than 1 indicate that the goal is more important. It was determined that a set of parameters resulted in a high Desirability rating (D=0.661) were Ethocel 1.67 gm, Propylene glycol 5.84 ml and carbomer 1.34gm used. The predicted response values corresponding to the later value of D were DT= 6.02sec, Folding endurance = 215, % drug release 68.06%. The model's prediction efficiency was confirmed by running the empirical values under ideal conditions.

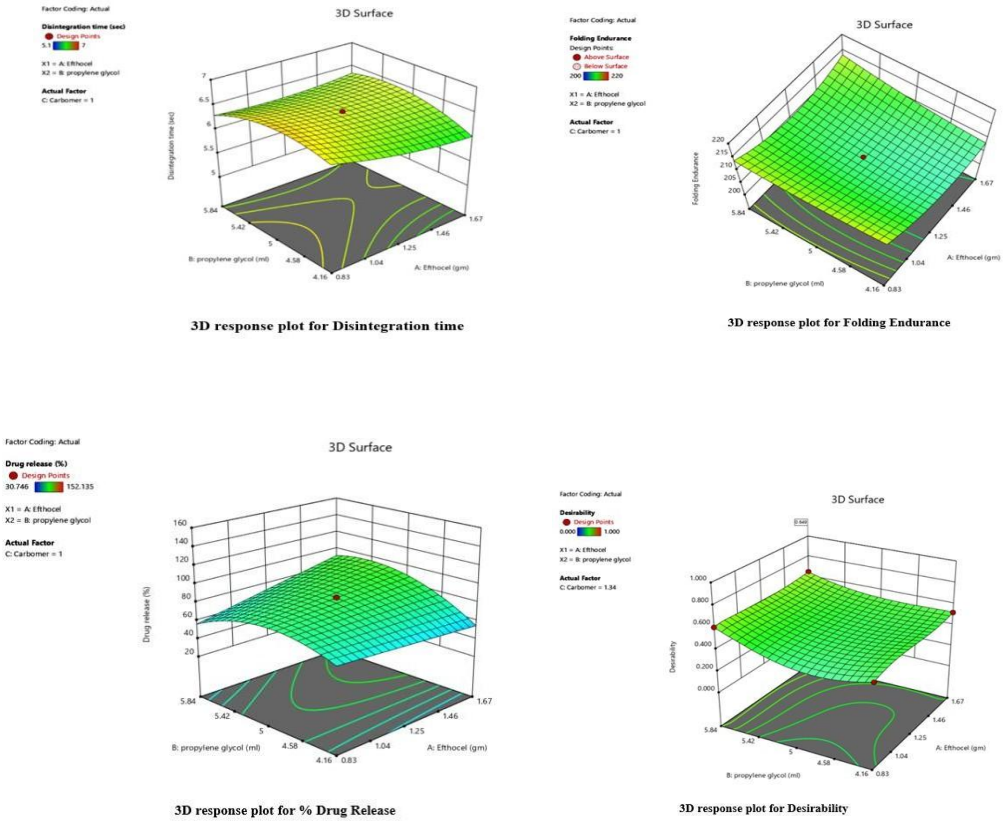

Figure 2 Response surface plot for Responses and Desirability

Drug polymer incompatibility study

FT-IR spectroscopy results

FTIR spectrum analysis used to examine the physical mixture of drug and polymer for any physical or chemical changes in the drug's properties. Curcumin's FTIR spectra were found to be in the range of 3423 cm-1 for the –OH group, 2923 cm-1 for the aromatic CH group(benzene), and 1509 cm-1 for C=C aromatic, 1627 cm-1 for C=O aromatic, and 1275 cm-1 for C-O-C stretching. Quercetin's FTIR spectra were found to be in the range of 3311 cm-1 for the –OH group, 2952 cm-1 for the aromatic CH group, and 1512 cm-1 for C=C aromatic, 1612 cm-1 for C=O aromatic, and 1286 cm-1 for C-O-C stretching. Lycopene's FTIR spectra were found to be in the region of 2929 cm-1 for such CH group and 1620 cm-1 for the C=C group. When comparing the spectra of formed peaks to the spectra of the drug and polymers, it was discovered that there was no substantial change in the initial peak of the drug and polymers, representing that has been no interaction between the drug and polymer. Figure 3 depicts the IR chromatogram.

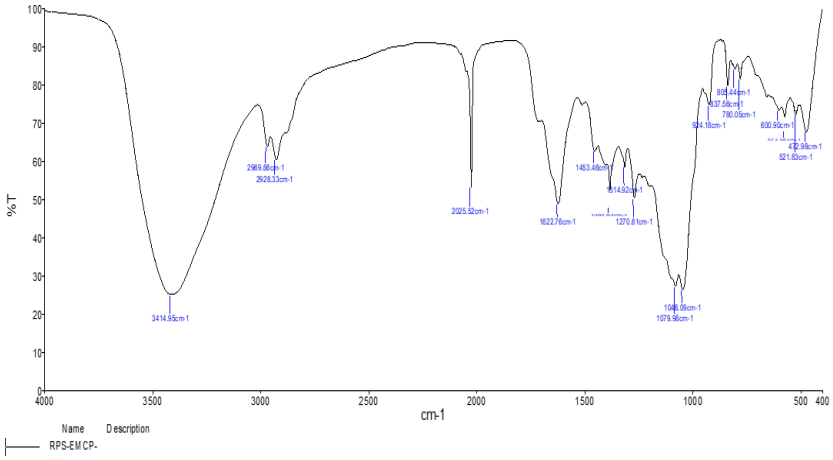

Figure 3: IR spectrum for Physical mixtures

DSC

DSC thermogram of Quercetin, curcumin, lycopene and polymer was studied. It was found that Curcuminshown endothermic peak at 184.81°C, Quercetin shown endothermic peak at 323.5°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 indicates that the medicine had no interaction with the polymer or excipients employed in the formulations' production. The DSC thermogram was shown in figure 4.

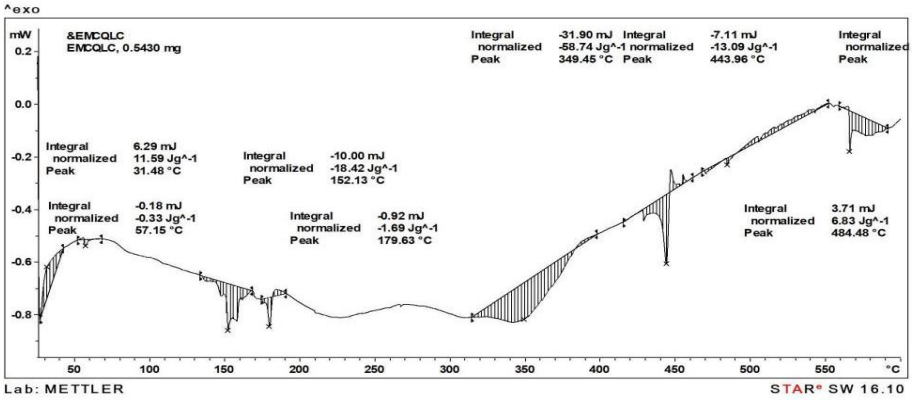

Figure 4: DSC Thermogram for formulated buccal patch

PHYSICAL INVESTIGATED OF BUCCAL PATCHES

Buccal patches thickness and folding endurances

As the weight of the polymer grows, so does the weight of the patches layer. Batches have a total weight of 347.43 mg. The thickness of patch film: As the polymer concentration rises, the patch film's thickness rises as well. The thickness of the buccal mucoadhesive patch formulation was determined to be in the 0.21 mm range. The low standard deviation numbers imply that the film is physically uniform. A patch folding endurance is a measure of its ability to survive rupture. The patch folding endurance was tested, and it was observed that as polymer concentration grows, so does the film's folding endurance. The folding endurance of the patch was assessed to be 217.33.

The folding endurance values were determined to be optimal, indicating that they had excellent physical and mechanical qualities. For formulations, the surface pH of buccal patches was determined to be in the range of 6.56. Table 2 summarises the findings. All of the films' surface pH was within the range of salivary pH. The surface pH of all formulations did not differ much. All of the formulations had a near neutral surface pH, indicating that they had less potential to irritate the buccal mucosa and should be relatively comfortable.

The mucoadhesive strength of buccal patches found to be 6.63 g. The disintegration time of drug loaded buccal patches was found to be 6.07 minutes. The Patches disintegration time was within the limit. Hence it was easily dissolved in saliva.

Table 2: Physical evaluation of optimized drug loaded Buccal patches

Buccal Patch	Curcumin + Quercetin+ Lycopene
Disintegration Time (min)	6.07
Folding endurance	217.33
Weight (mg)	347.43
Thickness (mm)	0.21
surface pH	6.56
Mucoadhesive strength (g)	6.63
Content uniformity (%)	
	Curcumin - 97.88
	Lycopene – 100.2
	Quercetin – 99.41

SEM analysis of drug loaded Buccal Patches

Figure 5 shows a cross-section of buccal patch compositions seen under a scanning electron. The cross-section of compositions inside the patch indicated a uniform and nonporous 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.

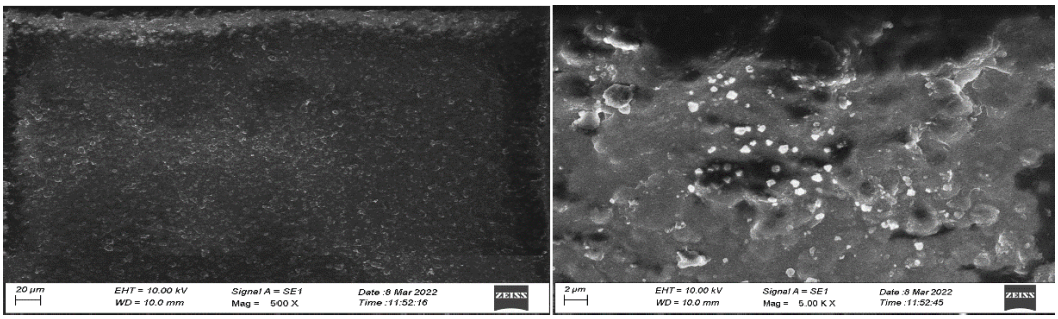

Figure: 5Scanning Electronic microscope Image for buccal patch

In vitro drug release study

In-vitro drug release of curcumin, quercetin and lycopene loaded optimized buccal patches was performed in pH 6.8 phosphate buffer. The results were shown in figure 6 and table 3. 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 drug and polymer ratio was the most critical factor impacting the release of drugs from buccal tablets. The drug release ranged from 73.5 % to 95.6 % in all formulations.

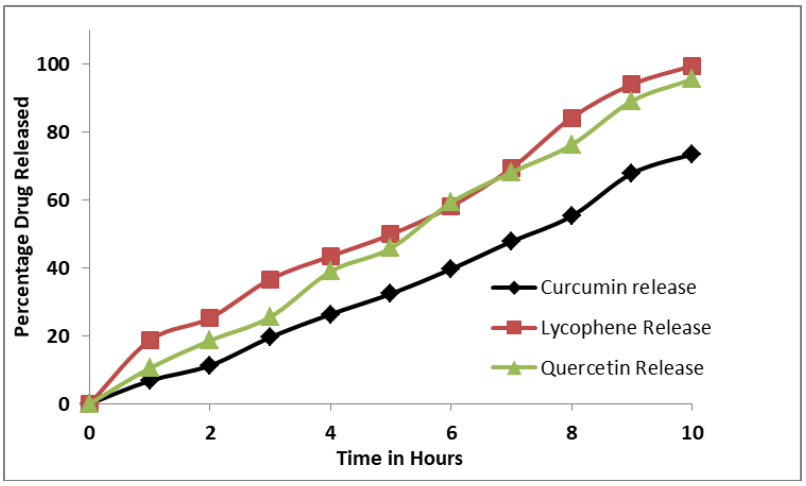

Figure 6 *In vitro* drug release study of buccal patch

Table: 3*In -vitro* Drug release study of drug loaded optimized buccal patch

Time (Hours)	Curcumin release	Lycopene release	Quercetin release
0	0.0	0.0	0.0
1	6.8	18.7	10.5
2	11.3	25.3	18.7
3	19.6	36.7	25.6
4	26.4	43.5	39.0
5	32.4	49.9	45.9
6	39.7	58.2	59.3
7	47.8	69.4	68.2
8	55.3	84.3	76.3
9	67.9	94.1	89.2
10	73.5	99.6	95.6

Stability Studies:

The optimized buccal patch samples were taken after30 (1 month), 60 (2 months), 90 (3months) days. The formulation (optimized) was tested for tensile properties, drug content, and percentage of drug release during 90 days at 40°C±0.5°C and 75±0.5 percent RH. Table - 4 summarizes the reports.

Table 4: Stability studies of drug loaded optimized buccal patches

Evaluation Parameter	After 30days	After 60 days	After 90 days
Colour and physical appearance	No change	No change	No change
%Drug content (Curcumin)	99.5±1.20	100.6±1.2	100.9±1.55
% Drug content (Lycopene)	101.7±0.62	100.3±1.97	101.8±1.01
% drug content (Quercetin)	102.1±0.76	99.9±2.03	101.5±1.09
% drug release (Curcumin)	73.5.±2.02	71.6±2.68	70.4±1.96
% drug release (Lycopene)	99.6.±1.5	96.9±1.88	95.3±1.59
% drug release (Quercetin)	95.6.±3.06	94.3±2.56	93.4±1.38

Cytotoxicity Study (MTT ASSAY)

MTT assay used to measure cell viability. Cell viability of prepared buccal patches was tested in KB cell lines at various concentrations. The developed buccal patches demonstrated cell viability was diminished in a dose-dependent potency at a concentration of 12.5, 25, 50, 100, and 200g/ml, with a notable difference between both 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 samples for the production of curcumin, quercetin and lyopene loaded buccal patches was found 2.808.

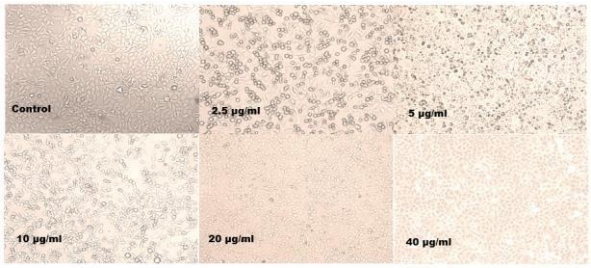

Figure 7 Cytotoxicity study for different concentration

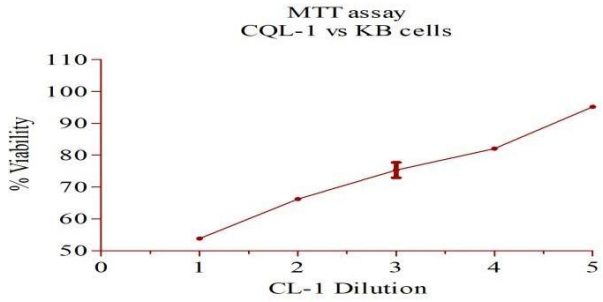

Figure 8 Cell Viability (%) of Drug Loaded Buccal Patch formulation

DISCUSSION

Preformulation studies are critical to the creation of a successful formulation. The FT-IR and DSC studies were used to determine drug excipient compatibility. FTIR is a non-destructive and fast method for determining the IR spectra of a pure medication as well as different patch formulations. It's utilized to figure out how medicines, synthetic, semi synthetic, and natural macromolecules interact. Formulations in their ultimate form many peaks were visible in the FTIR spectra, showing that the drug's chemical structure was preserved and responsible for the effective loading into the formulation. Curcumin, quercetin and lyopenehad no chemical interaction with the physical mixtures of polymers (Ethocel, methocel, carbomer, propylene glycol) utilised in this study. The buccal patches were made utilising the solvent casting process. Buccal patch formulations were prepared using central composite design method by design expert software version 12.0 (20 formulations). From this design optimized formulation was found out. The optimized conditions were Ethocel 1.67 gm, Propylene glycol 5.84 ml and carbomer 1.34gm used. DT = 6.02sec, Folding endurance = 215, and percentage drug release of 68.06 % were given the latter value of D, the estimated response values. The model's prediction efficiency was confirmed by running the experimental results under ideal conditions.

Buccal patches were given a physical examination. To evaluate the possibility of discomfort during in-vivo tests, the surface pH of the created patch was determined. Weight measure and thickness were found to be uniform, as evidenced by the low standard deviation value.[23]A physical examination of buccal patches were performed. The surface pH of the produced patch were measured to assess the probability of pain during in-vivo tests. The lower value number indicated that the weight measurement and thickness were both uniform.[24]Using quantities of ethocel and propylene glycol, drug release might be extended, resulting in reduced drug release. The prepared patches were tested for 180 days in order to determine their drug stability. The prepared buccal patches demonstrated excellent stability with no noticeable physiochemical alterations after specified intervals of 30 days. We performed in vitro assay to characterize the anticancer properties of drug loaded buccal patches using KB cell line as a cell culture media. In cell viability (MTT) assay performed against KB cell line. In the study evaluated by viability of KB cell line in the using prepared buccal patches at different concentration. The reduction of KB cell line was observed at 12.5µg/ml and IC50 value is 2.808.

CONCLUSION

The importance of combining curcumin, quercetin, and lycopene with in production of buccal patches was proven in this study. A portable measuring scale technique can be used to determine the mucoadhesive strength. According to the findings, the optimized formulations had good mucoadhesion, were irritation-free, and released the medication entirely by a diffusion process. As a result, the perspectives and approaches of buccal mucoadhesive patches can be considered a unique treatment for oral cancer.

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