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Development of Glutathione Loaded Mucoadhesive Drug Delivery System for Cosmeceutical Purpose

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ABSTRACT: The buccal mucosa provides a promising route for the delivery of However, challenges are encountered in developing sublingual mucoadhesive drug delivery formulations such as: prolonging drug retention, uniform drug content, desirable drug release profiles, adequate drug permeation and efficient delivery of reduced glutathione. The aim of this study was to develop glutathione loaded buccal film. The mucoadhesive films were prepared by solvent casting method. Several characterization studies including thickness, weight uniformity, folding endurance, surface pH and swelling were carried out on preliminary formulations to optimise formulations for in-vitro drug release and in-vitro permeation studies.

KEYWORDS: Glutathione, Buccal films, Goat Buccal mucosa, Sustained drug delivery.

I. INTRODUCTION

The term "cosmeceuticals" was introduced by dermatologist Dr Albert Kligman in 1984. Cosmeceuticals are products that act as both cosmetics and medicines and combine cosmetic products with pharmaceutical products. Typically applied by rubbing the product on the skin. Cosmeceuticals contain high amounts of active ingredients like vitamin C and hyaluronic acid. They are used for a variety of purposes like to remove wrinkles, decrease signs of aging, heal scars, prevent acne, keep the skin moist, and even used as sunscreen.[1]

Glutathione is a powerful antioxidant found in every cell in the body. It is made up of three types of molecules known as amino acids. Glutathione is an antioxidant produced in the body through enzymatic reactions, using the amino acids cysteine, L-glutamic acid and glycine. There are two different forms of glutathione: Reduced glutathione (GSH, or L-glutathione) is the active form. It repairs oxidative damage and oxidizes. Oxidized glutathione (GSSG) is the inactive form, which can be recycled back into active. [2] It has been called the Wonder drug for treating issues related to hyperpigmentation. It reduces acne, scars, blemishes and dark spots. It stimulates pheomelanin synthesis rather than the darker melanin. Pheomelanin is lighter in colour (yellow-red), so, an increase in the proportion of pheomelanin is associated with lighter skin colour. It interferes with the trafficking of melanogenic enzymes and inhibits tyrosinase activity, and melanosome transfer in various ways. Glutathione is produced exclusively in the cytosol and actively pumped into mitochondria. GSH is made available in cells in 3 ways; De novo synthesis via a two-step process catalysed by the enzymes glutamate, cysteine, ligase (GCL) and glutathione synthetase (requires ATP). Regeneration of oxidation GSSG to reduced GSH by glutathione reductase (requires NADPH). Recycling of cysteine from conjugated glutathione via GGTP (requires NADPH).[3]

Glutathione can act as antioxidant, anti-melanogenic agent, and for antiaging. Health Benefits of glutathione are as follows, it's a potent antioxidant directly binding to oxidative compounds that damage cell membranes, DNA and energy production. It directly neutralizes a wide range of oxidants, including superoxide, nitric oxide, carbon radicals, hydroperoxides, and lipid peroxides. Glutathione can act as detoxification agent, role of glutathione in your body ‘s detoxification system is vital. That is your natural processes sometimes need a boost from increased glutathione from your diet or supplements. During Phase1 detoxification, all sorts of toxins and xenobiotics are partially processed by specialized proteins inside mitochondria called cytochromes. In Phase 2 detoxification, various enzymes act directly on the toxins partially degraded and

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processed in Phase 1. These enzymes use glutathione to neutralize the toxins. Phase 3 detoxification is the elimination of toxins and xenobiotics. Toxins are removed from your body, mainly by the kidneys (urine) and liver (bile). Without glutathione, your body would not be able to neutralize and eliminate toxins effectively.Glutathione for Skin are concerned with acne, wrinkles, dryness, eczema, or puffy eyes, many are seeking flawless, youthful skin. Science says that glutathione is effective. Glutathione not only decreases the melanin (pigmentation) in your skin, but has also been found to decrease wrinkles and increase skin elasticity. Glutathione works on the skin pigment production by inhibiting tyrosinase, an enzyme involved in making melanin. [4]

Buccal drug delivery is an important route of drug administration and it is one of the novel drug delivery systems. The buccal mucosa is relatively permeable and provides affluent blood supply and permits a prolonged retention of a dosage form. Especially with the use of mucoadhesive polymers without much interference in processes such as mastication unlike the sublingual route.[5] Buccal film is defined as the dosage form which dissolves into the buccal mucosa or mouth and releases the medicament to provide local or systemic drug delivery and employs a water dissolving polymer (hydrocolloid bio adhesive polymer). These polymers allow the dosage form to adhere, hydrate and dissolve into the mouth.[6]

Advantages of buccal film:
Fast dissolution rate when compared to other conventional dosage forms.
Ease of administration and termination.
High patient compliance, cost effective.
Stable. Avoids hepatic first pass metabolism
Thereby increasing bioavailability [7]

II. DRUG AND EXCIPIENT PROFILE

Glutathione is involved in detoxification of both xenobiotic and endogenous compound. It facilitates excretion from cells (Hg), facilitate excretion from body (POPs, Hg) and directly neutralizes (POPs, many oxidative chemicals).
Curcumin has the ability to increase glutathione level and assist in restoring adequate levels of glutathione and improve activities of glutathione.
Vitamin C can used as antioxidant.
HPMC can be used as coating agent, film former, rate-controlling polymer for sustained release.
Ethanol can be used as antimicrobial, preservative, disinfectant, skin penetrant and solvent.
Citric acid can be used as flavouring agent, acidulant, sequestrant, antioxidant and synergist.
Propylene glycol used as plasticizer, humectant, disinfectant and antimicrobial agent
Peppermint oil can be used flavouring agent, carminative, and antibacterial.[8]
Carbopol is used as stabilizing agent, suspending agent, tablet binder

III. MATERIALS AND METHODS

SL.NO	INGREDIENTS	QUANTITY
1	GLUTATHIONE	500mg
2	CURCUMIN	54mg
3	HPMCK4M	300mg
4	CARBOPOL934	100mg
5	PROPYLENE GLYCOL	1ml
6	VITAMIN C	20mg
7	CITRIC ACID	5mg
8	PEPPERMINT OIL	0.1ml
9	ETHANOL	20ml

Extraction methods of curcumin are solvent extraction, hydro/steam distillation, ultrasound-assisted extraction, microwave assisted extraction, enzyme assisted extraction, pressurized liquid extraction and Soxhlet extraction

Soxhlet Extraction
1) Extract about 50 g of turmeric powder with 95% alcohol in a Soxhlet assembly until all the colouring matter is extracted.
2) Distill off alcoholic extract to semisolid brown coloured mass (about 4.5%)
3) Dissolve the extract in 50 ml of benzene and extract twice with equal volume of 0.1% sodium hydroxide solution.
4) Combine the alkaline extracts and acidify with dil. HCl. A yellow-coloured precipitate is formed. Allow it to settle for about 15 minutes.
5) After setting the precipitate concentrate the extract by boiling on water bath and at the same time dissolving precipitate in boiling water. During this process of boiling the resinous material would agglomerate and form lumpy mass.
6) Filter the solution in hot condition and concentrate filtrate to very small volume and finally cool to get curcumin (1.5%) [9]

Preformulation Studies
1) Physicochemical parameters
a) Organoleptic properties

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Organoleptic properties like color, odour and texture were evaluated.

b) Solubility study
Preformulation solubility analysis was done to select the suitable solvent system to dissolve the drug and also to test its solubility in the dissolution medium which was to be used. Solubility analysis of glutathione was done with ethanol, ammonia, methanol, water, acetone, diethyl ether, phosphate buffer.

2) Identification of pure drug:
a) Melting point determination

Melting point of the drug Glutathione was determined by taking small amount of glutathione in a capillary tube closed at one end and placed in a melting point apparatus and the temperature at which drug melts was recorded.

b) FTIR spectra of the drug

The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the wavelength region between 3500and 750 cm-1. The spectra Obtained for glutathione was compared with the reference standard.

c)Determination of λ_{max}

3) Compatibility studies of drug with curcumin using FTIR spectroscopy.

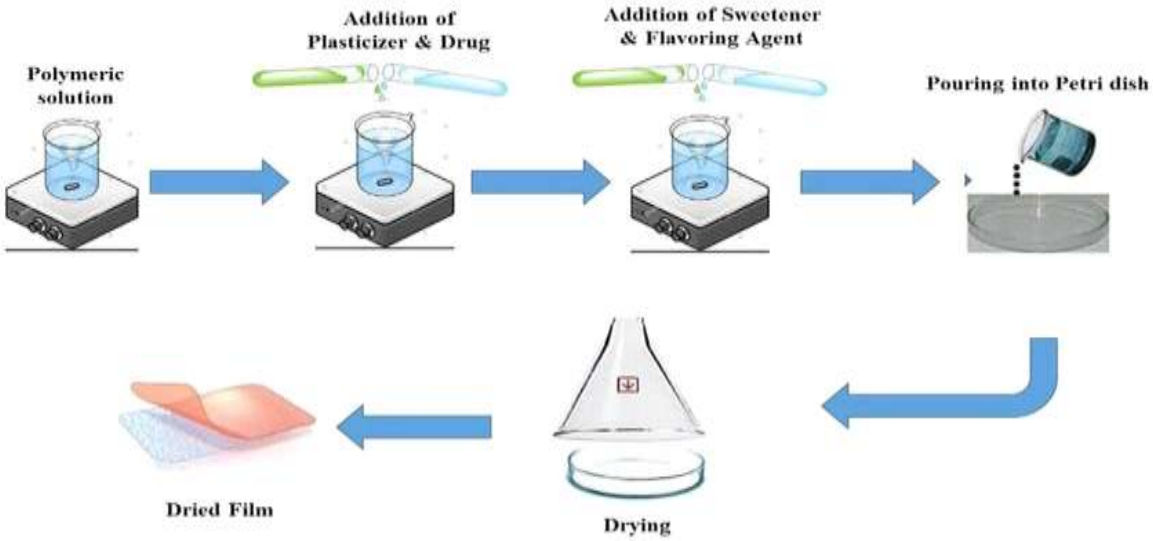
The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the wavelength region between 3500and 750 cm-1. The spectra obtained for the physical mixtures of glutathione with curcumin were compared to check compatibility of drug and curcumin.

4)Standard curve for Glutathione

Standard stock solution prepared and absorbance of Solutions of concentrations 3, 3.5, 4, 4.5, 5 µg/ml was measured in UV spectrophotometer at 204nm and a curve was plotted with concentration(x-axis) versus absorbance(y-axis) and from that graph, standard concentrations was determined.[10]

Development of Buccal film of Glutathione:
Methods of Film Preparation

Solvent casting method is the most widely used method for the manufacturing of buccal film due to its excellent uniformity in thickness, easy and low-cost processing. In this method, the drug and other excipients are dissolved in appropriate solvent to form a clear viscous solution and the formed solutions are mixed well. Then, solution is cast as a film and allowed to dry. The film is preferably air-dried or dried under oven, then the film is collected.



Film forming polymer (HPMC-300mg, CARBOPOL 934-100mg) and curcumin (54mg) was dissolved in solvent ethanol(20ml). Then required quantity of drug glutathione(500mg) added.1ml of propylene glycol added as plasticizer. Then vitamin C(20mg), citric acid(5mg),

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peppermint oil(0.1ml) added. The solution was mixed for 30minutes by using a magnetic stirrer. solution was transferred to petridish slowly drop by drop for uniform spreading. Kept for 24hours in room temperature for drying. After drying cut into definite shapes and packed in butter paper, kept in aluminium foil and stored in container. [11]

IV. EVALUATION

Characterization of the films:

Formulated films were subjected to the preliminary evaluation tests. Films with any imperfections, entrapped air, or differing in thickness, weight (or) content uniformity were excluded from further studies. Physicochemical properties such as thickness, weight uniformity, folding endurance, surface pH, and drug content uniformity of the prepared films were determined.

Physicochemical characteristics:

Physical appearance:

All the films were visually inspected for color, clarity, flexibility, and smoothness

Film thickness

The thickness of film is measured by micrometer screw gauge. The thickness should be evaluated at five different locations (four corners and one at center) and it essential to ascertain uniformity in the thickness of film directly related to accuracy of dose distribution in the film.

Weight uniformity

For the mass uniformity, six films from formulation were taken and weighed individually on electric balance. The average weight was calculated.

Folding endurance

Folding endurance gives the brittleness of a film. It is measured by manual repeated folding of film at some place till it broke. The number of times the film is folded without breaking is the folding endurance value.

Surface pH

Surface pH of the film can be determined by allowing a film of formulation to swell for two hours on an agar plate surface. The surface pH was measured by means of a pH paper placed on the surface of the swollen film

In-vitro release study

Dissolution studies were carried out in a USP dissolution apparatus (basket type) using 900ml of dissolution medium at 37± 0.5 °C, and a rotation speed of 50 rpm was used. An aliquot of sample was periodically withdrawn and replaced with fresh medium. The samples were filtered through whatman filter paper and analyzed spectrophotometrically

In-vitro buccal permeation study:

The in vitro buccal permeation study using goat mucosa is performed using a Franz diffusion cell at 37°C ±0.20. Goat buccal mucosa obtained from a local slaughter house has been used within 2h of slaughter. Freshly obtained goat buccal mucosa was mounted between the donor and receptor compartments. The film was attached with the mucosa and the compartments were clamped together. The film was placed in donor compartment and receptor compartment was filled 20 ml phosphate buffer (pH 6.8). One ml of the sample was withdrawn at 15 mins interval for a period of 2.5 hours and analyzed.

V. RESULT AND DISCUSSION

I.Extraction of curcumin from Curcuma longa:

Extraction of curcumin from Curcuma and crude curcumin was obtained

Extracted curcumin Soxhlet apparatus

II.Preformulation Studies:

1.Physico-chemical parameters

a) Organoleptic properties

SL NO	CHARACTER	PROPERTY OF THE DRUG
1	Colour	White
2	Odour	Strong pungent odour
3	Texture	Crystalline powder

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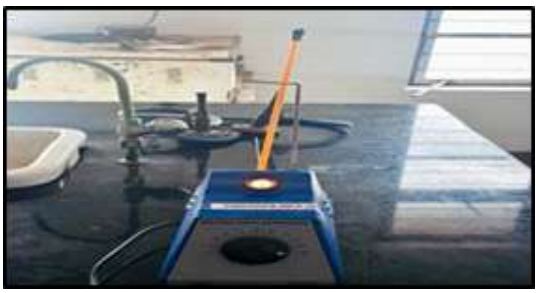
b) Solubility study

From the solubility study it was shown that drug glutathione was freely soluble in water, ethanol, liquid ammonia, insoluble in methanol, diethyl ether and acetone.

2.Identification of pure drug

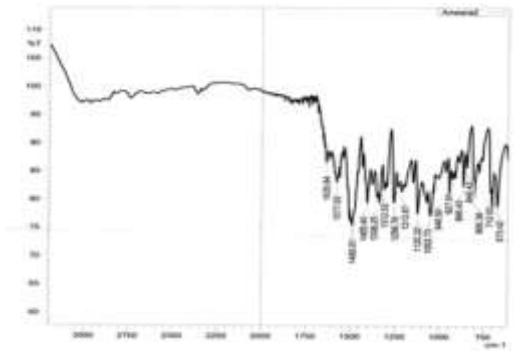
a)Melting point determination

Melting point was determined and it was found to be 195°C.



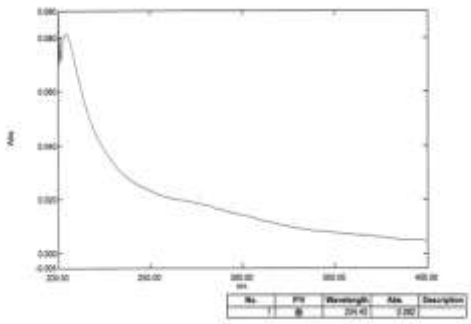
b) FTIR spectra of the drug

The Fourier transform infrared spectroscopy studies were carried out for pure drug (glutathione)



FTIR Spectrum of Glutathione

c)Determination of λmax

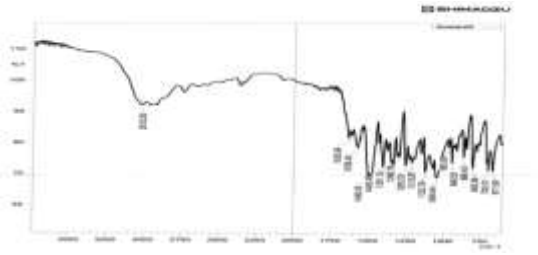


λmax of Glutathione

The λmax of the drug was found to be 204nm. The wavelength of the maximum absorption was noted and UV spectrum was recorded.

3)Compatibility studies of drug with curcumin using FTIR spectroscopy

The Fourier transform infrared spectroscopy studies were carried out for glutathione and curcumin. There were no changes in the major peaks of glutathione in the presence of curcumin. This revealed that the drug and the curcumin is compatible with each other.

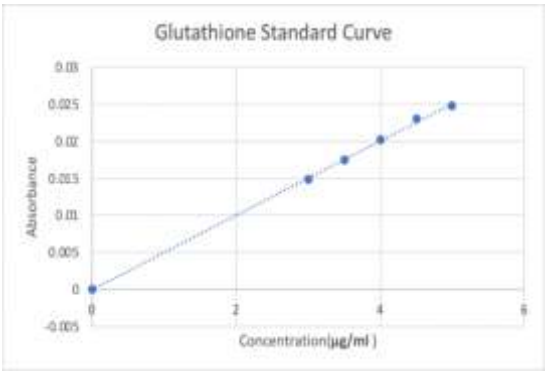


4)Standard curve for Glutathione

Standard stock solutions are prepared. Standard graph plotted by calibration curve method.

Spectrophotometric Data for the estimation of Glutathione:

Concentration(µg/ml)	Absorbance at 204 nm
0	0
3	0.0149
3.5	0.0175
4	0.0202
4.5	0.023
5	0.0248



Glutathione Standard Curve

III. Development of Buccal Film of Glutathione

Preparation of Buccal Film

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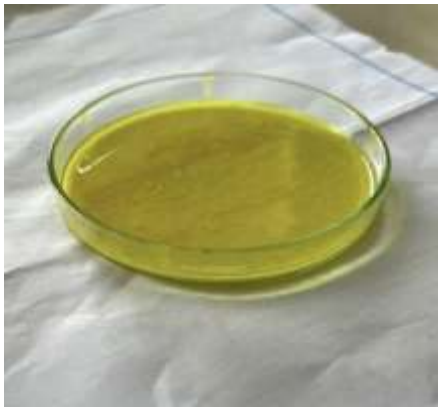
The formulation of buccal films of glutathione were prepared using polymers like HPMCK4M and Carbopol934.



Iv. Evaluation

1) Physicochemical characteristics:

a) Physical Appearance:

All polymer combinations used for fabrication of buccal films showed good film forming properties and reproducibility. The fabricated films were thin, flexible, elastic and smooth.

b) Film thickness:

The thickness was determined. The thickness of each film was measured at 5 different points and the average thickness was calculated. The thickness of film was found to be 0.22±0.15mm.

c) Weight Uniformity:

The weight uniformity was determined. The weight of buccal films of this formulation ranges from 0.20 mg to 0.23 mg.

d) Folding Endurance:

Folding endurance was determined. The folding endurance value for all films was > 200; it indicated that formulation have ideal film property.

e) Surface pH:

Surface pH of formulations was determined. The formulations were found to have pH between 6 to 7. This reveals that the prepared films may not cause any irritation to the buccal mucosa since the values are almost equal to the buccal pH.

2) Swelling Index:

Swelling index was determined. Any polymer with good swelling property is found to be a good candidate for bio adhesive strength. The faster this phenomenon occurs more rapidly will be the polymers adhering to its substrate. The results showed that formulation show good swelling property. The measurement of percentage swelling index of the film was found to be 92.8±0.5%.

3) In vitro release study

The cumulative % drug release data of this formulation has been shown in Table and graph is plotted between % cumulative drug release versus time as shown in figures.

Percentage Cumulative Drug Release:

Time(min)	%Cumulative Drug Release
10	22.12
15	25.6
30	30.02
60	37.4
90	44.03
120	51.5
180	64
240	78.5
300	89.02
360	96.5

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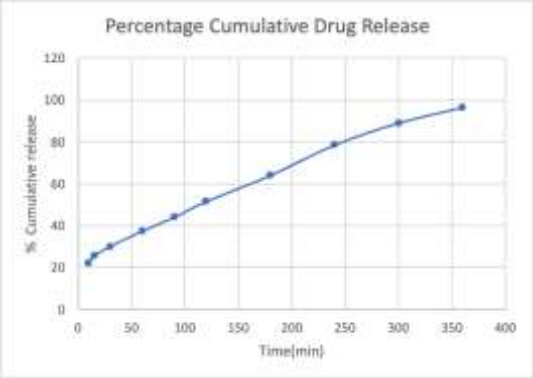


Multiple dissolution apparatus

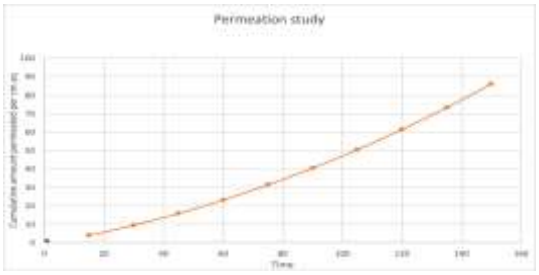
4) In vitro buccal permeation study:

Drug permeation from in vitro diffusion studies of formulation was shown. Formulation has showed maximum release (85.9%)

Franz diffusion cell Slaughtered goat

Measurement of invitro diffusion studies:

Time	%Cumulative Release
15	4.09
30	9.54
45	15.9
60	23.18
75	31.36
90	40.45
105	50.45
120	61.36
135	73.18
150	85.9



Permeation study

5) Stability studies

Sl. No	Parameter	Room Temperature
1	Color	No Change
2	Odour	No Change
3	pH	No Change
4	Texture	Fine
5	Smoothness	Smooth

The result of stability was shown in table. No change in color, odor, texture and smoothness was observed at mentioned conditions of stability.

VI. CONCLUSION

In the present study an attempt was made to develop and evaluate glutathione loaded buccal film. HPMC K4M was used as the main film forming polymer, Carbopol 934 was used as the copolymer, propylene glycol was used as the plasticizer curcumin was also added in order to enhance glutathione activity and film was formulated with the drug "Glutathione"

Before the formulations were made, the preformulation parameters were evaluated including organoleptic properties, solubility study, melting point, determination of λ max, FTIR, and compatibility study. Drug was found to be pure and compatible with the polymers used in the

formulations. FTIR studies concluded that there were no compatibility problems.

Buccal film was prepared by solvent casting method and all were evaluated. Several physiochemical characteristics such as physical appearance, film thickness, weight uniformity, folding endurance, in-vitro buccal permeation study, surface pH and swelling index were performed. The prepared films evaluated were smooth, flexible and elegant in appearance with uniform in weight, thickness and showing good folding endurance. In-vitro buccal permeation study and invitro drug release study was conducted shown satisfactory drug permeation and drug release.

Stability studies were performed and studies indicates that the formulation remains stable.

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