FORMULATION AND OPTIMIZATION OF PROLONGED RELEASE BUCCAL PATCH OF MELATONIN

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Submitted by

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DECLARATION

I SARAVANA KUMAR. M (261910013) hereby declare that the thesis entitled "**FORMULATION AND OPTIMIZATION OF PROLONGED RELEASE BUCCAL PATCH OF MELATONIN**" has been originally carried out by me under the supervision and guidance of **Dr. GRACE RATHNAM, M. Pharm., Ph.D.,** Department of Pharmaceutics, C. L. Baid Metha College of Pharmacy, Chennai-97 during the academic year 2019-2021. This work has not been submitted in any other degree at any other university and that all the sources I have used or quoted have been indicated and acknowledged by complete reference.

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CONTENTS

INTRODUCTION

Among the various routes of drug delivery, the oral route is perhaps the one mostly preferred by patients and clinicians. Based on our current understandings of biochemical and physiological aspects of absorption and metabolism, many drugs, cannot be delivered effectively through the conventional oral route, because after administration are subjected to pre-systemic clearance extensively in liver, which often leads to a lack of significant correlation between membrane permeability, absorption, and bioavailability [1].

Over the time, scientists and researchers in the drug development industries are focusing on alternate routes of administration to add to the potential of approved drug products, or to overcome the drawbacks of the oral route. To deliver drugs systemically via an alternate route of administration such as intranasal (IN), buccal, sublingual, pulmonary, vaginal, rectal, or transdermal (TD) [2].

Transmucosal routes of drug delivery which comprise of the mucosal linings of the nasal, rectal, vaginal, ocular, and oral cavity offer excellent opportunities and potential advantages over peroral administration for systemic drug delivery. These advantages include possible bypass of first pass effect, avoidance of pre systemic elimination within the GI tract and depending on the particular drug [3]. The sites of drug administration in the oral cavity include the floor of the mouth (sublingual), the inside of the cheeks (buccal) and the gums (gingival) [4].

In view of the systemic transmucosal drug delivery, the buccal mucosa is the preferred region as compared to the sublingual mucosa. One of the reasons is that buccal mucosa is less permeable and is thus not able to elicit a rapid onset of absorption and hence better suited for formulations that are intended for sustained release action.

Further, the buccal mucosa being relatively immobile mucosa and readily accessible, it makes it more advantageous for retentive systems used for oral transmucosal drug delivery. A relatively rapid onset of action can be achieved relative to the oral route, and the formulation can be removed if therapy is required to be discontinued [5].

CHARACTERISTICS OF AN IDEAL BUCCO-ADHESIVE SYSTEM [6]:

1. Quick adherence to the buccal mucosa and sufficient mechanical strength.
2. Drug release in a controlled fashion.
3. Facilitates the rate and extent of drug absorption.
4. Should have good patient compliance.
5. Should not hinder normal functions such as talking, eating, and drinking.
6. Possess a wide margin of safety both locally and systemically.
7. Should have good resistance to the flushing action of saliva.

ADVANTAGES OF BUCCAL DRUG DELIVERY SYSTEM [7]:

1. The buccal mucosa is relatively permeable with a rich blood supply, robust in comparison to the other mucosal tissues.
2. Bypass the first-pass effect and non-exposure of the drugs to the gastrointestinal fluids.
3. Easy access to the membrane sites so that the delivery system can be applied, localized, and removed easily.
4. Improve the performance of many drugs, as they are having prolonged contact time with the mucosa.
5. High patient acceptance compared to other non-oral routes of drug administration.
6. Increased residence time combined with controlled API release may lead to lower administration frequency.
7. Additionally significant cost reductions may be achieved, and dose-related side effects may be reduced due to API localization at the disease site.
8. Harsh environmental factors that exist in oral delivery of a drug are circumvented by buccal drug delivery.
9. It offers a passive system of drug absorption and does not require any activation.
10. Provides an alternative route for the administration of various hormones, narcotic analgesics, steroids, enzymes, cardiovascular agents etc.
11. It allows the local modification of tissue permeability, inhibition of protease activity and reduction in immunogenic response. Thus, delivery of therapeutic agents like peptides, proteins and ionized species can be done easily.

DISADVANTAGES OF BUCCAL DRUG DELIVERY SYSTEM [8]:

1. Limited absorption area- the total surface area of the membranes of the oral cavity available for drug absorption is 170 cm^2 of which ~50 cm^2 represents non-keratinized tissues, including buccal membrane.
2. Barrier properties of the mucosa.
3. The continuous secretion of the saliva (0.5-2 l/day) leads to subsequent dilution of the drug.
4. The hazard of choking by involuntarily swallowing the delivery system is a concern.
5. Swallowing of saliva can also potentially lead to the loss of dissolved or suspended drug and ultimately the involuntary removal of the dosage form.
6. Sometimes, the degradation of moisture sensitive drugs takes place by saliva.

PHYSICOCHEMICAL PROPERTIES OF THE ORAL MUCOSA:

The oral mucosa presents differently depending on the region of the oral cavity being considered. The masticatory mucosa covers those areas that are involved in mechanical processes, such as mastication or speech, and includes the gingival and hard palate. This masticatory region is stratified and has a keratinized layer on its surface, similar to the structure found at the epidermis, and covers about 25% of the oral cavity [9].

The specialized mucosa covers about 15%, corresponding to the dorsum of the tongue, and is a stratified tissue with keratinized as well as non-keratinized domains. Finally, the lining mucosa covers the remaining 60% of the oral cavity, consisting of the inner cheeks, floor of the mouth, and underside of the tongue. This lining epithelium is stratified and non-keratinized on its surface [10].

The buccal mucosa covers the inner cheeks and is classified as part of the lining mucosa, having approximately 40–50 cell layers resulting in an epithelium 500–600 μm thick (Fig. 1). The epithelium is attached to underlying structures by a connective tissue or lamina propia, separated by a basal lamina. These lining mucosa and the lamina propia regions provide mostly mechanical support and no major barrier for penetration of actives. The connective tissue also contains the blood vessels that drain into the lingual, facial, and retromandibular veins, which then open into the internal jugular vein [11].

This is one of the main advantages of buccal over oral delivery: absorption through the buccal epithelium avoids the gastrointestinal tract conditions, such as gastric pH, enzyme content, and the first pass effect due to direct absorption into the portal vein. Once a given drug molecule reaches the connective tissue, it may be readily distributed, thus the permeation barrier is across the whole thickness of the stratified epithelium.

The existence of membrane-coating granules in the epidermis has been well characterized and it is known to be the precursor of the keratin layer or stratum corneum. Even though the existence of approximately 2 μm in diameter cytoplasmic membrane- coating granules in the buccal epithelium has been proven, the permeation barrier is believed to be related to the presence of membrane coating granules in the buccal mucosa [12].

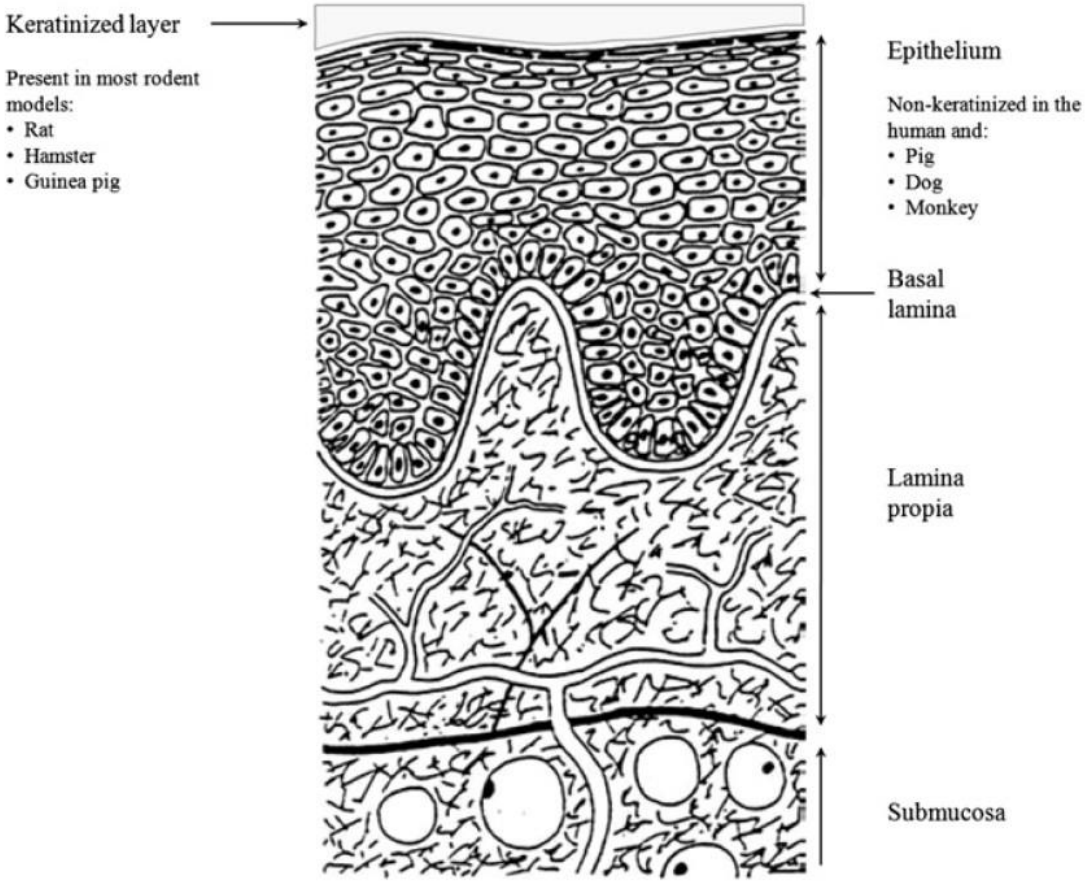


Fig-1: Diagram of a cross section of the buccal mucosa

PERMEABILITY OF ORAL MUCOSA:

Drugs administered via the oral cavity are absorbed into the reticulated and jugular veins and then drained into the systemic circulation, avoiding hepatic first-pass elimination of the

drugs. The superficial layers of the oral mucosa represent the primary barrier to the entry of substances from the exterior (although the lower layers have also been proposed to provide a significant barrier. There are two possible routes of drug absorption through the squamous stratified epithelium of the oral mucosa: transcellular and paracellular [13].

Permeation across the buccal mucosa has been reported to be mainly by the paracellular route through the intercellular lipids produced by membrane-coating granules. It has been argued, however, that the route taken depends on the physicochemical properties of the drug. Generally, small molecules that are predominantly lipophilic, with a log P of 1.6 – 3.3, are absorbed most rapidly; above this value their limited water solubility restricts their absorption [14].

Most drugs delivered successfully via the buccal or sublingual route are, therefore, small, and lipophilic, whereas large hydrophilic molecules are generally poorly absorbed. The amphiphilic nature of the intercellular lipids suggests that both a hydrophobic and hydrophilic pathway through the paracellular route is likely to exist; the situation may, therefore, be more complex than the relatively simple models sometimes described. Although passive diffusion is the main mechanism of drug absorption, specialized transport mechanisms have been reported to exist in other oral mucosa (that of the tongue) for a few drugs and nutrients; glucose and cefadroxil were shown to be absorbed in this way. The buccal mucosa is a potential site for the controlled delivery of hydrophilic macromolecular therapeutic agents (bio- pharmaceuticals) such as peptides, oligonucleotides, and polysaccharides. However, these high molecular weight drugs usually have low permeability leading to a low bioavailability, and absorption enhancers may be required to overcome this. Disease states in which the mucosa is damaged would also be expected to increase permeability.

MUCOADHESION:

Bioadhesion is the general term describing adhesion between any biological and synthetic surface. Mucoadhesion is a specific term describing the particular interaction of a mucosal membrane with a synthetic surface. The phenomenon of mucoadhesion has been explained by applying any of the five theories of adhesion [15].

Most of the mucoadhesive phenomena have two main stages that control the performance of the dosage form: the contact stage and the consolidation stage (Fig. 2). Since mucoadhesive films are dosage forms that are brought in contact with the biological membrane by the patient, the contact stage is initiated by the patient. During the contact process, the film will start dehydrating the mucus gel layer and will itself hydrate, initiating the interpenetration

of the polymeric chains into the mucus and vice versa. For mucoadhesive films, which usually are designed to remain for prolonged times in contact with the buccal mucosa, a second stage, the consolidation stage, needs to take place in order to maintain this bond. In the consolidation stage, the mucoadhesive strength will be determined by the polymer in the formulation, and how readily the dosage form hydrates upon contact with the mucus gel layer [16].

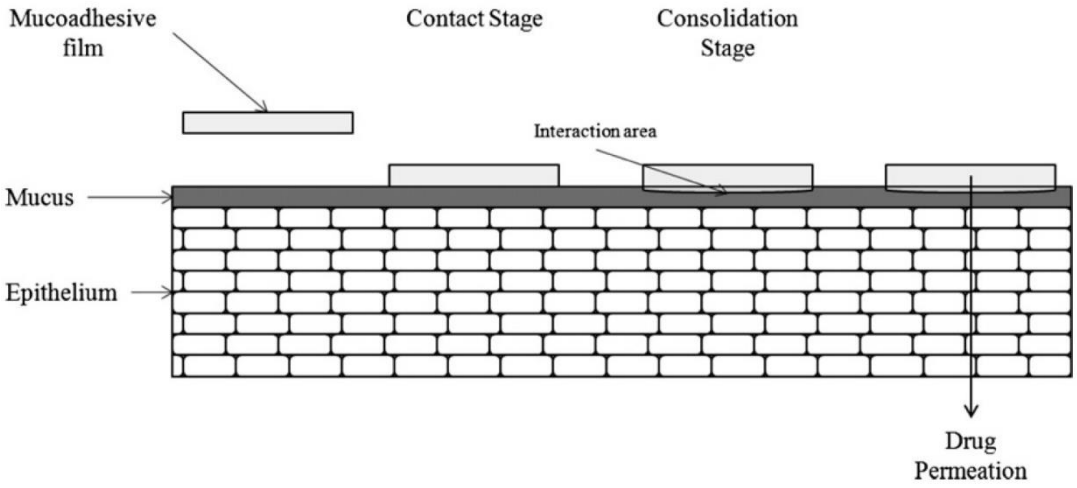


Fig-2: Contact and Consolidation stages of mucoadhesion

THEORIES OF MUCOADHESION:

Mucoadhesion is a complex process and numerous theories have been presented to explain the mechanisms involved a complete and comprehensive theory that can predict adhesion based on the chemical and/or physical nature of a polymer is not yet available. Five theories of adhesion that were originally developed to explain the performance of such diverse materials such as glues, adhesives, and paints, have been adopted to study the mucoadhesion[17].

1. Electronic Theory:

The electronic theory assumes that a double layer of electronic charge is formed at the interface as a result of different electronic characteristics of the mucoadhesive polymer and the mucus, and that attractive forces develop from the electron transfer across the electrical double layer. This system analogous to a capacitor: the system is charged when the adhesive and substrates are in contact and discharged when they are separated [18].

2. Adsorption Theory:

Adsorption theory states that a mucoadhesive polymer adheres to mucus because of the Vander Waals's interactions, hydrogen bonds, electrostatic attraction, hydrophobic interactions, or other related forces [19].

3. Wetting Theory:

The wetting theory emphasize the intimate contact between the mucoadhesive polymer and the mucus and primarily in liquid systems, it uses interfacial tension to predict spreading and subsequent adhesion. The spreading coefficient should be positive in order to adhere to a biological membrane. It was found that interfacial tension was proportional to X ½ , where 'X' is the Flory polymer-polymer interaction parameter. Low values of this parameter correspond to structural similarities between polymers and an increased miscibility [20].

4. Diffusion Theory:

The diffusion theory states that the chains of mucoadhesive polymer and mucin interpenetrate to a sufficient depth (in the range of 0.2 to 0.5 μm) to create a semipermanent bond through entanglement. The interpenetration is governed by diffusion coefficients and contact time, which are in turn dependent on the molecular weights, and flexibility of the chains. The probable penetration depth (L) can be estimated by the formula,

$$L = \sqrt{(tD_b)}^{1/2}$$

Where, 't' is the time of contact, and D_b is the diffusion coefficient of the bioadhesive material in mucus [21].

5. Fracture Theory:

The fracture theory analyzes the force that is required for the separation of two surfaces after adhesion. It is considered to be appropriate for the calculation of fracture strengths of the adhesive bonds involving rigid mucoadhesive materials and has frequently been applied to the analysis of tensile strength measurements. The maximum tensile strength produced during detachment can be determined by dividing the maximum force of detachment (F_m) by the total surface area (A_0) involved in the adhesion interactions. The equation can be written as:

$$S_m = F_m / A_0$$

These general theories are not particularly useful in establishing a mechanistic base to bioadhesives, but they do identify the variables important to the bioadhesion process [22].

FACTORS AFFECTING MUCOADHESION IN THE ORAL CAVITY:

1. Polymer-related factors:

i) Molecular weight:

In general, it has been shown that the bioadhesive strength of a polymer increases with molecular weights above 100,000 [23]. As one example, the direct correlation between the bioadhesive strength of polyoxyethylene polymers and their molecular weights, in the range of 200,000 to 7,000,000, has been shown by Tiwari et al. [24].

ii) Flexibility:

Bioadhesion starts with the diffusion of the polymer chains in the interfacial region. Therefore, it is important that the polymer chains contain a substantial degree of flexibility in order to achieve the desired entanglement with the mucus. A recent publication demonstrated the use of tethered poly(ethylene glycol)–poly(acrylic acid) hydrogels and their copolymers with improved mucoadhesive properties[25]. The increased chain interpenetration was attributed to the increased structural flexibility of the polymer upon incorporation of poly (ethylene glycol).

iii) Hydrogen bonding capacity:

In order for mucoadhesion to occur, desired polymers must have functional groups that are able to form hydrogen bonds. It is also confirmed that flexibility of the polymer is important to improve this hydrogen bonding potential.

iv) Cross-linking density:

The average pore size, the number average molecular weight of the cross-linked polymers, and the density of crosslinking are three important and interrelated structural parameters of a polymer network. Therefore, it seems reasonable that with increasing density of cross-linking, diffusion of water into the polymer network occurs at a lower rate which, in turn, causes an insufficient swelling of the polymer and a decreased rate of interpenetration between polymer and mucin.

v) Charge:

Some generalizations about the charge of bioadhesive polymers have been made previously, where nonionic polymers appear to undergo a smaller degree of adhesion compared to anionic polymers. Peppas and Buri have demonstrated that strong anionic charge on the polymer is one of the required characteristics for mucoadhesion [26]. It has been shown that some cationic polymers are likely to demonstrate superior mucoadhesive properties, especially

in a neutral or slightly alkaline medium. Additionally, some cationic high-molecular-weight polymers, such as chitosan, have shown to possess good adhesive properties.

vi) Concentration:

The importance of this factor lies in the development of a strong adhesive bond with the mucus and can be explained by the polymer chain length available for penetration into the mucus layer. When the concentration of the polymer is too low, the number of penetrating polymer chains per unit volume of the mucus is small, and the interaction between polymer and mucus is unstable [26]. In general, the more concentrated polymer would result in a longer penetrating chain length and better adhesion. However, for each polymer, there is a critical concentration, above which the polymer produces an "unperturbed" state due to a significantly coiled structure. As a result, the accessibility of the solvent to the polymer decreases, and chain penetration of the polymer is drastically reduced. Therefore, higher concentrations of polymers do not necessarily improve and, in some cases, actually diminish mucoadhesive properties [27].

vii) Hydration (swelling):

Hydration is required for a mucoadhesive polymer to expand and create a proper "macromolecular mesh" of sufficient size, and also to induce mobility in the polymer chains in order to enhance the interpenetration process between polymer and mucin. Polymer swelling permits a mechanical entanglement by exposing the bioadhesive sites for hydrogen bonding and/or electrostatic interaction between the polymer and the mucous network. However, a critical degree of hydration of the mucoadhesive polymer exists where optimum swelling and bioadhesion occurs [26].

2. Environmental factors:

The mucoadhesion of a polymer not only depends on its molecular properties, but also on the environmental factors adjacent to the polymer. Saliva, as a dissolution medium, affects the behavior of the polymer. Depending on both the saliva flow rate and method of determination, the pH of this medium has been estimated to be between 6.5 and 7.5. The pH of the microenvironment surrounding the mucoadhesive polymer can alter the ionization state and, therefore, the adhesion properties of a polymer. Mucin turnover rate is another environmental factor. The residence time of dosage forms is limited by the mucin turnover time. Movement of the buccal tissues while eating, drinking, and talking, and even during sleeeping is another concern which should be considered when designing a dosage form.

BUCCAL DOSAGE FORMS:

More recently, several buccoadhesive dosage forms were developed by many researchers with novel approaches emerging continuously and resulting in the clinical application of several drug molecules delivered through the buccal route rapidly, when requiring immediate effect, or in a controlled manner, if a prolonged release is needed [28].

1. Tablets
2. Particulate systems
3. Semisolid dosage forms: hydrogels
4. Wafers
5. Films/Patches

Films/Patches:

The development of buccal films has increased dramatically over the past decade as a promising alternative delivery for various therapeutic classes including proteins and peptides, analgesics, anti-inflammatory and anesthetic drugs. Great attention has been focused on this dosage form due to its high design flexibility, good adaptation to the mucosal surface, small size and reduced thickness, as well as patient compliance.

Moreover, buccoadhesive films are particularly convenient in patients that present swallowing difficulties, mainly in the pediatric and elderly fields. Thanks to their improved mechanical properties, they represent a less friable dosage form compared to most commercialized orally fast disintegrating tablets, which usually require special packaging.

Buccal films also reduce pain by protecting the wound surface and hence increase the treatment effectiveness. An ideal buccal film should be flexible, elastic, and soft yet strong enough to withstand breakage due to stress from activities in the mouth. Moreover, it should also possess good mucoadhesive strength so that it is retained in the mouth for the desired duration.

MANUFACTURE OF BUCCAL FILMS/PATCHES:

The main manufacturing processes involved in making mucoadhesive buccal films namely,

1. Film casting
2. Hot-melt extrusion.

Film casting:

The film casting method is undoubtedly the most widely used manufacturing process for making films found in the literature. This is mainly due to the ease of the process and the low cost that the system setup incurs at the research laboratory scale. The process consists of at least six steps: preparation of the casting solution; deareation of the solution; transfer of the appropriate volume of solution into a mold; drying the casting solution; cutting the final dosage form to contain the desired amount of drug; and packaging. During the manufacture of films, particular importance is given to the rheological properties of the solution or suspension, air bubbles entrapped, content uniformity, and residual solvents in the final dosage form.

The rheology of the liquid to be casted will determine the drying rates and uniformity in terms of the active content as well as the physical appearance of the films. During the mixing steps of the manufacturing process, air bubbles are inadvertently introduced to the liquid and removal of air is a critical step for homogeneity reasons. Films cast from aerated solutions exhibit an uneven surface and heterogeneous thickness [16].

Hot-melt extrusion of films:

In hot-melt extrusion, a blend of pharmaceutical ingredients is molten and then forced through an orifice (the die) to yield a more homogeneous material in different shapes, such as granules, tablets, or films. Hot-melt extrusion has been used for the manufacture of controlled-release matrix tablets, pellets, and granules, as well as orally disintegrating films. However, only a handful of articles have reported the use of hot-melt extrusion for manufacturing mucoadhesive buccal films.

BASIC COMPONENTS OF BUCCAL DRUG DELIVERY SYSTEM:

Drug substance:

The suitable active pharmaceutical ingredient or drug substance should be selected on the basis of its pharmacokinetic properties. The drug should be of following characteristics:

- The one time dose of drug should be small (dose $\leq$ 25 mg) [29].
- The drug should be having short biological half-life ranging from 2 to 8 hrs.
- The drugs showing first pass metabolism can be used for buccal drug delivery for avoiding the first pass metabolism.

Bioadhesive polymer:

The use of bio adhesive polymer determines the various parameters such as mucoadhesive strength, thickness, in-vitro release and the residence time of the drug delivery device. Generally, the polymers with high molecular weight are preferred because; they show effective release rate controlling properties. An ideal polymer should have following characteristics for achieving the optimized results:

- It should be inert.
- It should be compatible with the environment and drug.
- It should be adhered quickly with the mucus membrane and adherence should be long lasting for required time.

Backing membrane:

Backing membrane used for the formulations should be impermeable to drug as well as mucus in order to prevent the unnecessary drug loss from all sides of the device. The materials used for preparing backing membrane should be inert, insoluble or should have low water solubility. The commonly used materials in backing membrane include ethyl cellulose, carbopol, sodium alginate, HPMC, polycarbophil etc.

Plasticizers:

The plasticizers are used in order to improve the folding endurance of the delivery device. They provide enough flexibility to the dosage form for improving its patient acceptability and patient compliance. Few examples of commonly used plasticizers are PEG-400, PEG-600, dibutyl phthalate, propylene glycol etc.

Permeation enhancers:

These are the chemicals or liquids used to improve the permeation of drug from device into the mucus membrane. The permeation enhancers work by following mechanisms.

- By reducing the viscosity of mucus.
- By increasing the fluidity of lipid bilayer membrane.
- By countering the enzymatic barrier.
- By increasing the thermodynamic activity of drugs.

INSOMNIA:

Insomnia is a common complaint that can present independently or comorbidly with another medical disorder (eg, pain) or psychiatric disorder (eg, depression). Insomnia is the most prevalent sleep disorder and affects large proportions of the population on a situational, recurrent, or persistent basis. It carries a heavy burden for both patients and the health-care system as evidenced by its effect on quality of life, and on psychological, occupational, and economic domains. Insomnia is often unrecognized and untreated because of barriers to assessment and management [30].

EPIDEMIOLOGY:

Prevalence:

About 25% of adults are dissatisfied with their sleep, 10–15% report symptoms of insomnia associated with daytime consequences, and 6–10% meet criteria for an insomnia disorder. Insomnia is one of the most prevalent complaints in primary care; complaints increase with age and are twice as prevalent in women as in men.

Comorbidity:

A high rate of comorbidity exists between chronic insomnia and medical and psychiatric disorders. In the 2002 US National Health Interview Survey, individuals with insomnia were more than five times as likely to present anxiety or depression, and more than twice as likely to present congestive heart failures as individuals without insomnia.

Course and prognosis:

Insomnia can be a situational, recurrent, or persistent problem. Acute insomnia is often associated with life events or sleep schedule changes (eg, jet lag or shift work) and usually remits once the precipitating event has subsided. For some individuals, sleep disturbance can persist even after the initial cause has disappeared. Insomnia can follow an intermittent course, with recurrent episodes of sleep difficulties associated with stressful events. Even in persistent insomnia, night-to-night variability in sleep is often reported, with an occasional good night's sleep intertwined with periods of disrupted sleep. The prognosis of untreated insomnia is not well documented; however, chronic insomnia raises the risks for depression, hypertension, and, possibly, mortality in older adults. These associations reinforce the need to identify and treat insomnia early to prevent chronicity and morbidity [31].

Pathophysiological mechanisms:

Definitive pathophysiological mechanisms have not been identified, although several neurobiological abnormalities are associated with insomnia. Patients with insomnia show increased activation of the autonomic nervous system, as evidenced by sleep-related elevations in heart rate and heart rate variability, metabolic rate, body temperature, activity of the hypothalamic-pituitary adrenal axis activity, and norepinephrine secretion.

In one study, night-time blood pressure was higher in patients with insomnia than in controls. Changes in brain activity consistent with hyperarousal occur in insomnia.

Individuals with insomnia are more likely to have a family history of the disorder, which suggests a genetic vulnerability, a common environmental factor, or a learned component. Abnormalities related to sleep-wake regulatory genes have not yet been identified in insomnia.

PHARMACOTHERAPY:

Various drugs are used to treat insomnia, including over the-counter agents (OTCs; antihistamines, melatonin, and herbal preparations), prescription hypnotic drugs for insomnia (BzRAs, chronobiotic agents, and low-dose doxepin hydrochloride), and other prescription agents not specifically indicated for insomnia (antidepressants, antipsychotics, and anticonvulsants).

OTC agents:

Antihistamines used as sleep-inducing agents include diphenhydramine or doxylamine succinate, which are often combined with pain-relieving drugs such as paracetamol or ibuprofen.

Melatonin is a hormone produced by the pineal gland that contributes to reinforcement of circadian and seasonal rhythms. Synthetic melatonin is sold as a dietary supplement in the USA, but in some countries, it is deemed a prescription drug. Some evidence supports use of synthetic melatonin for insomnia related to circadian rhythm disorders such as delayed sleep phase and shift work sleep disorder. Side-effects can include drowsiness, dizziness, headache, nausea, and nightmares.

A range of herbal preparations are used for insomnia, most commonly valerian.

Hypnotic agents:

Most prescription hypnotic agents act as agonists at the benzodiazepine receptor; these

include several benzodiazepines and the structurally distinct BzRAs (Benzodiazepine receptor agonists). For example, zaleplon, zolpidem, zopiclone etc. newer BzRAs. Chronobiotic agents for insomnia include prolonged release melatonin and ramelteon.

Other prescription agents:

Antidepressants with sedating effects are some of the most commonly prescribed drugs for insomnia although data about efficacy are generally scarce. The doses of antidepressants typically used to induce sleep are substantially less than antidepressant doses. A sedating antidepressant might be appropriate for a patient with insomnia and major depression, either when used at a therapeutic dose or in combination with another antidepressant. Antidepressants might be considered for patients with a history of substance misuse or other contraindications to use of a controlled substance.

Antidepressants prescribed for insomnia include trazodone hydrochloride, mirtazapine, tricyclic antidepressants such as doxepin, and agomelatine. These agents can improve sleep in patients with comorbid depression and can have sleep-promoting effects in individuals with primary insomnia.

LITERATURE REVIEW

Silvia Rossi *et al.*, (2005) has described the main obstacles that drugs meet when administered via the buccal route derive from the limited absorption area and the barrier properties of the mucosa. The effective physiological removal mechanisms of the oral cavity that take the formulation away from the absorption site are the other obstacles that must be considered. The strategies studied to overcome such obstacles include the employment of new materials that, possibly, combine mucoadhesive, enzyme inhibitory and penetration enhancer properties and the design of innovative drug delivery systems which, besides improving patient compliance, favor a more intimate contact of the drug with the absorption mucosa [5].

John D Smart (2005) has reviewed the buccal formulations developed to allow prolonged localized therapy and enhanced systemic delivery, while avoiding first pass effects and the barrier to drug absorption especially for biopharmaceutical products. The bioadhesive polymers used in buccal delivery to retain a formulation and newer second generation bioadhesives have been discussed [13].

Javier O. Morales *et al.*, (2010) has reviewed the manufacture and characterization of mucoadhesive buccal films which has a number of advantages including bypassing the gastrointestinal tract and the hepatic first pass effect. Mucoadhesive films are retentive dosage forms and release drug directly into a biological substrate. The development of mucoadhesive buccal films has increased dramatically over the past. The ‘‘film casting process’’ involves casting of aqueous solutions and/or organic solvents to yield films suitable for this administration route. Over the last decade, hot-melt extrusion has been explored as an alternative manufacturing process and has yielded promising results. Characterization of critical properties such as the mucoadhesive strength, drug content uniformity, and permeation rate represent the major research areas in the design of buccal films [16].

Surender Verma *et al.*, (2011) reviewed the buccal drug delivery and its significant attention and momentum since it offers remarkable advantages. Over past few decades, buccal route for systemic drug delivery using mucoadhesive polymers to significantly improve the performance of many drugs has been of profound interest. This review article is an overview of buccal drug delivery systems encompassing a review of oral mucosa, formulation considerations for buccal

drug delivery system, theories and mechanism of mucoadhesion, different mucoadhesive formulations for buccal drug delivery and active ingredients delivered via the buccal route. Additionally, commercial technologies and future prospects of this route of drug delivery are discussed [7].

D. Zetner *et al.*, (2015) reviewed the pharmacokinetics of alternative administration routes of melatonin *in vivo*. Alternative administration routes were defined as all administration routes except oral and intravenous. 10 studies were included in the review. Intranasal administration exhibited a quick absorption rate and high bioavailability. Transdermal administration displayed a variable absorption rate and possible deposition of melatonin in the skin. Oral transmucosal administration of melatonin exhibited a high plasma concentration compared to oral administration. Subcutaneous injection of melatonin displayed a rapid absorption rate compared to oral administration. Transdermal application of melatonin has a possible use in a local application, due to slow absorption and deposition in the skin. Oral transmucosal administration may potentially be a clinically relevant due to avoiding first-pass metabolism. Subcutaneous injection of melatonin did not document any advantages compared to other administration routes [32].

Charles M Morin *et al.*, (2012) described Insomnia is a prevalent complaint in clinical practice that can present independently or comorbidly with another medical or psychiatric disorder. In either case, it might need treatment of its own. Of the different therapeutic options available, benzodiazepine-receptor agonists (BzRAs) and cognitive-behavioural therapy (CBT) are supported by the best empirical evidence. BzRAs are readily available and effective in the short-term management of insomnia, but evidence of long-term efficacy is scarce and most hypnotic drugs are associated with potential adverse effects. CBT is an effective alternative for chronic insomnia. Although more time consuming than drug management, CBT produces sleep improvements that are sustained over time, and this therapy is accepted by patients [30].

C. M. Ellis *et al.*, (1995) studied the hypnotic action of melatonin 5 mg p.o in 15 subjects with psychophysiological insomnia in a double blind controlled self-report questionnaire study. Effects on sleep and wakefulness were monitored by visual analogue scale and structured interview. Bedtime, sleep onset time, estimated total sleep and wake time, as well as self-rated sleep quality, were not altered by melatonin, and estimates of next-day function did not change.

The period of melatonin treatment was retrospectively correctly identified by 8 of 15 subjects. Despite unchanged ratings of night sleep quality on the last night of each treatment, 7 of 15 subjects reported that sleep had subjectively improved to a minor extent in the week of active treatment [33].

Rudiger Hardeland (2009) reviewed the hypnotic effects of melatonin and melatoninergic drugs are mediated via MT1 and MT2 receptors, especially those in the circadian pacemaker, the suprachiasmatic nucleus, which acts on the hypothalamic sleep switch. A major obstacle for the use of melatonin to support sleep maintenance in primary insomnia results from its short half-life in the circulation. Solutions to this problem have been sought by developing prolonged-release formulations of the natural hormone, or melatoninergic drugs of longer half-life, such as ramelteon, tasimelteon and agomelatine. With all these drugs, improvements of sleep are statistically demonstrable, but remain limited, especially in primary chronic insomnia [34].

Rob L. DeMuro *et al.*, (1995) studied the absolute bioavailability of oral melatonin tablets in 12 normal healthy volunteers. Subjects were administered, in a randomized crossover fashion, melatonin 2 mg intravenously and 2 and 4 mg orally. Blood was sampled over approximately eight (estimated) half-lives. Both the 2 and the 4 mg oral dosages showed an absolute bioavailability of approximately 15%. No difference in serum half-life was seen in any of the study phases. Oral melatonin tablets in dosages of 2 and 4 mg show poor absolute bioavailability, either due to poor oral absorption, large first-pass metabolism, or a combination of both. Further studies examining larger doses, in an attempt to saturate first-pass metabolism if it occurs,may be warranted [35].

Patrick Lemoine *et al.*, (2012) reviewed and summarizes published studies on Circadin's efficacy and safety (Summary of Product Characteristics and Medline search on 'Circadin' and 'insomnia'). The main significant and clinically relevant benefits are improvements in sleep quality and latency, next-day morning alertness and quality of life. The responses may develop over several days. An oral 2 mg dose once daily, for 3 months, has generally been well tolerated with no rebound, withdrawal or 'hangover' effects and no safety concerns on concomitant therapy with antihypertensive, antidiabetic, lipid-lowering or anti-inflammatory drugs. Untoward effects of hypnotics on cognition, memory, postural stability and sleep structure are not seen with Circadin. Given as a first-line prescription, with 13 weeks' posology and the lack

of rebound effects, Circadin has the potential to improve quality of life in insomnia patients aged 55 years and older and avoid long-term use of hypnotics [36].

Bhusnure O.G *et al.*, (2017) have developed and validated a spectrophotometric method to estimate Melatonin in tablet dosage form according to Quality by Design (QbD) approach as per ICH Q8 (R2) guidelines. QbD approach was carried out by varying various parameters and these variable parameters were designed into Ishikwa diagram. The critical parameters were determined by using principle component analysis as well as by observation. The estimated critical parameters in zero order spectroscopic method were Methanol, sample preparation tablet, wavelength 222 nm, slit width: 1.0, scan speed medium, and sampling interval: 0.2, The above methods were validated according to ICH Q2(R1) guidelines. Proposed methods can be used for routine analysis of Melatonin in tablet dosage form as they were found to be robust and specific [37].

G. S. Asane *et al.*, (2013) reviewed bioadhesive polymers that adhere to the mucin/epithelial surface are effective and lead to significant improvement in oral drug delivery. Improvements are also expected for other mucus-covered sites of drug administration. Bioadhesive polymers applications in the eye, nose, and vaginal cavity as well as in the GI tract, including the buccal cavity and rectum. This article lays emphasis mainly on mucoadhesive polymers, their properties, and their applications in buccal, ocular, nasal, and vaginal drug delivery systems with its evaluation methods [38].

Ana Flo *et al.*, (2016) formulated and evaluated melatonin in different vehicles to assess the influence of different vehicles on the permeation of Melatonin through buccal and skin tissues. Transmucosal results showed that sodium carboxymethylcellulose 4 % and glycerin 8 % was the best and OB (orabase®) the worst vehicle. Poloxamer 407 20% w/v to water and Poloxamer lecithin oganogel 10% w/v and isopropyl palmitate (10% w/v) followed similar behaviour. Photostability studies revealed high percentage of degradation of melatonin in solution which was also similar when was loaded in OB. The rest of formulations showed low rates of degradation. C940 or M68 (Montanov 68) and NaCMC can be proposed as formulations for a potential systemic effect of MLT by skin and buccal mucosa routes, respectively. However, if the intended objective is to obtain local action in the skin and buccal mucosa, the proposed formulations are M68 or P407 and PLO [39].

Pakorn Kraisit *et al.*, (2016) prepared the hydroxypropyl methylcellulose (HPMC)/polycarbophil (PC) mucoadhesive blend film and to investigate the main and interaction effect of HPMC and PC mixtures on the physicochemical and mechanical properties of blend films using a simplex lattice mixture design approach. The cubic and quadratic models were selected to analyze mucoadhesive properties in terms of work of adhesion and maximum detachment force, respectively. It was shown that HPMC/PC blend film had higher mucoadhesive properties than pure HPMC film. The suitable models for analyzing swelling index of blend films at various times were assessed. The puncture strength, % elongation and hydrophilicity of films were also examined. The pure HPMC film displayed more homogeneous and smoother structures compared with the blend film, as observed by SEM and AFM. Intermolecular hydrogen bonding between HPMC and PC was detected using FTIR and XRD. Therefore, the blend film shows high potential for use as a buccal delivery system [40].

Rachna Kumria *et al.*, (2018) formulated and evaluated chitosan-based buccal bioadhesive films of zolmitriptan. Factorial design (3^2) is constructed and conducted in a fully randomized manner to study all 9 possible experimental runs. The films were prepared by solvent casting method by varying the content of chitosan (X_1) with levels of (0.25, 0.50, 0.75 mg) and polyvinyl alcohol (X_2) with levels of (1, 2, 3 mg). The effect of these two independent variables on swelling index (Y_1), percent drug release in 15 min (Y_2) and 5 h (Y_3), and mucoadhesive strength (Y_4) of prepared films was evaluated. The mucoadhesion increased with an increase in factor X_1 and decreased when the factor X_2 was increased. This study concludes that the chitosan-based buccal film (F7) having chitosan 0.75 mg and PVA 1 mg was optimized and could be used in both prophylaxis and acute treatment of migraine, although need to be proved in vivo [41].

Amelia M. Avachat *et al.*, (2013) developed mucoadhesive buccal films using tamarind seed xyloglucan (TSX) as novel mucoadhesive polysaccharide polymer for systemic delivery of Rizatriptan through buccal route using 3^2 full factorial design. TSX (X_1) 2 %, 4 %, 6 % and glycerin (X_2) 4 %, 8 %, 12% were selected as factors with responses of tensile strength (Y_1), bioadhesion force (Y_2), and % drug release at 2h (Y_3). *Ex vivo* diffusion studies were carried out using Franz diffusion cell, while bioadhesive properties were evaluated using texture analyzer with porcine buccal mucosa as model tissue. Out of 9 formulations, the formulation

F5 having TSX 4% and glycerin 8% was chosen as optimal formulation. This study suggests that tamarind seed polysaccharide can act as a potential mucoadhesive polymer for buccal delivery of a highly soluble drug like Rizatriptan benzoate [42].

Surya N. Ratha Adhikari *et al.*, (2010) have formulated and evaluated buccal patches for the delivery of atenolol using sodium alginate (600 to 900 mg) with various hydrophilic polymers like Carbopol 934 P (100 to 300 mg), sodium carboxymethyl cellulose (100 – 300 mg), and hydroxypropyl methylcellulose (100 to 300 mg) in various proportions and combinations were fabricated by solvent casting technique. Various physicomechanical parameters like weight variation, thickness, folding endurance, drug content, moisture content, moisture absorption, and various *ex vivo* mucoadhesion parameters like mucoadhesive strength, force of adhesion, and bond strength were evaluated. An *in vitro* drug release study was designed, and it was carried out using commercial semipermeable membrane. All these fabricated patches were sustained for 24 h and obeyed first-order release kinetics. *Ex vivo* drug permeation study was also performed using porcine buccal mucosa, and various drug permeation parameters like flux and lag time were determined [43].

Mohamed S.Pendekal *et al.*, (2011) prepared a monolayered buccal patch containing Tizanidine hydrochloride using the emulsification solvent evaporation method. The polymers Eudragit RS 100 or Eudragit RL 100 (6, 10, 15 mg) and chitosan (0.5, 1, 2 mg). Polymer solutions in acetone 30 % w/w were combined with a THCl aqueous solution (in some cases containing chitosan) by homogenization at 9000rpm for 2min in the presence of triethyl citrate as plasticizer and cast in novel Teflon molds. Physicochemical properties such as film thickness, in vitro drug release and in vitro mucoadhesion were evaluated after which permeation across sheep buccal mucosa was examined in terms of flux and lag time. Out of 15 formulation F5 was concluded as superior. Formulations prepared using a Eudragit polymer alone exhibited satisfactory physicomechanical properties but lacked a gradual in vitro drug release pattern. Incorporation of chitosan into formulations resulted in the formation of a porous structure which did exhibit gradual release of drug [44].

Anroop B. Nair *et al.*, (2012) reviewed the *in vitro* techniques to evaluate buccal films, there are no official standardized methods for its evaluation. Significant efforts have been made to demonstrate and improve the efficacy, potency and safety of buccal film using in vitro, ex vivo

and in vivo assessments. Besides the physical properties of the film, several other parameters such as residence time, mucoadhesion, drug release, in vitro and in vivo buccal permeation profiles and absorption kinetics of the drug are examined while characterizing the prepared buccal films. This review provides an overview about the various parameters that are considered and assessed as a part of formulation development to ensure quality product with desired characteristics [45].

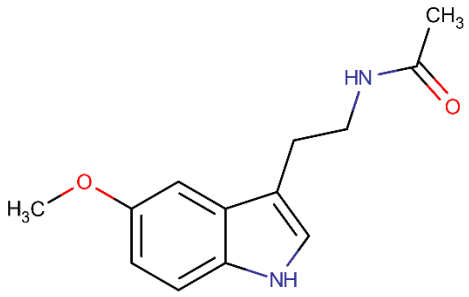
Anroop B. Nair *et al.*, (2018) developed and evaluated oral mucoadhesive film with palonosetron. Films were prepared by solvent casting method using Proloc 15 and Eudragit RL 100 polymers. The composition of polymers (3, 5, 7.5 %) and plasticizers (2.5, 3, 5 %) were optimized and evaluated for physicomechanical properties, mucoadhesion, swelling, drug release and permeation across mucosal membrane. The drug loaded films (F1-F4) demonstrated desirable physical properties, mechanical strength and mucoadhesion. Rapid hydration of films was observed which may provide prompt mucoadhesion of film with the buccal mucosa. A biphasic drug release profile was noticed in films (F1-F4), with greater amount being released in 2 h. Ex vivo studies using films (F3 and F4 with 0.25 mg and 0.5 mg palonosetron per cm^2, respectively) showed greater transport when drug concentration was high. Scanning electron microscopy image shows that drug loaded film possesses morphological features of an ideal film [46].

DRUG PROFILE

Drug : Melatonin

Synonym : N-acetyl-5-methoxytryptamine

Chemical structure



IUPAC Name : N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide

Molecular Formula : $C_{13}H_{16}N_2O_2$

Molecular Weight : 232.28

Category : Psycholeptics

Indication : Used for insomnia, jet lag, and circadian rhythm disorders

Description : A white to off-white crystalline powder.

Solubility : Slightly soluble in water, soluble in acetone, ethyl acetate and methanol.

Melting point : 117 °C

Pharmacodynamics:

Melatonin is a hormone normally produced in the pineal gland and released into the blood. The essential amino acid L-tryptophan is a precursor in the synthesis of melatonin. It helps regulate sleep-wake cycles or the circadian rhythm. Production of melatonin is stimulated by darkness and inhibited by light. High levels of melatonin induce sleep and so consumption of the drug can be used to combat insomnia and jet lag. MT1 and MT2 receptors may be a target for the treatment of circadian and non-circadian sleep disorders because of their differences in pharmacology and function within the SCN. SCN is responsible for maintaining the 24-hour cycle which regulates many different body functions ranging from sleep to immune functions [47].

Pharmacokinetics:

Absorption:

Melatonin was rapidly and extensively absorbed, with a T_{max} range of 1.5-3 h in humans. But oral bioavailability was low in humans, averaging around 15% across studies. The pharmacokinetics in humans appear to be non-linear and likely as a result of saturable first pass hepatic metabolism [48].

Distribution:

A value of 0.55 L/kg was reported for humans in the literature, suggesting reduced tissue distribution compared with animals. However, volume of distribution has been reported to vary with age, and values of 1.8-2.5 L/kg have also been reported for adult and prepubertal subjects in the literature. Melatonin was also shown to bind to human plasma proteins (albumin > alpha1-acid glycoprotein > high density lipoprotein with weak binding to other proteins) over the concentration range 0.2-2 nm.

Metabolism:

Melatonin is rapidly and primarily metabolised by the liver and cleared from the body. The major metabolic pathway determined in humans involves 6-hydroxylation in the liver via the hepatic microsome P-450 system to yield 6- hydroxymelatonin. The second, less significant pathway is 5-demethylation to yield the melatonin precursor, N-acetyl serotonin. Both 6-hydroxymelatonin and N-acetylserotonin are ultimately conjugated to sulphate and glucuronic acid and excreted in the urine as their corresponding 6-sulphatoxy and 6-glucoronide derivatives.

Elimination:

In humans 70-90% of urinary radioactivity was identified as the sulphate and/or glucuronide conjugates of 6-hydroxymelatonin. Melatonin is rapidly metabolized and eliminated, with 90% of administered radioactivity excreted within 24 h of dosing in humans. The apparent elimination half-lives in humans were 40-50 min.

Commercially available products:

Circadin 2 mg

Slenyto 1 mg and 5 mg

EXCIPIENTS PROFILE [49]

HYDROXY PROPYL METHYL CELLULOSE K4M

Non-proprietary names:

BP: Hypromellose

JP: Hydroxypropylmethylcellulose

PhEur: Hypromellosum

USP: Hypromellose

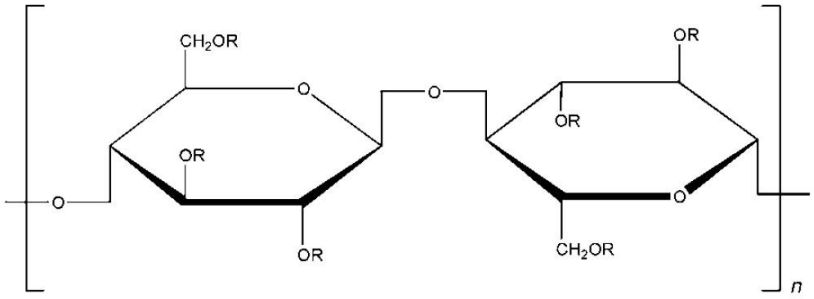
Synonyms:

Hydroxypropyl methylcellulose, HPMC, Methocel, methylcellulose propylene glycol ether, methyl hydroxypropylcellulose, Metolose, Tylopur.

Description:

An odorless, tasteless, white or creamy white colored fibrous or granular powder.

Structural formula:



where R is H, CH_3, or $CH_3CH(OH)CH_2$

Chemical name:

Cellulose, 2-hydroxypropylmethyl ether, cellulose hydroxypropylmethyl ether

Molecular weight:

Approximately 86,000

Functional category:

Coating agent, film former, tablet binder, stabilizing agent, suspending agent, Viscosity increasing agent, and emulsion stabilizer.

Density:

Bulk Density -0.341 g/cm^3

Tapped Density -0.557 g/cm^3

True Density -1.326 g/cm^3

Solubility:

Soluble in cold water forming viscous colloidal solution, insoluble in chloroform, alcohol and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol.

Viscosity:

4000 mPas

Stability and storage conditions:

Very stable in dry conditions. Solutions are stable at PH 3.0-11.0. Store in a tight container, in a cool place

Incompatibilities:

Extreme PH conditions, oxidizing materials.

Safety:

Human and animal feeding studies have shown HPMC to be safe.

Applications:

Hypromellose is widely used in oral, ophthalmic and topical pharmaceutical formulations. In oral products, hypromellose is primarily used as a tablet binder, in film-coating, and as a matrix for use in extended-release tablet formulations.

CARBOPOL 974P

Non-proprietary names:

BP: Carbomers

PhEur: Carbomera

USPNF: Carbomer

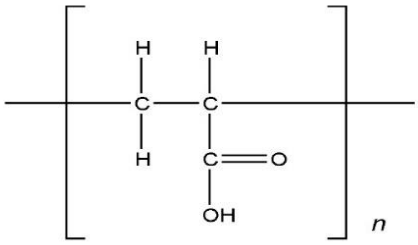
Synonyms:

Acritamer, acrylic acid polymer, Carbopol, carboxy polymethylene, polyacrylic acid, carboxyvinyl polymer, Pemulen, Ultrez.

Description:

Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a slight characteristic odour.

Structural formula:



Chemical name:

Carbomer

Molecular weight:

Approximately 7×10^5 to 4×10^9 Da

Functional category:

Bioadhesive, emulsifying agent, release-modifying agent, suspending agent, tablet binder, viscosity-increasing agent.

Density:

Bulk Density -0.341 g/cm^3

Tapped Density -0.557 g/cm^3

True Density -1.326 g/cm^3

Solubility:

Soluble in water and, after neutralization, in ethanol (95%) and glycerin.

Viscosity:

300 - 115000 mPas

Stability and storage conditions:

Carbomers are stable, hygroscopic materials. Exposure to excessive temperatures can result in discoloration and reduced stability. Carbomer powder should be stored in an airtight, corrosion resistant container in a cool, dry place.

Incompatibilities:

Incompatible with phenol, cationic polymers, strong acids, and high levels of electrolytes.

Safety:

Carbomers are generally regarded as essentially nontoxic and non-irritant materials; there is no evidence in humans of hypersensitivity reactions to carbomers.

Applications:

Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents. Carbomer resins have also been investigated in the preparation of sustained-release matrix beads, as enzyme inhibitors of intestinal proteases in peptide-containing dosage forms, as a bioadhesive for a cervical patch and for intranasally administered microspheres, in magnetic granules for site-specific drug delivery to the esophagus and in oral mucoadhesive controlled drug delivery systems.

POLYETHYLENE GLYCOL 400

Non-proprietary names:

BP: Macrogols

PhEur: Macrogola

USPNF: Polyethylene glycol

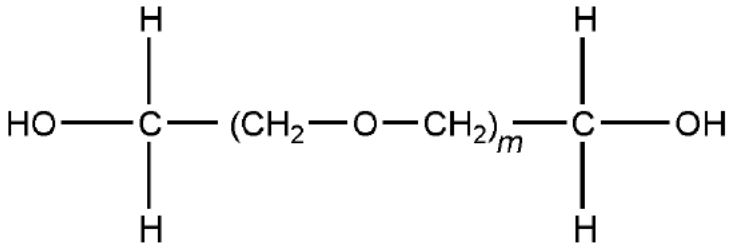
Synonyms:

Carbowax, Carbowax Sentry, Lipoxol, Lutrol E, PEG, Pluriol E, polyoxyethylene glycol.

Description:

Liquid grades (PEG 200–600) occur as clear, colourless or slightly yellow-colored, viscous liquids. They have a slight but characteristic odour and a bitter, slightly burning taste. PEG 600 can occur as a solid at ambient temperatures.

Structural formula:



Chemical name:

α-Hydro-ω-hydroxypoly(oxy-1,2-ethanediyl)

Molecular weight:

PEG 400 - Approximately 380–420

Functional category:

Ointment base; plasticizer; solvent; suppository base; tablet and capsule lubricant.

Density:

1.11–1.14 g/cm^3 at 258C for liquid PEGs.

Solubility:

Liquid polyethylene glycols are soluble in water, acetone, alcohols, benzene, glycerin, and glycols.

Viscosity:

PEG 400 - 105–130 mPas

Stability and storage conditions:

Polyethylene glycols are chemically stable in air and in solution. It should be stored in well closed containers in a cool, dry place.

Incompatibilities:

Incompatible with some coloring agents, phenol, tannic acid, and salicylic acid.

Safety:

Polyethylene glycols are widely used in a variety of pharmaceutical formulations. Generally, they are regarded as nontoxic and nonirritant materials.

Applications:

Polyethylene glycols (PEGs) are widely used in a variety of pharmaceutical formulations including parenteral, topical, ophthalmic, oral, and rectal preparations. It has been used experimentally in biodegradable polymeric matrices used in controlled-release systems.

ETHYL CELLULOSE

Non-proprietary names:

BP: Ethylcellulose

PhEur: Ethylcellulosum

USPNF: Ethylcellulose

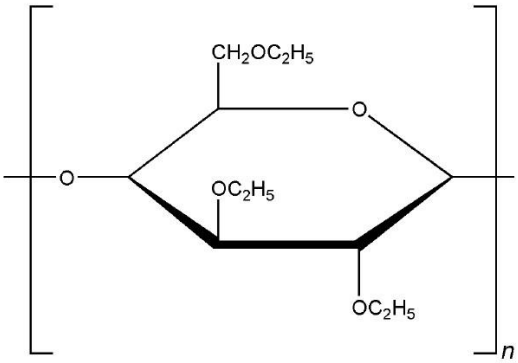
Synonyms:

Aquacoat ECD, Aqualon, E462, Ethocel, Surelease.

Description:

Ethylcellulose is a tasteless, free-flowing, white to light tan colored powder.

Structural formula:



Chemical name:

Cellulose ethyl ether

Molecular weight:

Approximately 454.5

Functional category:

Coating agent, flavoring fixative, tablet binder, tablet filler, viscosity-increasing agent.

Density:

Bulk Density -0.4 g/cm^3

Solubility:

Ethylcellulose is practically insoluble in glycerin, propylene glycol, and water.

Viscosity:

7 to 100 mPa s

Stability and storage conditions:

Ethylcellulose is a stable, slightly hygroscopic material. Ethylcellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. Ethylcellulose should be stored at a temperature not exceeding 32ºC in a dry area.

Incompatibilities:

Incompatible with paraffin wax and microcrystalline wax.

Safety:

Ethylcellulose is widely used in oral and topical pharmaceutical formulations. It is also used in food products.

Applications:

The main use of ethylcellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethylcellulose, dissolved in an organic solvent or solvent mixture, can be used on its own to produce water-insoluble films. Higher-viscosity ethylcellulose grades tend to produce stronger and more durable films. Ethylcellulose films may be modified to alter their solubility, by the addition of hypromellose or a plasticizer.

AIM AND OBJECTIVE

Aim:

The aim of the present investigation is to formulate prolonged release buccal patches containing melatonin of 2 mg.

Melatonin when orally administered has low bioavailability of ~15% due to extensive first pass metabolism, and it has a very short half-life of about ~45min. So, the aim is to develop a prolonged release buccal patch of melatonin and to improve its bioavailability.

Objective:

- To formulate buccal patches of Melatonin using various polymer concentrations of Hydroxy propyl methyl cellulose, and Carbopol.
- To study the influence of drug polymer ratio on drug release.
- To optimize the buccal patch using design of experiments.
- To evaluate the patches for their physicochemical parameters like appearance, thickness, weight uniformity, folding endurance, drug content, surface pH, swelling index.
- To conduct in vitro dissolution studies, *ex vivo* permeation studies, and stability studies.

PLAN OF WORK

1. **Literature review**

2. **Preformulation studies**
    a) Description of the drug
    b) Incompatibility studies
    c) Calibration curve of Melatonin

3. **Formulation of Melatonin Buccal patches**
    a) Preparation of HPMC and Carbopol patches
    b) Preparation of Backing layer

4. **Optimization of Melatonin buccal patches**
    a) Using Design of Experiment software

5. **Evaluation of patches**
    a) Appearance of the film
    b) Weight variation
    c) Thickness of the patch
    d) Folding endurance
    e) Swelling Index
    f) Surface pH
    g) % Moisture loss
    h) Drug content uniformity
    i) In vitro dissolution study
    j) Ex-vivo permeation studies
    k) Ex-vivo Mucoadhesion time

6. **In Vitro Release Kinetics**

MATERIALS AND METHODS

LIST OF MATERIALS USED AND MANUFACTURERS

Table - 1: List of materials used and manufacturers

S.No	Materials	Manufacturers
1.	Melatonin	Flamma Group, Italy
2.	HPMC K4M	Merck Limited, Mumbai
3.	Carbopol 974P	SD Fine-Chem Limited, Mumbai
5.	Poly ethylene glycol 400	Fischer Scientific Chemicals, Mumbai
6.	Ethyl cellulose E15	Vipul Chem, china
7.	Ethanol	Merck Limited, Mumbai

LIST OF INSTRUMENTS USED AND MANUFACTURERS

Table - 2: List of instruments used and manufacturers

S.No	Instruments	Manufacturers
1.	Digital Balance	Infra, India
2.	Digital pH meter	Elico Ltd, India
3.	Mechanical Stirrer	Remi, India
4.	Dissolution apparatus	Electrolab TDT OP, India
5.	Vernier caliper	Mitutoyo, Japan
6.	Franz Diffusion cell apparatus	Orchid Scientific Pvt Limited, India
7.	UV spectrometer	Shimadzu UV 1800, Japan

METHODOLOGY

PREFORMULATION STUDY

Description of Drug

Physicochemical properties of drugs such as state, colour, odour was physically examined and compared with the reported description of drugs.

Drug polymer compatibility study

Fourier transform Infra-red (FT-IR) was the tool for solid state characterization of pharmaceutical solid. FT-IR Spectroscopy of pure drug, and physical mixture were carried out on Shimadzu FT-IR 8400S model to investigate any possible interaction between the drug and the utilized excipients. The samples were finely grounded with KBr to prepare the pellets under a hydraulic pressure of 600 psi and a spectrum was scanned in the wavelength range of 4000 and 500 cm^{-1} using Shimadzu FT-IR spectrophotometer.

PREPARATION OF STANDARD CURVE

The wave length of maximum absorbance of Melatonin was found to be 222 nm using Phosphate buffer pH 6.8 as blank. 25 mg of melatonin was weighed and transferred to a 50 ml volumetric flask and made upto the volume using methanol. From the resulting solution 1mL were pipetted out into separate 25 mL volumetric flask and made upto the volume using pH 6.8 Phosphate buffer to represent 20 µg/mL of the drug. From this solution 1, 2, 3, 4, and 5 mL into separate 10 mL volumetric flasks and made upto the volume using pH 6.8 Phosphate buffer to represent 2, 4, 6, 8, and 10 µg/mL of the drug. The absorbance of the solutions was measured at 222 nm taking 6.8 Phosphate buffer as blank using UV-Visible spectrophotometer. The calibration curve was then plotted taking concentration (µg/mL) along X-axis and absorbance along Y- axis.

PREPARATION OF BUCCAL PATCH

The buccal film was prepared by solvent casting method. First the accurately weighed quantity of polymer HPMC K4M was dissolved in required quantity of ethanol:water (1:1) mixture while stirred by a mechanical stirrer. The accurately weighed quantity of carbopol 974P was dissolved in required quantity of distilled water and then neutralized by 10 % NaOH solution to get a transparent viscous solution. Then the carbopol solution was poured into the HPMC solution while stirring to get a carbopol HPMC polymer dispersion. Then the accurately

weighed quantity of melatonin was dissolved in a minimum quantity of ethanol and added to the polymer dispersion while stirring. Then weighed quantity of plasticizer PEG 400 was added to the drug-polymer dispersion while stirring and left to stir for 30 min to get a homogenous solution. After 30 min of stirring the solution was left idle until the air bubbles were removed and then casted into a petri dish and left for air drying for 24 h, resulting in a thin film after the solvent evaporation. The composition of the prepared buccal patches were shown in table - 4.

PREPARATION OF BACKING LAYER

The backing layer was prepared by dissolving the accurately weighed quantity of ethyl cellulose in ethanol while stirring to get a 5% w/v solution and then weighed quantity of 2% v/v plasticizer PEG 400 was added while stirring and left to stir for 15 min. Then the ethyl cellulose solution was casted on to a petri dish and left for 24 hours to dry, resulting a thin hydrophobic layer after solvent evaporation. Then the hydrophobic layer was attached to the film by using 5% w/v PVP solution as binder.

DRUG LOADED IN THE PATCH

Diameter of petri dish = 9.6 cm

Radius of the petri dish (r) = 4.8 cm radius

Total Surface Area of petri dish= πr^2 = 3.14 X 4.8 X 4.8 = 72.3 cm^2

Now, Dose was 2 mg in 2 cm X 2 cm = 4 cm^2

4 cm^2 contain 2 mg of drug.

Number of 4 cm^2 films obtained from the main film = 72.3/4 = 18.07

Approximately 18 films of 4 cm^2 can be obtained.

Thus, the amount of drug should be incorporated in the area = 18.07 × 2 = 36.14 mg

So, 72.3 cm^2 contains 36.14 mg of drug.

OPTIMIZATION OF BUCCAL FILM

Design of experiment (DOE)

A two factor and three-level factorial design was used as the experimental design. The independent variables studied were amount of HPMC K4M (X_1) and Amount of Carbopol 974P (X_2). Time taken for 50% drug release (Y_1), Drug release at 8^{th} hour (Y_2), Mucoadhesion time (Y_3) were considered as dependent variables which were shown in table - 3.

Experimental design

The factorial design is a technique that allows identification of factors involved in a process and assesses their relative importance. In addition, any interaction between factors chosen can be identified. Construction of a factorial design involves the selection of parameters and the choice of responses. Experimental runs were designed by Design Expert 11.0.1 [Stat Ease. Inc.] Software following full factorial method. 3^2 full factorial design was applied for examining two variables (factors) at three levels with a minimum of 9 runs shown in table - 4. Totally nine melatonin buccal patch formulations were prepared employing selected combinations of the two factors as per 3^2 Factorial and evaluated to find out the significance of combined effects of the two factor to select the best combination required to achieve the desired melatonin buccal patch.

Table - 3: Factors and Factor levels investigated in factorial experimental design

Factors: Formulation Variables	**Levels (mg/patch)**		
	-1	**0**	**+1**
HPMC K4M	300	600	900
Carbopol 974P	120	240	360
Response	**Goal**		
Time taken for 50% drug release	Minimize		
Drug release at 8th hour	Maximize		
Mucoadhesion time	Maximize		

Table - 4: The formulation design matrix for the film in mg/patch

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9
HPMC K4M (mg)	300	600	600	900	300	900	600	300	900
Carbopol 974P (mg)	360	360	240	240	240	360	120	120	120
PEG 400 (mg)	300	300	300	300	300	300	300	300	300
Melatonin (mg)	36.14	36.14	36.14	36.14	36.14	36.14	36.14	36.14	36.14
Ethanol:water (ml)	30	30	30	30	30	30	30	30	30
Water (ml)	30	30	30	30	30	30	30	30	30

Optimization

To understand the influence of formulation variables on the quality of formulations with a minimal number of experimental trials and subsequent selection of formulation variables to develop an optimized formulation using established statistical tools for optimization.

Mathematical modeling, evaluation of the ability to fit to the model and response surface modeling were performed with employing Design-Expert® software (Version 11). In a full factorial design, all the factors are studied in all the possible combinations. Hence, 3^2 factorial designs were chosen for the current formulation optimization study.

EVALUATION OF PATCH

Appearance of the film

The overall appearance of the patch was checked visually.

Weight variation

Three films of 4 cm^2 size were cut randomly, individually the patch were weighed on electronic balance and the mean weight was calculated.

Thickness of patch

The thickness of patch was directly related to drug content uniformity so it was essential to find uniformity in the thickness of the film. It can be measured by calibrated digital Vernier Calipers. The thickness was measured at different spots of the patch and average was taken as film thickness.

Drug content

Spectrophotometric method was used to assess the uniformity of drug distribution through measuring drug content at different parts of the same film. Three 4 cm^2 of each film were weighed individually, dissolved in 20 ml methanol, and the solution was then filtered through filter paper and the concentration of melatonin was measured spectrophotometrically at 222 nm. Each preparation was tested in triplicates, and the percentage drug content was calculated from the following equation,

$$\% \text{ Drug content} = \frac{\text{Actual amount}}{\text{Theoretical amount}} \times 100$$

Folding Endurance

The folding endurance of the patch was used to estimate the mechanical strength of the patch to withstand the folding or the ability to withstand the brittleness. It was measured by repeatedly folding a patch at the same line before it breaks. The folding endurance was the number of times the film was folded without breaking. Higher the folding endurance value greater was the strength of the patch [50].

Swelling property

Phosphate buffer pH 6.8 was prepared to check the swelling property of the patch. The initial weight of the patch was determined and placed in the preweighed stainless steel mesh. The system was dipped in the Phosphate buffer pH 6.8. The increase in the weight of the patch was noted by weighing the system at regular intervals [50]. The degree of swelling was determined by the formula,

$$\text{Degree of swelling} = \frac{[\text{Final weight } (W_t) - \text{Initial weight } (W_o)]}{[\text{Initial weight } (W_o)]} \times 100$$

Surface pH

Patch was slightly wet with help of water. The pH was measured by bringing the electrode in contact with the surface of the patch. The study was performed on three patch of each formulation and average was taken [50].

Percent moisture loss

lt was done to check the integrity of patch at dry condition and hygroscopicity of patch. Three patch of 4 cm^2 size were cut out and weighed accurately. Then the patch were rested in a desiccator Containing fused anhydrous calcium carbonate. After 3 days the patches are removed, weighed and percentage weight loss are calculated. Average percentage moisture loss three patch was calculated [50].

$$\% \text{ Moisture loss} = \frac{\text{Final weight - Initial weight}}{\text{Final weight}} \times 100$$

Ex vivo mucoadhesion time

The ex vivo mucoadhesion/retention time of the oral buccoadhesive films was determined using goat cheek mucosa. Goat check pouch of size 3 x 3 cm^2 was cut and pasted on the inner side of the beaker using double-sided adhesive tape. The film of size 4 cm^2 was cut, and its surface was made wet using a drop of Phosphate buffer pH 6.8. Films were pasted on the surface of the goat pouch by applying a gentle force for 10 sec. Phosphate buffer pH 6.8

(500 ml), maintained at 37 ±1°C, was poured into the beaker and stirred at 150 rpm to simulate buccal conditions. All the experiments were performed in triplicate [51].

In vitro dissolution test

As there was no official method prescribed for *in vitro* drug release study of buccal patches. A method mentioned and used in previous studies was carried out. The patch was pasted on to the inner side of the vessel using double side adhesive tape. Dissolution was carried out by using prewarmed pH 6.8 Phosphate buffer as dissolution medium. A suitable volume of the sample was withdrawn at every 1 hour. The dissolution parameter was maintained as below Apparatus: USP Type II paddle, Medium: 900 ml of Phosphate buffer pH 6.8, Speed: 50 RPM, Temperature: 37ºC ± 0.5ºC, Time: 8 hours, Sampling interval: 1hr. The absorbance of the resulting solution was measured by UV spectrometer at 222 nm [51].

Ex-vivo Permeation study

Tissue preparation

Buccal mucosa was obtained from freshly sacrificed goat at a local ranch. The mucosa was transported to the laboratory in an isotonic buffer solution pH 7.4 and used within 2h of animal sacrifice. The majority of underlying connective tissues was removed with the help of a scalpel blade and then the remaining buccal mucosa was carefully trimmed with surgical scissor to a proximately uniform thickness of about 500 μm. It was then used for permeation study.

Permeation study

The Ex-vivo buccal permeation study was carried out for best optimized formulation. The permeation study of melatonin through the excised layer of goat buccal mucosa was performed using Franz diffusion cell at 37 ± 0.5^{O} C. Fresh goat buccal mucosa was mounted between the donor and receptor compartments. The buccal patch was placed with the core facing the mucosa, and the compartments were clamped together. The donor compartment was filled with 5ml of phosphate buffer pH 6.8. The receptor compartment was filled with phosphate buffer pH 6.8± 0.5 and the hydrodynamics in the compartment was maintained by stirring with a magnetic bead at uniform slow speed. The amount of drug permeated through the buccal mucosa was determined by withdrawing samples at predetermined time intervals and analyzed for drug content by UV spectrophotometer at 222 nm [50].

DRUG RELEASE KINETICS

The matrix systems were reported to follow the zero order release rate and the diffusion mechanism for the release of the drug. To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted into, Zero order, First order, Higuchi matrix, Hixson crowell and peppa's model. In this by comparing the r values obtained, the best fit model was selected [50].

Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation

$$Q_t = Q_o + K_o t$$

Where Q_t was the amount of drug dissolved in time t, Q_o was the initial amount of drug in the solution and K_o was the zero order release constant.

First order kinetics

To study the first order release kinetics the release rate data were fitted to the following equation.

$$\text{Log } Q_t = \log Q_o + k1_t/2.303.$$

Where Q_t was the amount of the drug released in time t, Q_o was the initial amount of the drug in the solution and K1 was the first order release constant.

Higuchi model

Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. And the equation was

$$Q_t = K_H\text{-}t^{1/2}$$

Where Q_t was the amount of drug released in time t, K_H was the Higuchi dissolution constant.

Korsmeyer and Peppa's model

To study this model the release rate data are fitted to the following equation.

$$M_t/M_\alpha = K.tn$$

Where M_t/M_α was the fraction of drug release, K was the release constant, t was the release time and n was the Diffusional exponent for the drug release that was dependent on the shape of the matrix dosage form.

Hixson and Crowell erosion equation

$$Q_O^{1/3} - Qt^{1/3} = K_{HC}t$$

Where,

Q_t = Amount of drug released at time t

Q_O = Initial amount of drug

K_{HC} = Rate constant for Hixson Crowell equation

RESULTS AND DISCUSSION

PREFORMULATION STUDY:

Description of Drug

The appearance of the Melatonin was visually observed. It was found that it was a white powder and it complies with the IP.

Drug polymer compatibility study

The compatibility of drug in the formulation was confirmed by comparing FT-IR spectra of pure drug with FT-IR of its drug with excipients shown in Fig – 3 and 4.

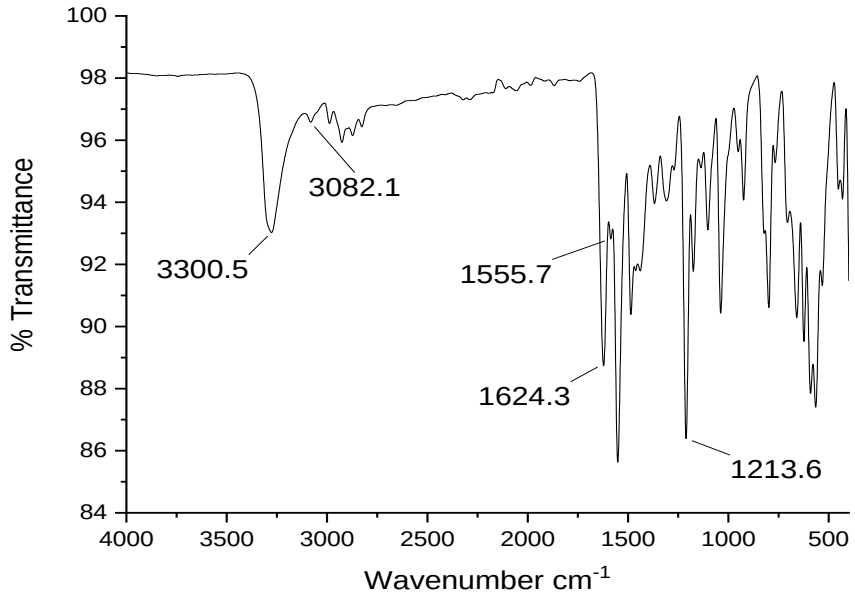


Fig-3: FT-IR spectra of Melatonin

Table - 5: IR spectra interpretation of melatonin

S.No	Wavenumber cm^{-1}	Interpretation
1	3300.5	N – H
2	3082.1	C – H
3	1555.7	C - N
4	1624.3	N – H bending
5	1213.6	C-O-C stretching

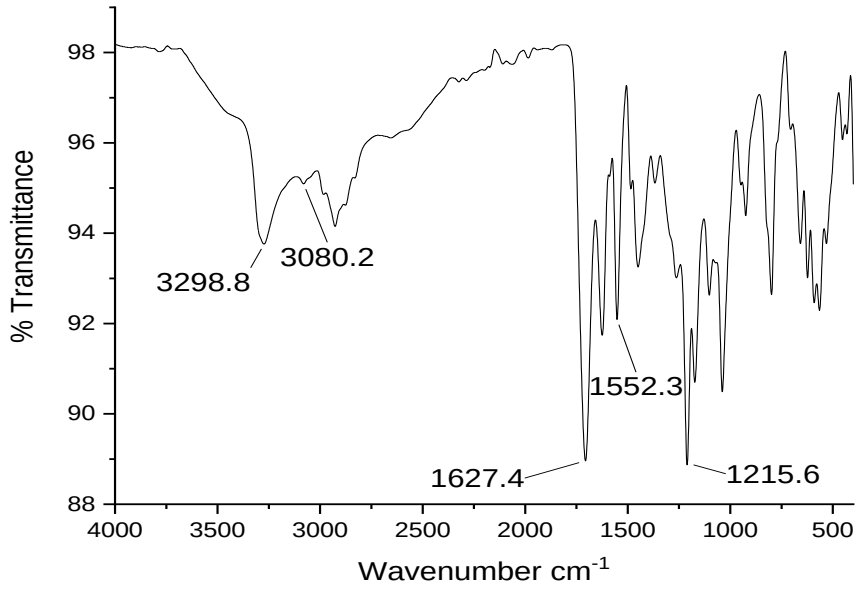


Fig - 4: FT-IR spectra of Melatonin with all excipients

Table - 6: IR spectra interpretation of melatonin with all excipients

S.No	Wavenumber cm^{-1}	Interpretation
1	3298.8	N – H
2	3080.2	C – H
3	1552.3	C - N
4	1627.4	N – H bending
5	1215.6	C-O-C stretching

Inference:

The FTIR spectra of melatonin with all excipients showed no shift and no disappearance of the characteristic peaks of melatonin suggesting that there is no interaction between the drug and the excipients.

PREPARATION OF STANDARD CURVE

The standard curve of melatonin in Phosphate buffer pH 6.8 is given in table - 7 and Fig - 5.

Table - 7: Data for calibration curve of melatonin in Phosphate buffer pH 6.8

Concentration (µg/mL)	Absorbance at 222nm
2	0.130
4	0.340
6	0.530
8	0.752
10	0.950

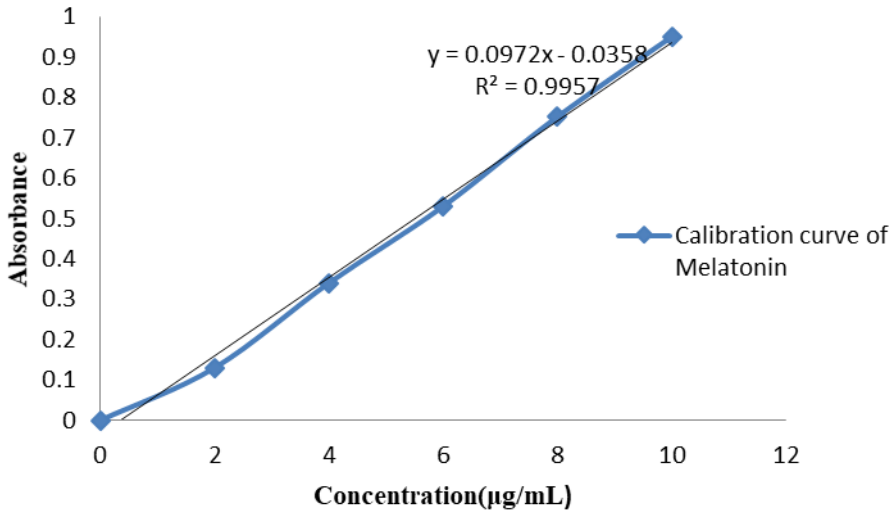


Fig- 5: Calibration curve of melatonin in Phosphate buffer 6.8

It was found that the solution of melatonin in Phosphate buffer pH 6.8 shows linearity (R^2 = 0.9957) in absorbance at concentrations of 2 -10 (µg/mL) and obey Beer Lambert Law.

EVALUATION OF PATCHES:

Appearance of the film

The overall appearance was found to be clear and transparency was good which showed that the drug has distributed uniformly.

Weight variation

Three films of size 4 cm^2 were cut randomly, individually the patch were weighed on electronic balance and the mean weight was calculated. Weight of patches was ranging from 26.99±0.7 to 97.31±0.5 mg. Weight of patches was found to be increasing proportion of polymer. The results were shown in table - 8.

Table - 8: Weight Variation of patches

Formulation	Weight (mg) (n=3)
F1	44.34 ± 0.6
F2	71.22 ± 0.3
F3	62.57 ± 0.7
F4	89.13 ± 0.8
F5	35.89 ± 0.2
F6	97.31 ± 0.5
F7	53.68 ± 0.6
F8	26.99 ± 0.7
F9	80.25 ± 0.5

Thickness of patch

Thickness of all the patches was found to be in the range of 0.09±0.83 to 0.27±0.85 mm. As the total amount of polymer increases the thickness of the patches were found to be increased. The results were shown in table – 9 and Fig - 6.

Table - 9: Thickness of patches

Formulation	Thickness (mm) (n=3)
F1	0.14 ± 0.33
F2	0.21 ± 0.89
F3	0.18 ± 0.57
F4	0.25 ± 0.64
F5	0.11 ± 0.97
F6	0.27 ± 0.85
F7	0.17 ± 0.69
F8	0.09 ± 0.83
F9	0.24 ± 0.76

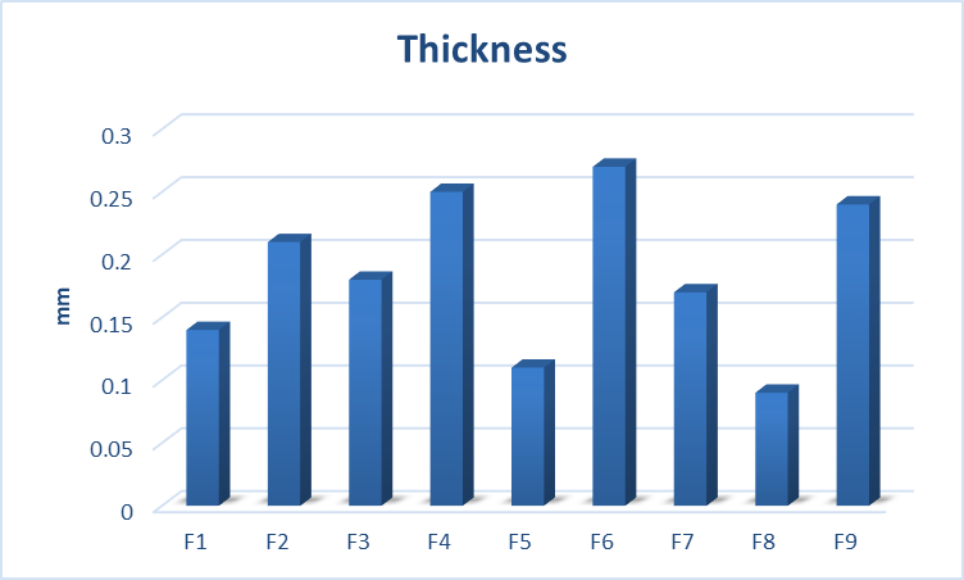


Fig – 6: Thickness of patches

Drug content

All the batches of the patches contain 98.15±0.3 to 101.51±0.1 % of drug which indicate that there is no loss of drug during preparation of the patch. All the batches of the patches exhibit drug content within limit 98 to 102 % which is within the desirable range due to the equal distribution of drug in the solution. The results were shown in table - 10.

Table - 10: Drug content of patches

Formulation	Drug content (%) (n=3)
F1	99.82 ± 0.8
F2	101.57 ± 0.6
F3	98.25 ± 0.9
F4	98.54 ± 0.9
F5	99.10 ± 0.5
F6	98.43 ± 0.4
F7	100.56 ± 0.2
F8	101.51 ± 0.1
F9	98.15 ± 0.3

Surface pH

Surface pH for all batches was between 6.5±0.03 to 7.1±0.01 which were due to pH of the drug solution as well as the polymer, hence no mucosal irritations was expected and ultimately achieves patient compliance. The results were shown in table - 11.

Table - 11: Surface pH of patches

Formulation	Surface pH (n=3)
F1	6.5 ± 0.01
F2	6.8 ± 0.02
F3	6.4 ± 0.01
F4	6.8 ± 0.03
F5	6.8 ± 0.02
F6	7.1 ± 0.01
F7	6.6 ± 0.04
F8	6.5 ± 0.03
F9	6.7 ± 0.02

Folding Endurance

Folding endurance is the index of ease of handling the patches. As the amount of polymer increases the folding endurance was found to be increased. Folding endurance for the patches was found to be 412±19 to 530±14. All patches exhibited folding endurance above 300 proving the flexible nature of the patch. The results were shown in table - 12 and Fig - 7.

Table - 12: Folding endurance of patches

Formulation	Folding endurance (n=3)
F1	493 ± 16
F2	412 ± 19
F3	502 ± 15
F4	519 ± 19
F5	485 ± 16
F6	530 ± 14
F7	483 ± 16
F8	470 ± 15
F9	506 ± 18

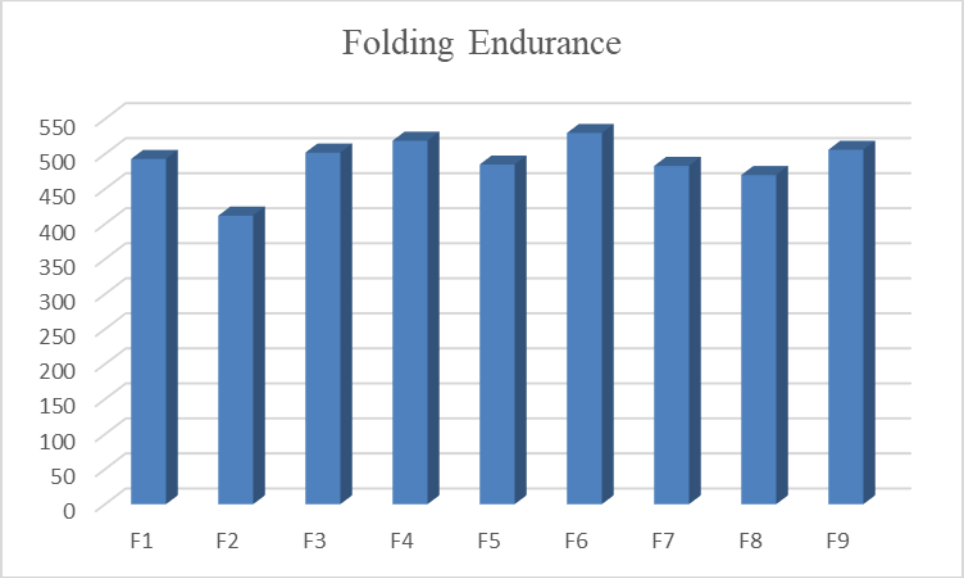


Fig - 7: Folding endurance of patches

Swelling property

Swelling index shows the moisture uptake and swelling behavior of buccal patches. All the patches were subjected to swelling studies. The results indicated that all the patches exhibited appreciable swelling nature. The swelling index increasing with polymer concentration for HPMC K4M. Also it increases with increasing content of carbopol 974P. The results were shown in table - 13 and Fig - 8.

Table - 13: Swelling index of patches

Formulation	Swelling index (%) (n=3)
F1	224.08 ± 4.5
F2	250.56 ± 3.4
F3	244.87 ± 2.2
F4	297.02 ± 3.1
F5	212.69 ± 2.8
F6	312.43 ± 3.4
F7	225.56 ± 2.4
F8	202.34 ± 2.0
F9	285.09 ± 3.6

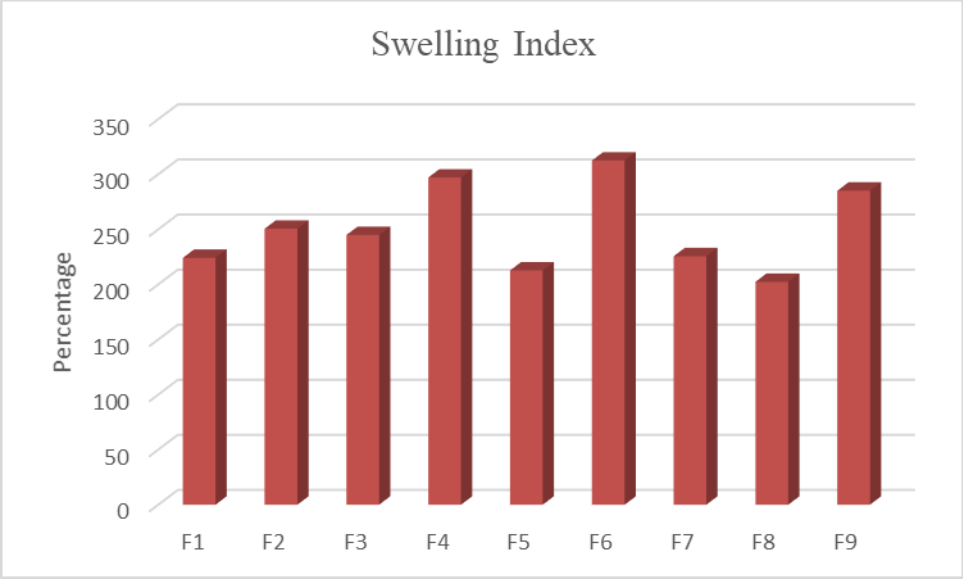


Fig - 8: Swelling index of patches

Percent moisture loss

The percentage moisture loss of all batches were between 1.25±0.02 to 2.41±0.12 %, which was carried out to ensure physical stability or integrity of buccal films. The increase in polymer concentration increases % moisture loss. This shows that there is no considerable change in the physical stability and integrity of patches. The results were shown in table - 14 and Fig - 9.

Table - 14: Percentage moisture loss of patches

Formulation	Moisture loss (%) (n=3)
F1	1.68 ± 0.02
F2	2.13 ± 0.09
F3	2.05 ± 0.07
F4	2.28 ± 0.04
F5	1.46 ± 0.06
F6	2.41 ± 0.12
F7	1.91 ± 0.08
F8	1.25 ± 0.02
F9	2.21 ± 0.01

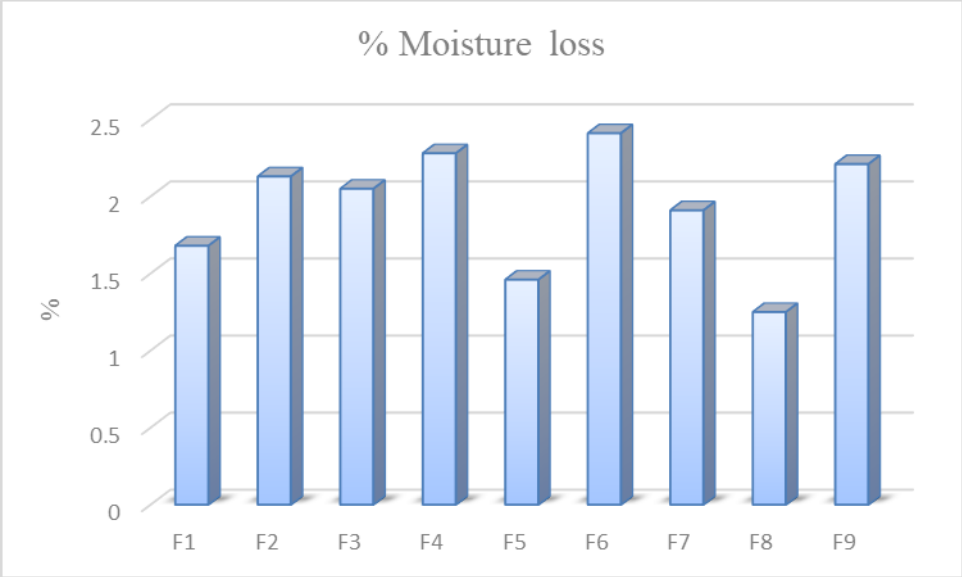


Fig - 9: Percentage moisture loss of patches

Ex vivo mucoadhesion time

Mucoadhesion time of the patches were ranging from 390±09 to 616±18 min. It shows that increasing in HPMC K4M concentration increases the mucoadhesion time significantly, but increasing carbopol 974P concentration shows very less appreciation in the mucoadhesion time. Formulation F1, F5, F8 doesn't meet required mucoadhesion time for 8 h due to low HPMC K4M concentration. The results were shown in table – 15 and Fig - 10.

Table - 15: Mucoadhesion time of patches

Formulation	Mucoadhesion time (min) (n=3)
F1	427 ± 13
F2	530 ± 17
F3	518 ± 12
F4	592 ± 14
F5	415 ± 11
F6	616 ± 18
F7	510 ± 13
F8	390 ± 09
F9	572 ± 15

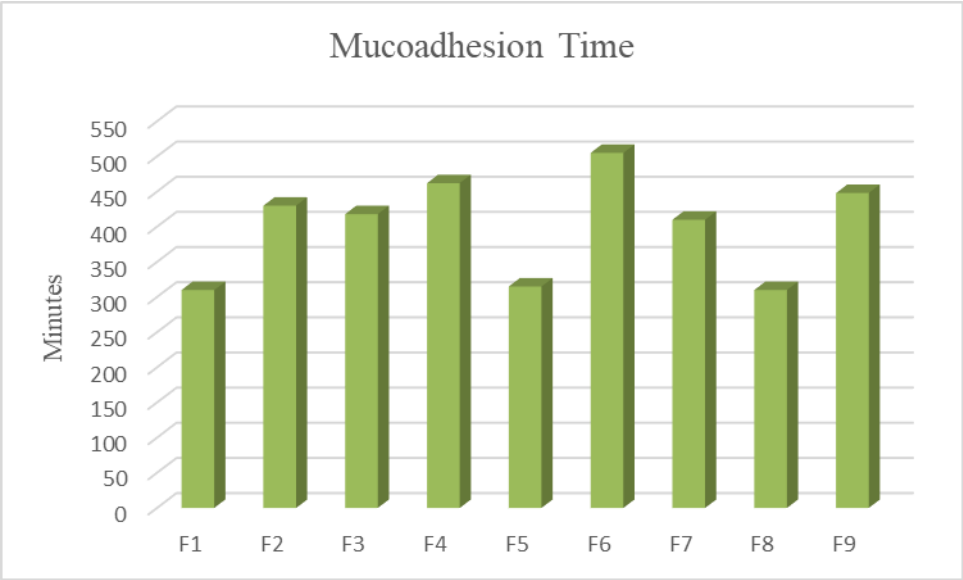


Fig - 10: Mucoadhesion time of Patches

In vitro dissolution test

The *in vitro* drug release studies were done for all the batches in Phosphate buffer pH 6.8 using Dissolution apparatus USP type II. The release data were given in the table - 16 and Fig - 11 and 12.

Dissolution Parameters

Dissolution medium : Phosphate buffer pH 6.8 (900ml)

Paddle speed : 50 rpm

Apparatus : Dissolution apparatus USP type II

Temperature : 37ºC ± 0.5ºC

Withdrawal time : 8h with 1h interval

Volume withdraw : 5 ml

Table - 16: Cumulative percentage drug release of patches

Time (hour)	**Cumulative percent drug release**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1	12.72	8.06	11.05	7.56	12.88	6.83	12.01	12.91	7.23
2	21.02	19.78	21.03	18.36	22.14	16.42	20.91	22.31	19.21
3	33.41	30.12	32.21	29.21	33.72	28.66	32.86	34.01	29.91
4	48.24	45.86	46.53	44.92	49.79	43.17	47.61	51.08	45.03
5	58.55	56.17	56.29	53.92	61.06	53.31	57.37	62.45	54.30
6	67.74	64.98	65.53	62.99	68.01	63.11	66.80	70.10	64.66
7	80.73	73.60	76.44	71.89	81.18	71.06	78.15	84.97	72.27
8	94.45	88.23	91.67	80.76	96.74	77.13	94.32	99.34	84.65

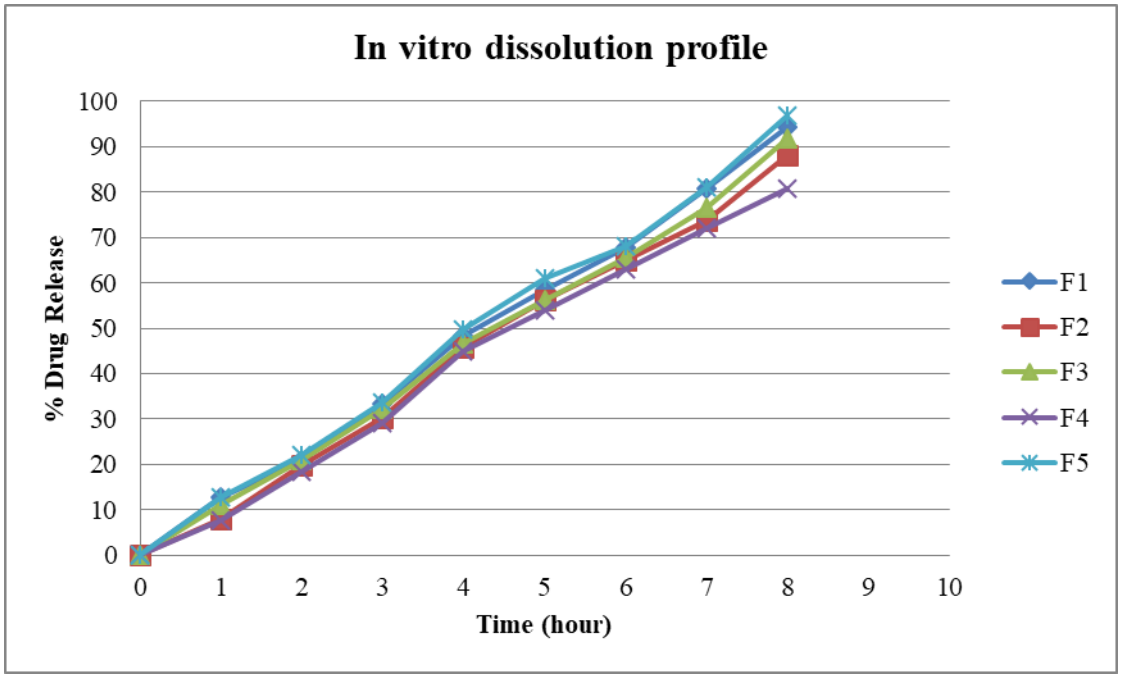


Fig - 11: Dissolution profile of F1, F2, F3, F4 and F5

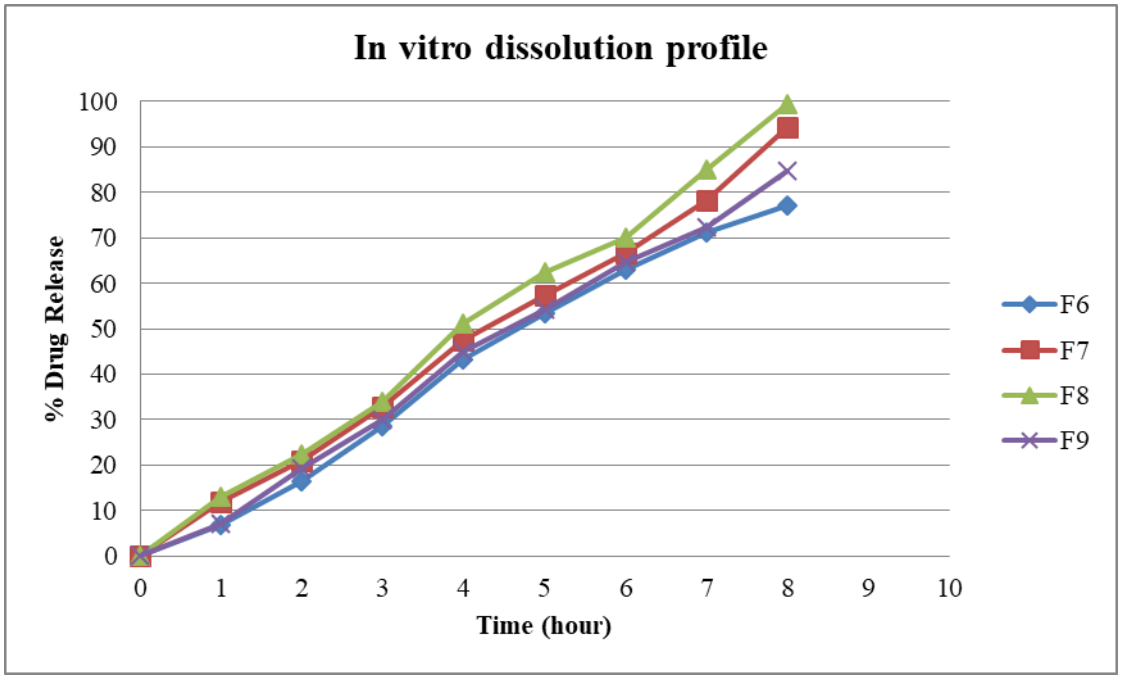


Fig - 12: Dissolution profile of F6, F7, F8 and F9

Ex-vivo Permeation study

Ex vivo drug permeation through fresh Goat buccal mucosa using Franz diffusion cell and the results were given in table - 17 and Fig - 13.

Permeation study parameters

Donor compartment : Phosphate buffer pH 6.8

Receptor compartment : Phosphate buffer pH 6.8

Apparatus : Diffusion cell

Withdrawal time : 8 h with 1 h interval

Volume withdrawn : 5mL

Table - 17: *Ex vivo* Percentage drug permeation of patches

Time (hours)	**Percentage drug permeation**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1	8.64	5.66	9.98	4.80	8.83	2.43	9.67	11.35	4.31
2	18.09	15.09	16.67	13.96	17.11	11.90	17.23	19.36	17.76
3	31.23	28.19	29.21	24.22	26.78	21.78	27.69	29.63	25.31
4	44.67	40.06	41.34	38.99	34.29	33.56	40.11	47.43	39.23
5	53.54	51.19	53.11	47.93	56.06	45.80	53.41	57.82	46.32
6	64.74	59.45	60.33	55.65	68.34	53.34	62.12	73.40	59.03
7	78.73	70.60	73.16	66.49	77.19	62.65	75.97	80.57	68.17
8	86.15	82.34	84.98	72.79	90.41	69.30	86.22	92.61	77.59

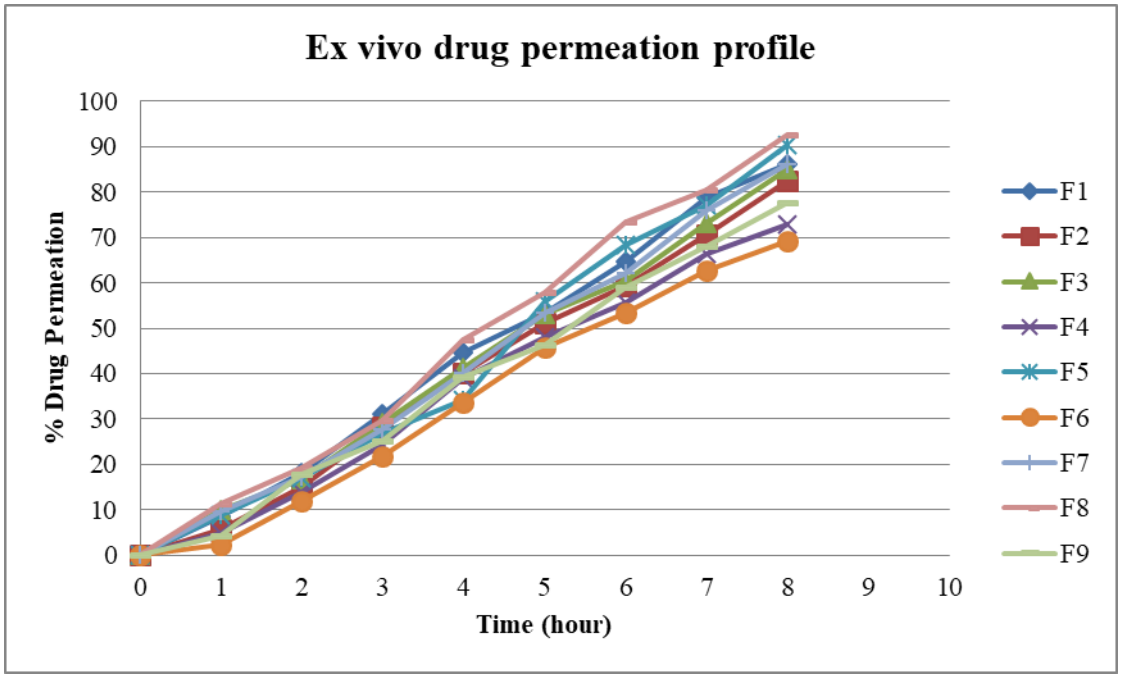


Fig - 13: *Ex vivo* drug permeation profile

OPTIMIZATION:

On the basis of defined constraints for each independent variable, the Design Expert® Software version 11 automatically generated the optimized formulation. The experiments were performed and the responses were obtained. The data were shown in table - 18.

Table - 18: Results of independent variable and corresponding dependent variables

Trials	Factor 1	Factor 2	Response 1	Response 2	Response 3
	HPMC K4M	Carbopol 974P	Time taken for 50 % drug release	Drug release at 8th hr	Mucoadhesion time
	mg	mg	min	%	min
F1	300	360	252	94.45	427
F2	600	360	264	88.23	530
F3	600	240	264	91.67	518
F4	900	240	276	80.76	592
F5	300	240	246	96.74	415
F6	900	360	282	77.13	616
F7	600	120	258	94.32	510
F8	300	120	234	99.34	390
F9	900	120	270	84.65	572

Time taken for 50 % drug release

This 3D surface graph (Fig - 14) illustrates that increasing the concentration of HPMC K4M increases the time taken for 50 % drug release, on the other hand increasing the concentration of carbopol 974P also increases the time for 50% drug release but comparatively lesser than HPMC K4M.

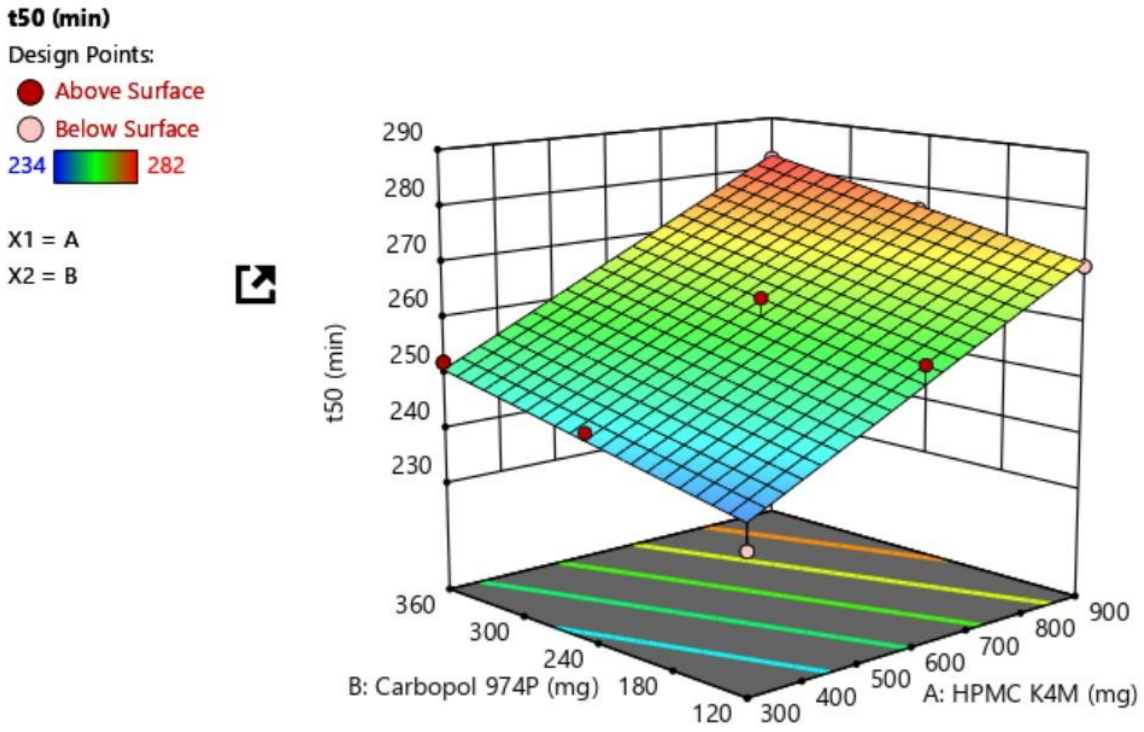


Fig - 14: Effect of HPMC K4M and Carbopol 974P on time taken for 50 % drug release

Drug release at 8h

This 3D surface graph (Fig - 15) illustrates that increasing both the polymer concentrations results in decreased drug release. Increasing the HPMC K4M concentration has more release retarding tendency. Thus the formulations containing higher amounts of HPMC k4M has very less drug release at 8^{th} hour.

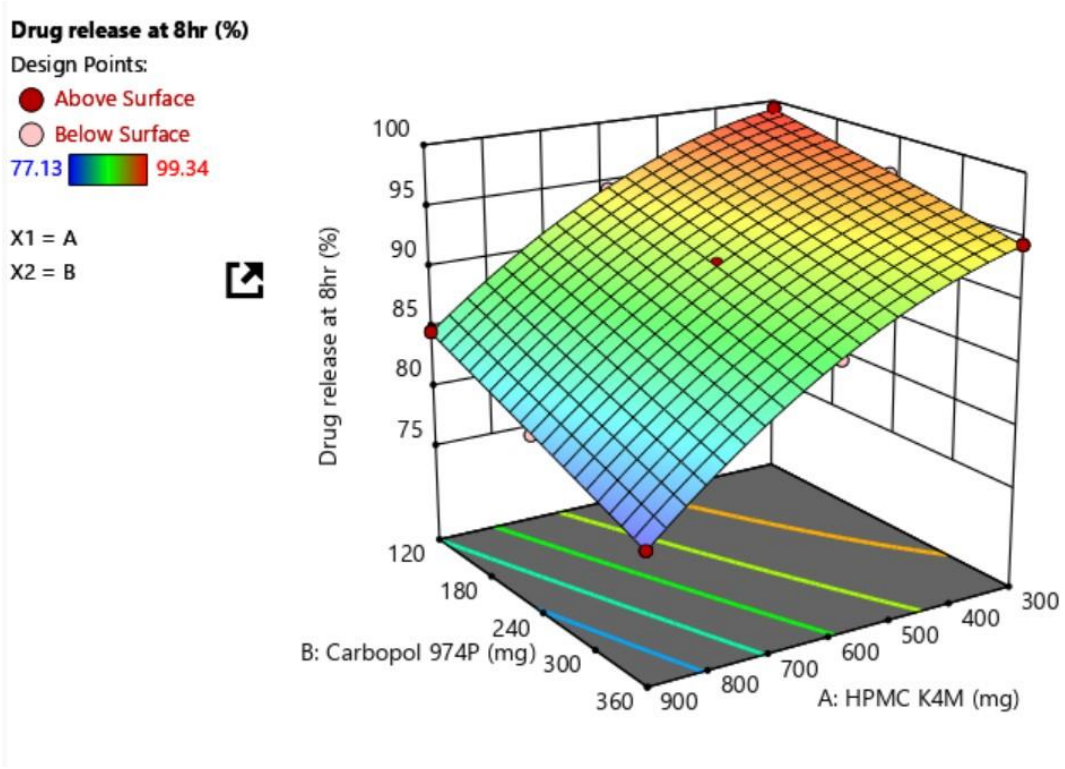


Fig - 15: Effect of HPMC K4M and Carbopol 974P on drug release

Mucoadhesion time

This 3D surface graph (Fig - 16) illustrates that the highest concentration of both polymers has higher mucoadhesion time. Increasing HPMC K4M concentration increases mucoadhesive time of the patches considerably.

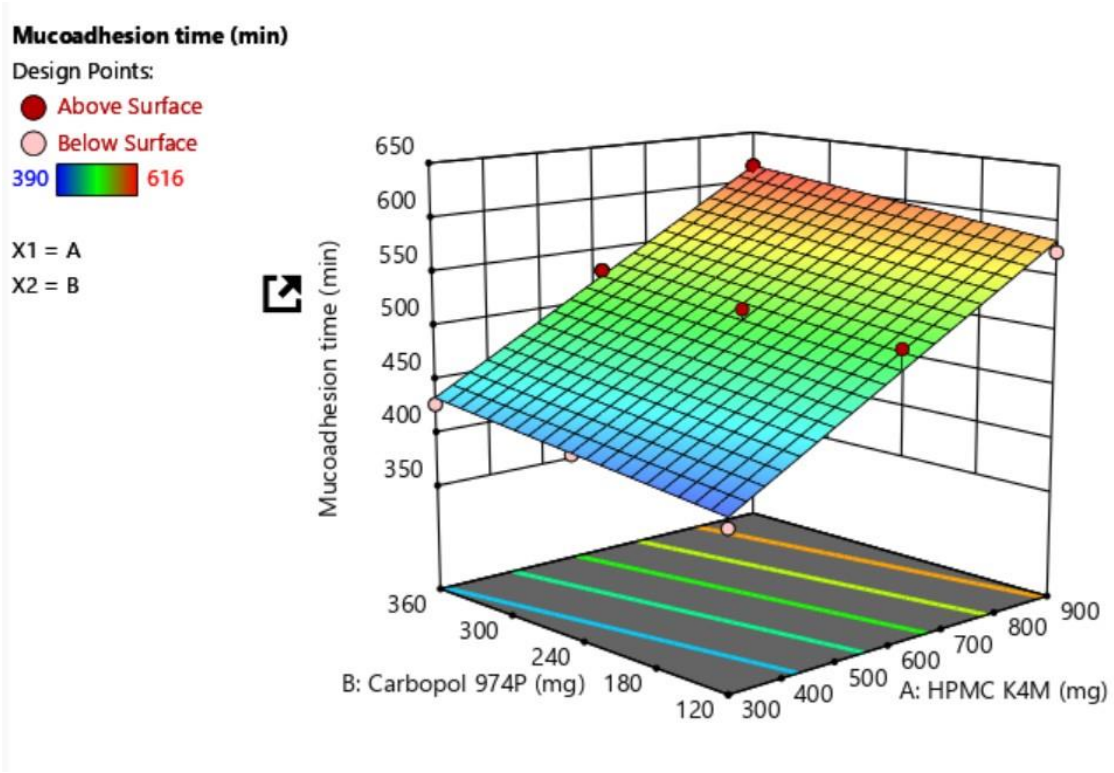


Fig - 16: Effect of HPMC K4M and Carbopol 974P on mucoadhesion time

ANOVA:

Table - 19 represents the statistical parameters such as adjusted R^2, predicted R^2, model P values, adequate precision and % CV. Based on table - 19 the responses time taken for 50% drug release, drug release at 8 h and mucoadhesion time was well fitted to the linear and quadratic model with P value of < 0.0500. Table - 19 shows adjusted R^2 for Y_1, Y_2 and Y_3 which is in reasonable agreement with the predicted R^2. Adequate precision measures the signal-to-noise ratio.

A ratio greater than 4 is desirable ratio indicating an adequate signal. This model can be used to navigate the design space. The results show that 90% of response variations in t50, drug release at 8 h and mucoadhesion time could be described by Factorial design as a function of main composition. So it can be concluded that linear model was suitable model for analysis. The results were shown in table - 19.

Table – 19: Response model and statistical parameters obtained from ANOVA

Responses	Adjusted R_2	Predicted R_2	Model P value	Adequate precision	% CV
Time taken for 50 % drug release	0.9587	0.9267	< 0.0001	24.9457	1.17
Drug release at 8 h	0.9992	0.9978	< 0.0001	128.9092	0.2348
Mucoadhesion time	0.9800	0.9666	< 0.0001	32.7227	2.26

Point prediction:

The melatonin buccal patches were formulated and responses were measured. The software generated the optimized formulation and predict the response based on the constraint. Then batch was formulated based on the suggested formulation and response were observed. The observed values of responses were compared to the predicted values of the response and % error was calculated to validate the method. The observed value of Y_1, Y_2 and Y_3 were in a close agreement to the predicted one. By this the validity of optimization procedure was proven. The point prediction has been shown in table - 20.

Desirability of optimum formulation was 0.922. When desirability value is between 0.8 and 1, the formulation quality is regarded to be acceptable and excellent. When this value Is < 0.63, the formulation quality is regarded as poor.

Table - 20: Optimum formulation derived by Factorial design

Factor	HPMC K4M	Caropol 974P	Desirability
Optimum formulation	629.74	120.00	0.922

Table – 21: Point Prediction for melatonin buccal patches

Point Prediction	Time taken for 50 % drug release (min)	Drug release at 8 h (%)	Mucoadhesion time (min)
Predicted	256.253	93.724	499.99
Observed	261	92.59	512
% error	1.85	1.2	2.4

% error = (observed value-predicted value)/predicted value x 100

Table - 22: Evaluation of Optimized melatonin buccal patches

Evaluations	Optimized Formulation results
Weight (mg)	56.21 ± 0.5
Thickness (mm)	0.18 ± 0.43
Drug content (%)	98.17 ± 0.7
Folding endurance	513 ± 12
Swelling index (%)	240.14 ± 2.0
Surface pH	6.9 ± 0.03
Percentage moisture loss (%)	2.04 ± 0.02
Mucoadhesion time (min)	512 ± 12

Table - 23: In - Vitro release of optimized formulation

Time (hour)	Drug release (%)
1	11.89
2	20.65
3	34.76
4	46.21
5	55.93
6	62.85
7	74.30
8	92.59

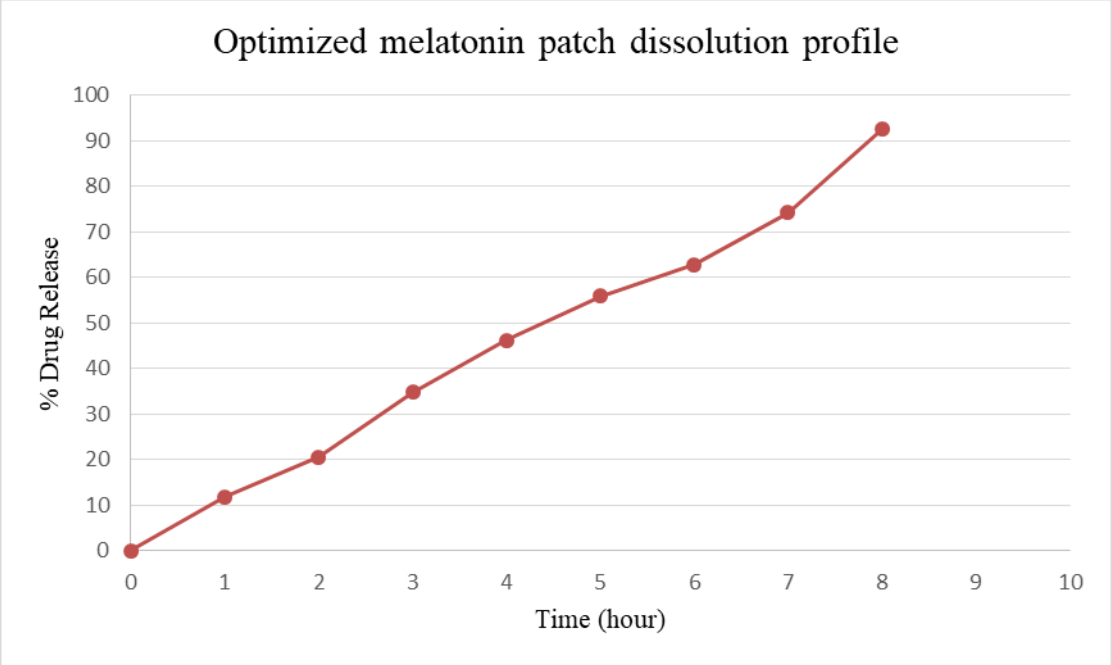


Fig - 17: Optimized melatonin patch dissolution profile

The optimized melatonin buccal patch shows a cumulative percent drug release at 8 h of 92.59 %.

Table - 24: Percentage drug permeation of optimized formulation

Time (hour)	Drug permeation (%)
1	8.59
2	17.87
3	25.06
4	37.15
5	49.43
6	58.37
7	70.56
8	85.93

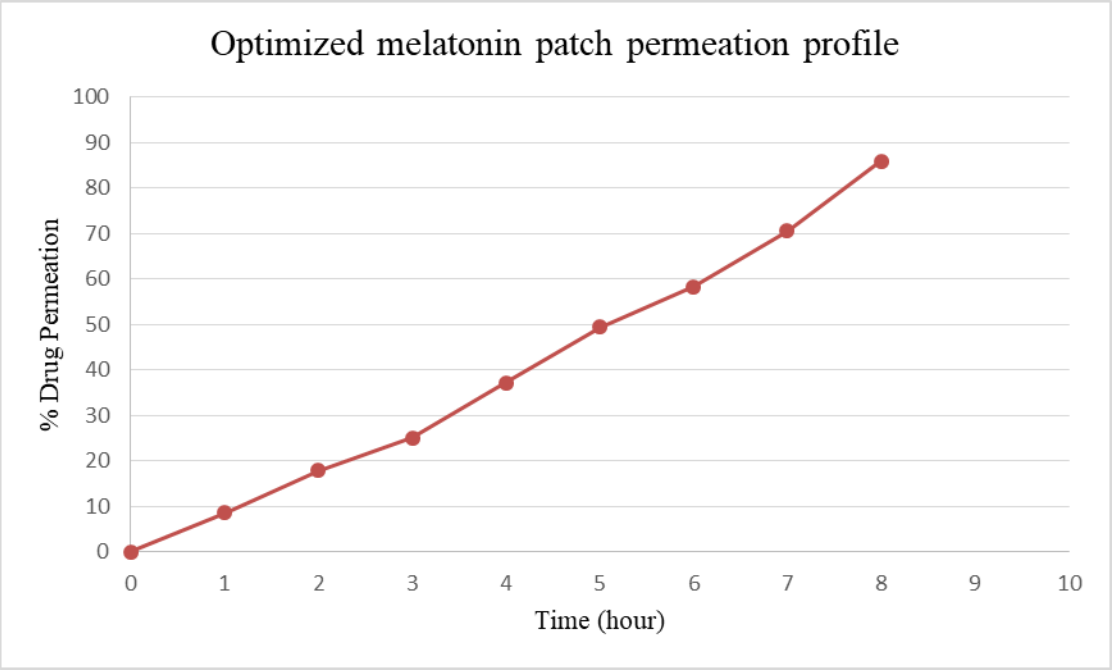


Fig - 18: Optimized melatonin patch permeation profile

The optimized melatonin buccal patch exhibits an 85.93 % permeation at 8 h on goat buccal mucosa, the permeation profile was relatively steady and the amount consistently permeated with duration of time. The results were shown in table - 24 and Fig - 18.

DRUG RELEASE KINETICS OF OPTIMIZED MELATONIN PATCH:

The drug release kinetics for the optimized formulation was calculated and the results obtained are presented in table - 25.

Table - 25: Kinetic modelling of drug release

Formulation	Zero order R^2	First order R^2	Higuchi R^2	Hixson Crowell R^2	Korsmeyer peppas R^2	n value
Optimized Melatonin patch formulation	0.9906	0.8227	0.9681	0.9638	0.9943	0.9787

Examination of correlation coefficient (R^2) value indicated that the drug release followed a diffusion-controlled mechanism for the optimized melatonin buccal patch from the R^2 value. To study the drug release kinetics, data obtained from In-Vitro drug release studies are plotted in various kinetic models. The curve fitting results of the release rate profile of the designed formulation gave an idea on the mechanism of drug release. Based on the "n" value 0.9787 for the optimized formulation, the drug release was found to follow super case II transport. This value indicates a coupling of the diffusion and erosion mechanism and indicates that the drug release was controlled by more than one process. Also, the drug release mechanism was best explained by zero order, as the plots showed the highest linearity, as the drug release was best fitted in zero order kinetics, it indicated that the rate of drug release was concentration independent. The kinetics were shown in the following Fig - 19 to 23.

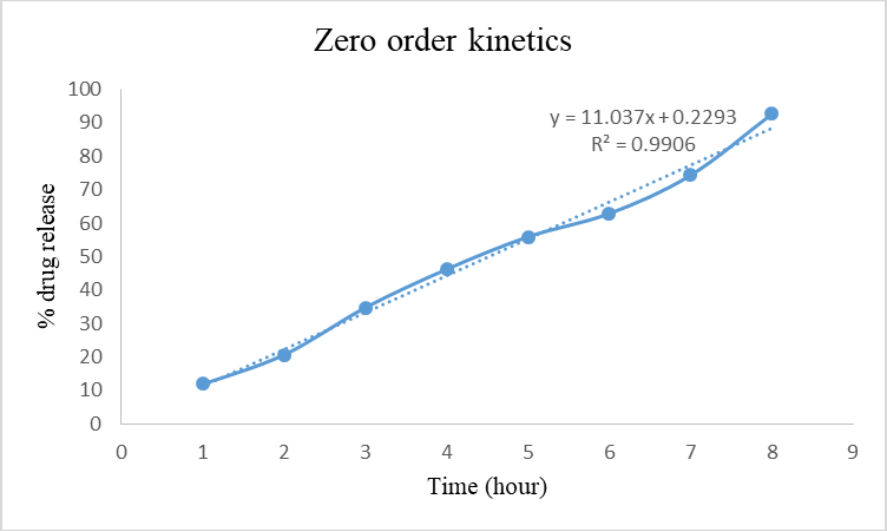


Fig - 19: Zero order release for the optimized formulation

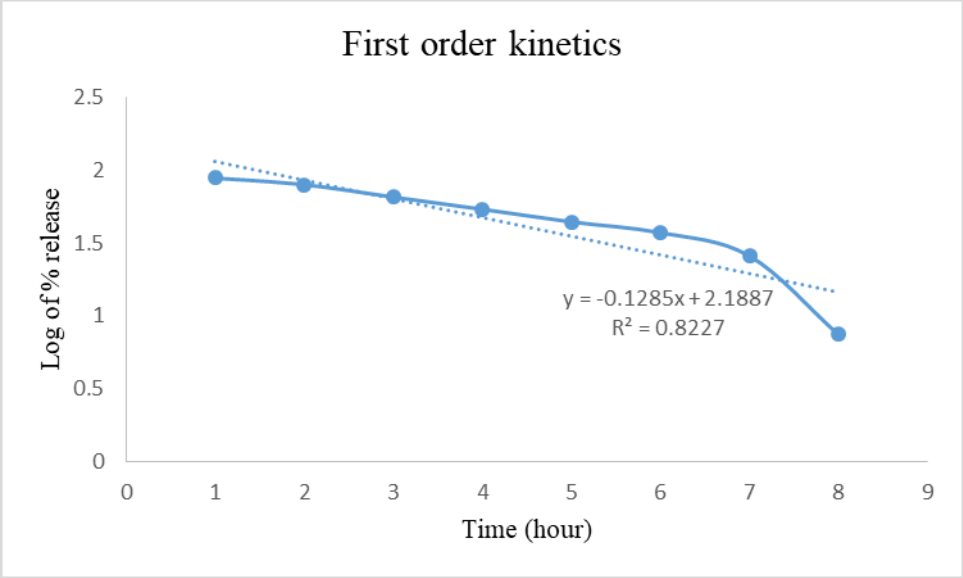


Fig - 20: First order release for the optimized formulation

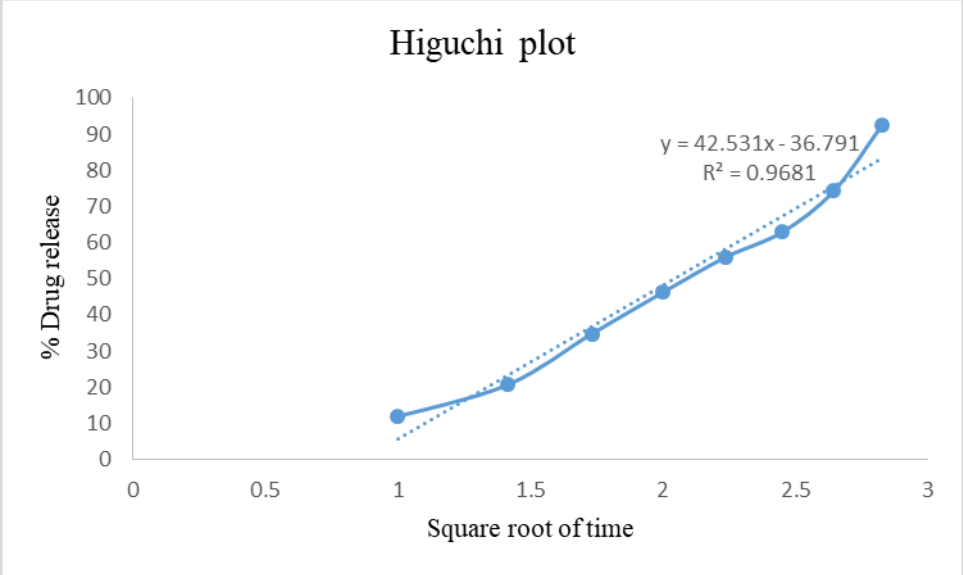


Fig - 21: Higuchi plot for the optimized formulation

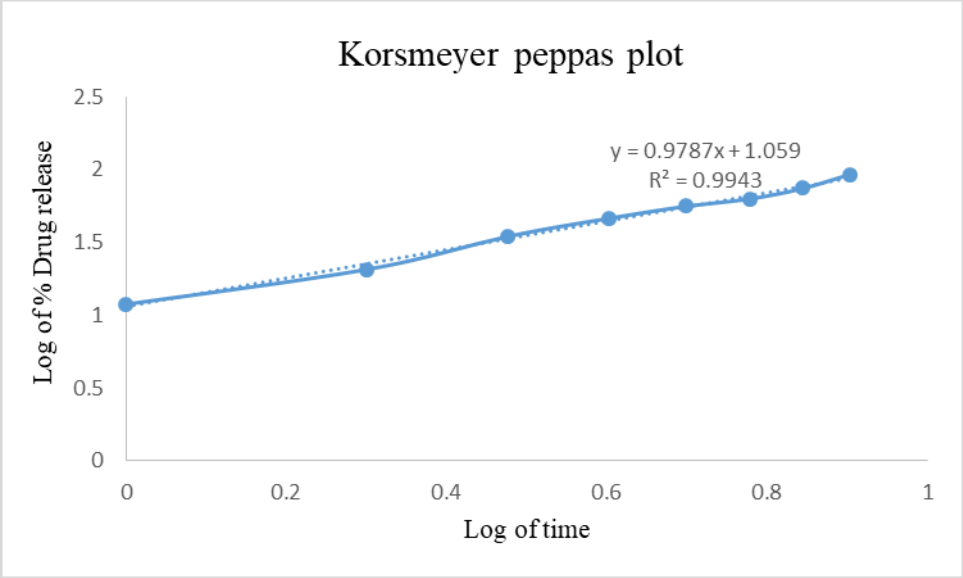


Fig - 22: Korsmeyer peppas model for the optimized formulation

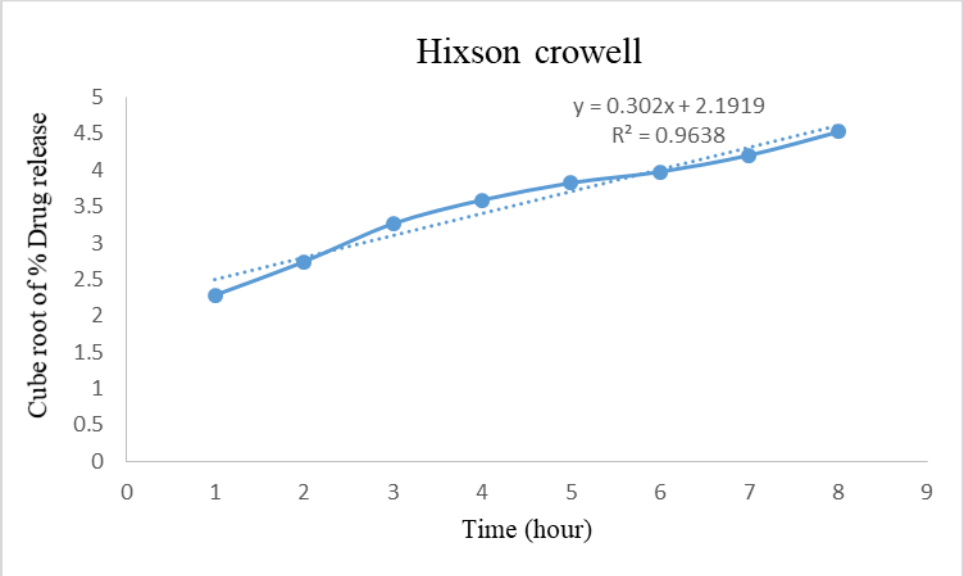


Fig - 23: Hixson plot for the optimized formulation

SUMMARY AND CONCLUSION

Melatonin is a hormone which is used for the treatment of chronic insomnia, jet lag and regulation of circadian rhythm. Since it has a very low oral bioavailability of less than 15% and very short half-life of less than 45 min, therefore mucoadhesive buccal patch formulation was investigated.

In the present work successful attempt was made to formulate prolonged release melatonin buccal patch by solvent casting method using hydrophilic polymers HPMC K4M, Carbopol 974P and Polyethylene glycol 400 as plasticizer. The drug polymer compatibility was verified by FT-IR studies. The standard curve of melatonin in pH 6.8 phosphate buffer was prepared.

A two factor and three-level factorial design was used to optimize the formulation, where concentration of HPMC K4M (X_1) and Carbopol 974P (X_2) taken as two independent variables and dependent variables were T50 (Y_1), drug release at 8h (Y_2), mucoadhesion time (Y_3). Totally 9 trial buccal patches were prepared and evaluated for weight variation, thickness, drug content, folding endurance, swelling property, surface pH, percent moisture loss, *ex vivo* mucoadhesion time, *in vitro* dissolution test and *ex vivo* permeation study.

It was observed that increasing the HPMC K4M concentration has a significant increase in mucoadhesion time and decrease in drug release.

Then using the design expert software the optimized formulation was obtained. The best polymer composition was found to be HPMC K4M (629.74 mg) and Carbopol 974P (120 mg).

The optimized formulation shows satisfactory results in the parameters such as thickness, hardness, drug content, swelling index, mucoadhesion time, in vitro dissolution and diffusion studies. It shows zero order drug release profile depending on the regression value and shown required mucoadhesion time of 512 min as well as a satisfactory release of 92.59 % at 8h with good mechanical properties. A hydrophobic backing layer was attatched to the patch for unidirectional release. Slow, controlled and maximum release of melatonin over a period of 8 h was obtained from the optimized buccal patch.

Buccal delivery has been extensively investigated for both local and systemic therapy of various drug molecules by different delivery approaches. Currently, there are only few commercial formulations available or under clinical trials.

This low commercial success is probably due to the high production cost. Nevertheless, the recent technological advances in mucoadhesive presents new opportunities and is likely to pave way for several other molecules into clinical use. In this study, a full factorial (3^2) design was constructed to optimize the mucoadhesive buccal patch of melatonin.

The suggested formulation could successfully achieve prolonged drug release and maintain drug concentration required for inducing and maintaining sleep, which consequently enhance patient compliance.

REFERENCES

1. Harris D, Robinson JR. Drug delivery via the mucous membranes of the oral cavity. J Pharm Sci 1992; 81:1-10.
2. Mathias N R, Hussain A M. Non-invasive systemic drug delivery: developability considerations for alternate routes of administration. J Pharm Sci 2010; 99:1-20.
3. Shojaei Amir H. Buccal mucosa as a route for systemic drug delivery. J Pharm Sci 1998; 1(1):15-30.
4. Hoogstraate Janet A J, Wertz P W. Drug delivery via the buccal mucosa. Pharm Sci Technolo Today 1998; 1(7): 309-316.
5. Rossi Silvia, Giuseppina Sandri, Carla M Caramella. Buccal drug delivery: a challenge already won?, Drug Discov Today Technol 2005; 2(1): 59-65.
6. Yajaman S, Ketousetuo Kuotsu, Bandyopadhyay A K. Buccal bioadhesive drug delivery - a promising option for orally less efficient drugs. J Control Release 2006; 114: 15-40.
7. Surender Verma, Mahima Kaul, Aruna Rawat Sapna Saini. An overview on buccal drug delivery system. Int J Pharm Sci Res 2011; 2(6): 1303-1321.
8. Miller N S, Montakarn Chittchang, Thomas P Johnston. The use of mucoadhesive polymers in buccal drug delivery. Adv Drug Deliv Rev 2005; 57:1666-1691.
9. Wertz P, Squier C. Cellular and molecular basis of barrier function in oral epithelium. Crit Rev Ther Drug Carrier Syst 1991; 8: 237–269.
10. Squier C, Hill M. Oral mucosa, in: A. Ten Cate (Ed.), Oral Histology, Development, Structure, and Function, Mosby Incorp, 1989; 319–356.
11. Gandhi R, Robinson J. Oral cavity as a site for bioadhesive drug delivery. Adv Drug Deliv Rev 1994; 13: 43–74.
12. Squier C. Membrane coating granules in nonkeratinizing oral epithelium. J Ultrastruct. Res 1977; 60: 212–220.
13. John D smart. Buccal drug delivery. Expert Opin Drug Deliv (2005) 2(3):507-517.
14. Florence at, Attwood da. Buccal and sublingual absorption. In: Physicochemical Principals of Pharmacy. Basingstoke: Palgrave UK; 1998; p392.
15. Chickering D, Mathiowitz E. Definitions, mechanisms, and theories of bioadhesion, in: Mathiowitz E, Chickering D, Lehr C (Eds.), Bioadhesive drug delivery systems: Fundamentals, Novel Approaches, and Development, New York: Marcel Dekker Inc; 1999.

16. Javier O Morales, Jason T McConville. Manufacture and characterization of mucoadhesive buccal films. Eur J Pharm Biopharm. 2011; 77: 187–199.
17. Duchene D, Touchard F, Peppas NA. Pharmaceutical and medical aspects of bioadhesive systems for drug administration. Drug Dev Ind Pharm. 1988; 14: 283-318.
18. Derjaguin BV, Smilga VP. The electronic theory of adhesion in: adhesion: fundamentals and practice. University of Nottingham, England: Gordon and Breach; 1966.
19. Tabor D. Surface forces and surface interaction, J Colloid Interface Sci 1977; 58: 2-13.
20. Paulsson M. Controlled release gel formulations for mucosal delivery. Ph.D. Thesis, Faculty of Pharmacy, Sweden: Uppasala University; 2001
21. Jasti Drs B, Li X, Cleary G. Recent advances in mucoadhesive drug delivery systems. Business briefing: Pharmtech 2003; 194-197.
22. Lee JW, Park JH, Robinson JR. Bioadhesive based dosage forms: the next generation, J Pharm Sci 2000; 89: 850- 866.
23. Ravi G Pawar, Sagar D Pawar, Banerjee S K, Gadhave M V, Gaikwad D D. Novel buccoadhesive polymers: a review. Res J Pharm Technol 2012; 5-5-15.
24. Tiwari D, Goldman D, Sause R, Madan P L. Evaluation of polyoxyethylene homopolymers for buccal bioadhesive drug delivery device formulations. AAPS Pharm Sci 1 (1999) E13.
25. Huang Y,Leobandung W, Foss A, Peppas N A. Molecular aspects of muco and bioadhesion: tethered structures and site-specific surfaces. J Control Release 2000; 65: 63– 71.
26. Peppas N A, Buri P A. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues, J Control Release 1985; 2: 257–275.
27. Solomonidou D, Cremer K, Krumme M, Kreuter J, Effect of carbomer concentration and degree of neutralization on the mucoadhesive properties of polymer films, J Biomater Sci, Polym. Ed. 12 2001; 1191–1205.
28. E. Russo. A focus on mucoadhesive polymers and their application in buccal dosage forms. J Drug Deliv Sci Technol 2015; 1-13.
29. Singh R, Sharma D, Garg R. Review on mucoadhesive drug delivery system with special emphasis on buccal route: an important tool in designing of novel controlled drug delivery system for the effective delivery of pharmaceuticals. J Dev Drugs 2017; 6: 169.

30. Charles M Morin, Ruth Benca. Chronic insomnia. Lancet 2012; 379: 1129–41
31. Breslau N, Roth T, Rosenthal L, Andreski P. Sleep disturbance and psychiatric disorders: a longitudinal epidemiological study of young adults. Biol Psychiatry 1996; 39: 411–18.
32. Zetner D, Andersen L P H, Rosenberg J. Pharmacokinetics of alternative administration routes of melatonin: a systematic review. Drug Res 2015.
33. Ellis E M, Lemmens G, Parkes J D. Melatonin and insomnia. J sleep Res 1996; 5: 61-65.
34. Rudiger Hardeland. New approaches in the management of insomnia: weighing the advantages of prolonged-release melatonin and synthetic melatoninergic agonists. Neuropsychiatr Dis Treat 2009; 5: 341–354.
35. Rob L DeMuro, Anne N Nafziger. The absolute bioavailability of oral melatonin. J Clin Pharmacol 2000; 40: 781-784.
36. Patrick Lemoine, Nava Zisapel. Prolonged-release formulation of melatonin (Circadin) for the treatment of insomnia. Expert Opin Pharmacother 2012; 13(6):895-905.
37. Bhusnure O G, Gandge N V, Gholve S B, Giram P S. QbD approach for analytical method development and validation of melatonin by spectroscopic method. J Pharm Res 2017; 11(5): 464-471.
38. Asane G S, Nirmal S A, Rasal K B, Naik A A, Mahadik M S. Polymers for mucoadhesive drug delivery system: a current status polymers for mucoadhesive drug delivery system. Drug Dev Ind Pharm 2008; 34:1246–1266.
39. Ana Flo. Melatonin delivery: transdermal and transbuccal evaluation in different vehicles. Pharma Res 2016.
40. Pakorn Kraisi, Sontaya Limmatvapirat, Jurairat Nunthanid, Pornsak Sriamornsak, Manee Luangtana Anan. Preparation and characterization of hydroxypropyl methylcellulose/polycarbophil mucoadhesive blend films using a mixture design approach. Chem Pharm Bull 2016.
41. Rachna Kumria, Bandar E Al-Dhubiab, Jigar Shah, Anroop B Nair. Formulation and evaluation of chitosan-based buccal bioadhesive films of zolmitriptan. J Pharm Innov 2018.

42. Amelia M Avachat, Kishore N, Gujar, Kishor V Wagh. Development and evaluation of tamarind seed xyloglucan-based mucoadhesive buccal films of rizatriptan benzoate. Carbohydr Polym 2013; 91: 537– 542.
43. Surya N Ratha, Adhikari Bhabani S Nayak, Amit K Nayak, Biswaranjan Mohanty. Formulation and evaluation of buccal patches for delivery of atenolol. AAPS Pharm Sci Tech 2010 11(3).
44. Mohamed S, Pendekaln, Pramod K, Tegginamat. Formulation and evaluation of a bioadhesive patch for buccal delivery of tizanidine. Acta Pharmaceutica Sinica B 2012; 2(3): 318–324.
45. Anroop B Nair, Rachna Kumria, Sree Harsha, Mahesh Attimarad, Bandar E Al-Dhubiab, Ibrahim A Alhaider. In vitro techniques to evaluate buccal films. J Control Release. 2013; 166: 10–21.
46. Anroop B. Nair, Bandar E Al-Dhubiaba, Jigar Shah Patel Vimal, Mahesh, Attimarada, Sree Harsha. Development and evaluation of palonosetron loaded mucoadhesive buccal films. J Drug Deliv Sci Technol 2018.
47. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 896, Melatonin. Retrieved January 6, 2022 from https://pubchem.ncbi.nlm.nih.gov/compound/Melatonin.
48. Australian Public Assessment Report for Melatonin; AusPAR Circadin Melatonin Commercial Eyes Pty Ltd PM-2008-2125-1; 8-13
49. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Handbook of pharmaceutical excipients. 6th ed.London: Pharmaceutical Press; 2009. p 110-521.
50. Lodhi M. Formulation and evaluation of buccal film of ivabradine hydrochloride for the treatment of stable angina pectoris. Int J Pharm Res 2013; 3(1): 47–53.
51. Kumria R, Nair AB, Goomber G, Gupta S. Buccal films of prednisolone with enhanced bioavailability. Drug Deliv. 2016; 23(2):471-8.

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CERTIFICATE OF PARTICIPATION

This is to certify that Dr/ Mr/Ms SARAVANA KUMAR M

has attended webinar entitled ***"Ethical considerations in animal experimentation and research"*** on 3rd July, 2020, organised by Department of Pharmacology in association with Department of Pharmacy Practice, C.L.Baid Metha College of Pharmacy, Chennai.

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