DEVELOPMENT AND CHARACTERIZATION OF DENTAL PATCHES CONTAINING AMOXICILLIN TRIHYDRATE, METRONIDAZOLE AND LIDOCAINE HYDROCHLORIDE FOR THE TREATMENT OF PERIODONTAL AND OTHER DENTAL DISEASES

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Certificate

This is to certify that DEEPAYAN CHANDA (Class Roll No: 001711402005, Examination Roll No: M4PHA19007 and Registration No: 140831 of 2017-2018) has carried out the research work entitled **"DEVELOPMENT AND CHARACTERIZATION OF DENTAL PATCHES CONTAINING AMOXICILLIN TRIHYDRATE, METRONIDAZOLE AND LIDOCAINE HYDROCHLORIDE FOR THE TREATMENT OF PERIODONTAL AND OTHER DENTAL DISEASES "** independently with proper care and attention under my supervision and guidance in the Pharmaceutics Research Laboratory in the Department of Pharmaceutical Technology, Jadavpur University. He has incorporated his findings into this thesis of the same title, being submitted by him, in partial fulfilment of the requirements for the degree of Master of Pharmacy from Jadavpur University. I appreciate his endeavour to do the project and his work has reached my gratification.

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DECLARATION

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of his Master of Pharmaceutical Technology studies. All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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Dedicated to my Family and my Guide

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION-

Disease of a dental region are found to be most reported problem in several parts of the world, found in both male and female of various age groups .In the recent years the problems of dental region has been increased large amounts in the last few years. According the report more than 65% of people in the world has been suffering from problems related to dental region. Most of the people have been suffering from most of the oral diseases like periodontal infections, dental caries, dry socket (Parkash, 2000). In most of the cases it has been found that not proper maintenance of oral hygiene increased the chances of bacterial endocarditis and other diseases of the cardiovascular region in patients who were experienced periodonitis and not proper maintenance of maternal oral hygiene showed new born with low birth weight. As per the report provided by Government of India& World Health Organization collaborative programme, more than 49% of school children are found that they have dental caries and above 80% of person above 18 years of age are suffering from periodontal and other dental diseases. It has been also observed that wide amount of misconduct makes the mouth a microbial paradise, microbes mainly grows on the cheek, back of the tongue and in the moist, oxygen less area between the tooth surface and the nearby buccal tissues (Morriso et al.1999, Lieff et al.2004). Tooth extraction cannot be avoided in the majority of the cases and hence, complications after surgery has been observed such that- excessive bleeding, delayed wound healing, dry socket syndrome etc, are found to be headache. There are several numbers of products which are available in the market which are prescribed in the oral cavity and dental region. The high percentage of dental caries and periodontal disease is observed especially in the new- born babies and patients above 60 years of age. A variety of dosage form have been available in the market for the treatment of dental disorders but very less importance has been given on the sustained delivery of the products to the oro-dental cavity (Genco et al.1969). Chemotherapy of periodontal and other dental diseases faced several formidable challenges and obstacles. We know that oral cavity has unique anatomical and physiological features such as higher amount fluid production (saliva and gingival fluids) and relative motions or friction between the dosage form and tissues and food, the retention of dosage form is poor and clearance of active agents is rather large, resulting in the need for frequent dosing. The product which are avaliable in the pharma market for the treatment of various dental diseases and procedures which are adopted to counter the diseases are and these problems provide wide array of patient noncompliance. Hence, for the new products to be successful in the market it is must provide attention to correct this attributes and focus should be on sustained or targeted drug delivery (Lindhe et al.1983).

1.2. Diseases of the Dental Region-

There are more than 240 various type of disease that may cause hazard in the oral cavity. The most significant diseases that have been found in young adults are dentinoma, dental caries, periodontitis, dentinal sclerosis, root resorption, aphthous stomatitis (mouth ulcers), gingivitis, fungal infection, chronic bacterial infection etc. If these diseases are not treated they may cause

various complications in different parts of the body. Sometimes these problems can even cause death of the patient. Recent developments in molecular biology, microbiology, immunology and genetics have lead researchers to focus more on the relationship between oral and systemic diseases with a more scientifically oriented approach (Antonia et al., 2010). So, there is a need for targeting the dental region to provide better treatment. Common diseases of dental region are written below-

a) Periodontal Disease-

Periodontitis is a localized inflammation caused by infection of various bacteria and there is a formation periodontal pocket associated with sub gingival plaque. These periodontal pockets are ideal for the growth and multiplication of harmful microorganisms. So, the disease requires immediate care, otherwise teeth may be lost. The therapeutic efficacy can be achieved by destroying bacteria, by mechanical cleaning of plaque using abrasive materials, systemic as well as topical administration of antimicrobial agents and antibiotics. In periodontal diseases it has been observed that nearby areas of gum, supporting structures of the teeth become infected, and as a result teeth become very loose, the proper strength to hold the teeth in the oral cavity diminished. Periodontal disease mainly includes a number of pathological conditions which cause inflammation and damage of the gums (gingival) and the supporting structures of the teeth (Muhammad Azhar Sheikh et al., 2012). Typically periodontal diseases refer to as gingivitis (inflammation of the gingival area) and periodonitis (inflammation of the periodontal ligament).The periodontal diseases generally cause degeneration and it is neoplastic in nature. Primarily the disease is localized in the gingival but then it develops to as the marginal periodonitis (Denise C. Bowe et al., 2011). The periodontal pocket provides suitable conditions for the colonization of gram negative bacteria as well as obligates anaerobes like *Porphyromonas gingivalis*, *Bacteroides* spp., *Capnocytophaga spp*. and *Actinobacillus actionomycetemcomitns* the bacteria accumulate in the periodontal pocket that develops between the roots of affected teeth and soft tissues. Periodontal disease, bleeding of gums and infections of oral cavity increased risk of dysphasia and leads to patient noncompliance. Bleeding of oral cavity cause bacterial infection (bacteraemia) which may lead serious and fatal health related problem known as infective endocarditis. Inflammation of the oral cavity, swelling of gums makes impossible for the drug to reach at the site of action, so very large dose are required to provide sufficient pharmacological action. For the care of oral-dental infection, gingival bleeding, pus- formation and local ulceration, it is necessary to give drug which act quickly and provide highest potency at site of action (Dugal et al., 2010).

b) Occlusal Trauma-

1. Primary occlusal trauma

2. Secondary occlusal trauma

Figure 1 illustrates list of host factors substantially influence the development of dental biofilms. The mouth facilitates the growth of a characteristic resident microbiota. The composition of the oral microbiota is influenced by various causes, like temperature, pH, atmosphere, as well as by the host defenses and host genetics, endogenous nutrients for biofilm formation. In health, the resident oral microbiota forms a symbiotic relationship with the host, regulated by active host–microbe cross talk. This resident microbiota is sensitive to perturbations in the host environment, especially to changes in nutrient supply and pH, so that previously minor components of the microbiota can become more competitive (and vice versa), resulting in reorganization of biofilm community structure.

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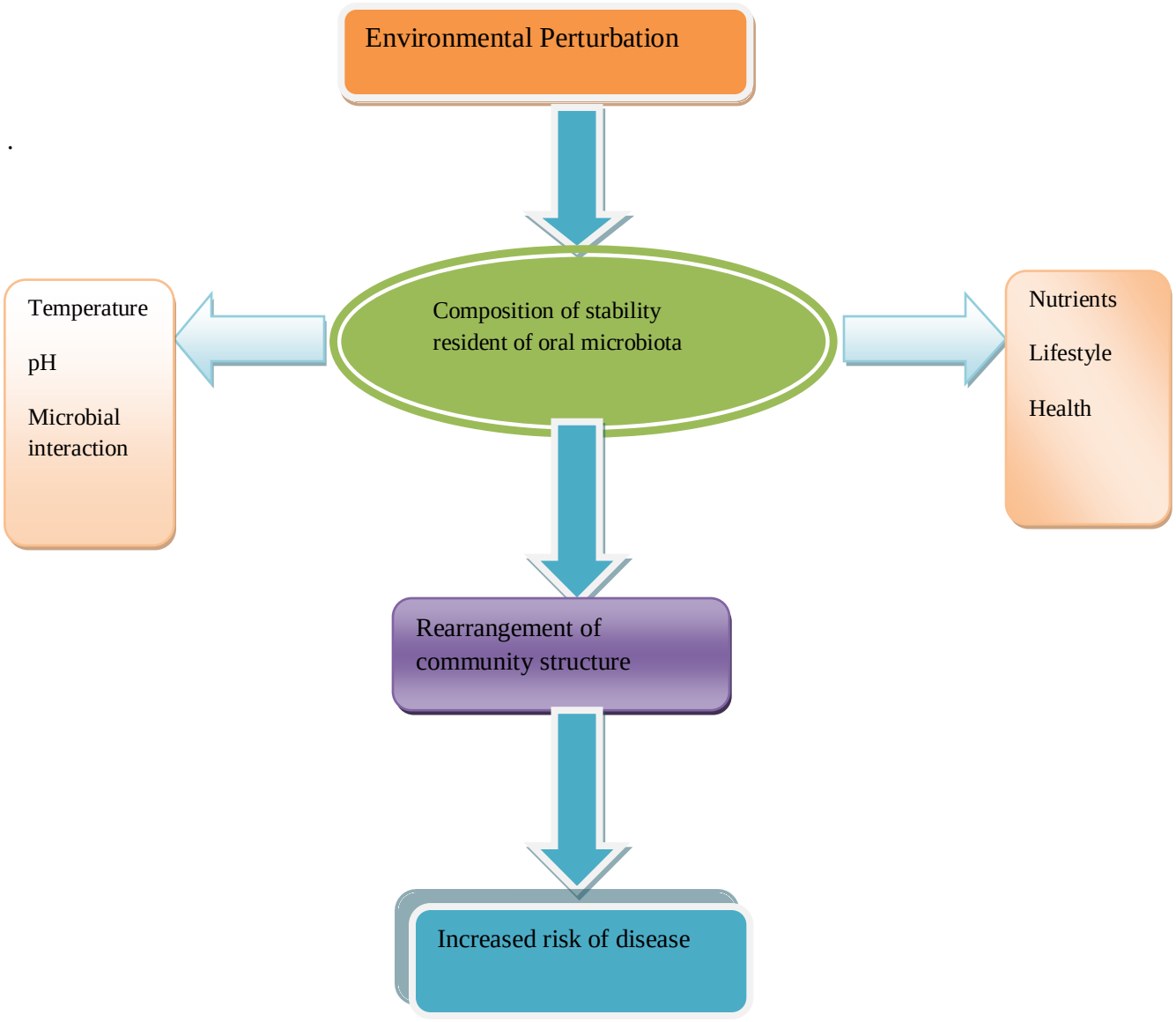

Figure 1: List of host factors that influence dental biofilms (Marsh and Martin, 2009).

The host environment dictates the composition and gene expression of the resident microbiota. Changes in oral environmental conditions can disrupt the normal symbiotic relationship between the host and its resident microbes, and increase the risk of disease (Bradshaw et al. 1989).

To counter the periodontal disease, treatment needs to be carried out by maintaining of proper oral hygiene along with the basic periodontal therapy. Normally, systemic therapy with antimicrobial agents is widely used method to counter periodontal diseases. (Slots 2002; Herrera et al. 2008). In last two decades, the research studies on periodontitis proves that combination of antimicrobial treatment along with mechanical treatment (full mouth scaling and root canal treatment) will be one of the best way for the treatment of periodontal diseases, than the mechanical treatment alone (Kaner et al. 2007). In the systemic treatment, the choices of drug are amoxicillin; amoxicillin-clavulanic acid and metronidazole generally recommended as the first line treatment in aggressive periodontal illness (Matarazzo et al. 2008; Ahuja et al. 2006). Drug deliveries through either by topical or systemic route have their own limitations and drawbacks. However, the mucoadhesive drug delivery systems have currently become a remarkable area for drug delivery research owing their potential to optimize localized drug delivery, by retaining dosage forms at the site of action or within the absorption site.

c) Dental Caries-

Dental caries is a process by which the colonizing bacteria which is present in the enamel produce bacterial acid that leaches the enamel and thus promoting tooth decay forming dental caries. Dental caries then progresses to the inner pulp of the teeth which contains the nerves and blood vessels of the teeth. As the name suggest endodontic diseases are referred to those diseases that affects the inner portion. This infection to the inner portion is accompanied by the production of puss which leads to pulp tissue necrosis. Conventional way of treating endodontic includes root canal treatment and pulpotomy (Ken Yaegaki, Jeffrey Coil et al., 2006).

d) Aphthous Stomatitis- Canker sores which are commonly referred by Aphthous ulcers or recurrent Aphthous Stomatitis (RAS) are inflamed lesions of the mucous lining of the mouth that may involve the cheeks, tongue, lips, gums and roof or floor of the mouth. Usually it is painful and is associated with redness, swelling, and sometimes occasional bleeding from the affected area(s).This disease can range from mild to severe and, in extreme cases, even hinder the food ingestion, thus making the person more prone to malnutrition. The cause of RAS is unknown, although several factors are suspected including genetics, Stress, nutritional deficiencies, diet,

hormonal changes, infectious agents (both bacterial and viral) and immunological disorders. As the cause of the disease is not fully known, it is difficult to find a proper cure and current treatments are aimed towards ameliorating the symptoms. There are 3 clinical presentations of RAS: aphthous minor, aphthous major and herpetiform ulcers. Aphthae minor and major which are limited to non-keratinized mucosa whereas herpetic form ulcers appear on both non-keratinized and keratinized mucosa (Crispian Scully & John Greenman 2008).

e) Alveolar Osteitis-

It is a complication of wound healing following extraction of a tooth. Alveolar osteitis is also known as dry socket. The term alveolar refers to the alveolus, which is the part of the jawbone that surrounds the teeth, and "bone inflammation" by the term osteitis. After dental extractions Alveolar osteitis remains one of the most common postoperative complications (Addy M et al., 1996). Various factors important in causing dry socket are: Insufficient blood supply to the alveolus, Preexisting infection. (Granuloma, periodontal or pericoronal infection) use of large amounts of local anesthetics, leading to vasoconstriction. Post-operative bleeding, Trauma to alveolus during extraction, Infection during or after extraction, Root/bone fragments or foreign bodies left in the socket, Excessive irrigation and curettage, fibrolytic or proteolytic activity in the clot, Loss of clot due to patient's negligence, Patient actions like sucking liquids, sneezing, coughing, rinsing water post extraction, Predisposing factors in patient, such as smoking, poor general health (Higashi et al., 1991). Mandibular molars particularly the third molars are more prone to Dry socket. This condition is associated with excruciating pain, foul breath, unpleasant taste, empty socket and gingival inflammation and lymphadenopathy. Various complications and problems associated with dry socket are pain; Delayed healing after tooth extraction, infection, interference with other needed dental procedures (Higashi et al., 1991).

f) Xerostomia-

The phenomenon of dry mouth resulting from reduced or absent salivary flow is termed as Xerostomia. Xerostomia is a common complaint among older adults and a study suggested that 30 percent of population aged 65 and above experience Xerostomia. Various reasons may lead to Xerostomia. It is a common as side effects of wide variety of medications, therapeutic radiation to head and neck, systemic diseases and diseases involving the salivary glands. When salivary hypo function and Xerostomia occur, transient and permanent oral and extra oral disorders can develop. Individuals may have problems with eating while Xerostomia due to dry mouth and, speaking and swallowing. Loss of sensation or altered taste followed by oral burning or soreness is quite common. Diseases which affect the salivary glands are Sjogren's syndrome, Sarcoidosis, and Amyloidosis and hence cause Xerostomia. Xerostomia can be cause by systemic diseases like Diabetes, HIV infections, chronic graft-vs.-host disease after allogenic bone marrow transplant, emotional Stress and mental depression. Use of medications like Antihistamines,

Antidepressants and Antipsychotics, Antihypertensive, Anti-anxiety agents, Diuretics, Anti Parkinsonism drugs, Anti emetics, Bronchodilators, Sedatives. (A.M. Robinso, et al. 2001).

g) Halitosis-

Persistent genuine halitosis is suffered among 85% of patients; microorganisms are responsible for the odour that originates from the mouth. It is likely that there is a complex interaction between several oral bacteria species (mainly gram-negative anaerobic flora) because no single specific bacterial infection has invariably been associated with halitosis. Halitosis was found to be associated with the bacterium *Solobacterium moorei*. Amino acids like cysteine, methionine, tryptophan, arginine and lysine that are biotransformed into hydrogen-sulphide, methyl-mercaptan, indole, putrescine and cadaverine respectively responsible for the odiferous products that cause halitosis (Arvind Venkatesh et al., 2012).

Micro organisms associated with Halitosis- *Centipeda periodontii*, *Eikenella corrodens*, *Enterobacteriaceae*, *Fusobacterium nucleatum* subsp. *Nucleatum*, Fusobacterium nucleatum subsp. Polymorphum, Fusobacterium nucleatum subsp. *Vincentii*, Fusobacterium *periodonticum, Porphyromonas endodontalis,* Porphyromonas gingivalis, Prevotella (Bacteroides) *melaninogenica,* Prevotella intermedia, *Bacteroides (Bacteroides*) *loescheii, Solobacterium moorei, Tannerella forsythia* (*Bacteroides forsythus*), *Treponema denticola.*

Causes-

1. Gingivitis, periodonitis, acute necrotizing ulcerative gingivitis, pericoronitis, abscesses
2. Systemic disease (inflammatory/infectious disorders, cutaneous, (gastrointestinal and haematological disease), malignancy, local causes.
3. Poor maternal oral hygiene.
4. Increased metabolic activity of microbes during sleep that is associated by a physiological reduction in salivary flow, lack of nocturnal physiologic oral cleansing (e.g. movement of the facial and oral muscles) and variable oral hygiene procedures prior to sleep.
5. Halitosis occur either as a result of the ingestion of certain food and drinks, such as spices, garlic, onion, durian, cabbage, cauliflower and radish, or of habits such as smoking tobacco, drinking alcohol, is usually transient, often caused by sulphur-containing volatile agents and is considered to arise both from intra-oral (food debris) and extra-oral (respiratory) origins. (Jones MN et al., 1994).

1.3 Mucoadhesion:

'Adhesion' is defined by the scientist as the molecular force of attraction in the area of contact between unlike bodies that act to hold them together' and 'bioadhesion' is simply those adhesive

phenomena where at least one of the adherents is biological. The materials are attached to each other by interfacial forces for an extended period of time.

When a neutral or synthetic polymer is fixed in a biological setting, it is often termed "bioadhesion", furthermore if this adhesion occurs on mucosal membranes it is termed as "mucoadhesion" (Andrews et al. 2009; Hearnden et al. 2012). Mucoadhesion has been widely promoted as way of achieving site-specific drug delivery through the incorporation of mucoadhesive hydrophilic polymers within pharmaceutical formulation along with active pharmaceutical ingredients (API). For mucoadhesion to occur, a succession phenomenon is required. The first step of mucoadhesion is concerned with intimate contact between mucoadhesive polymer and membrane, either from the good wetting of the mucoadhesive surface or from the swelling of the mucoadhesive. In the second stage, after contact is established, interpenetration of the chain of the mucoadhesive with those of the mucus takes place.

1.3.1 Advantages of mucoadhesive drug delivery system:

- Drugs can be easily administered to the patients who are unconscious.
- It avoids hepatic first pass metabolism.
- Drugs which are destroyed by the acidic environment the gastric juice can be given by this route.
- It is easy to administer and it is easy to removal.
- Permits localization of the drug in the oral cavity for longer period of time.
- Drugs which have poor bioavailability can be administered by this route.
- Drugs which have short biological half life can be administered in this route ex- Nitroglycerine (2h).
- The presence of saliva helps large amount of water for dissolution of drug unlike in case of transdermal and rectal route.

1.3.2 Disadvantages of mucoadhesive drug delivery system:

- Low permeability in the buccal membrane when it is compared with sublingual membrane.
- Dilution of drugs due to the continuous secretion of saliva (0.5-2 litres/ day).

1.3.3 Properties of a drug candidate for oral mucoadhesive drug delivery:

There are following properties of a drug candidate must be considered for selection of a drug for mucoadhesive drug delivery system which are discussed below-

- Molecular size of drug should be 75-600 Dalton.
- Drug should either be lipophilic or hydrophilic.
- Drug should be stable at salivary pH.

1.3.4 Anatomy & Physiology of oral mucosa:

The oral cavity consists of following parts like- lips, cheek, tongue, hard palate, soft palate and floor of the mouth (Dowty et al. 1992). Most of the oral mucosa is covered by compacted epithelium cells. Below the epithelium there are layers such as- the basement membrane, lamina propia and sub mucosa and also many sensory receptors including the taste receptors of the tongue (Figure 2). There are three types of oral mucosa can be found in the oral cavity.

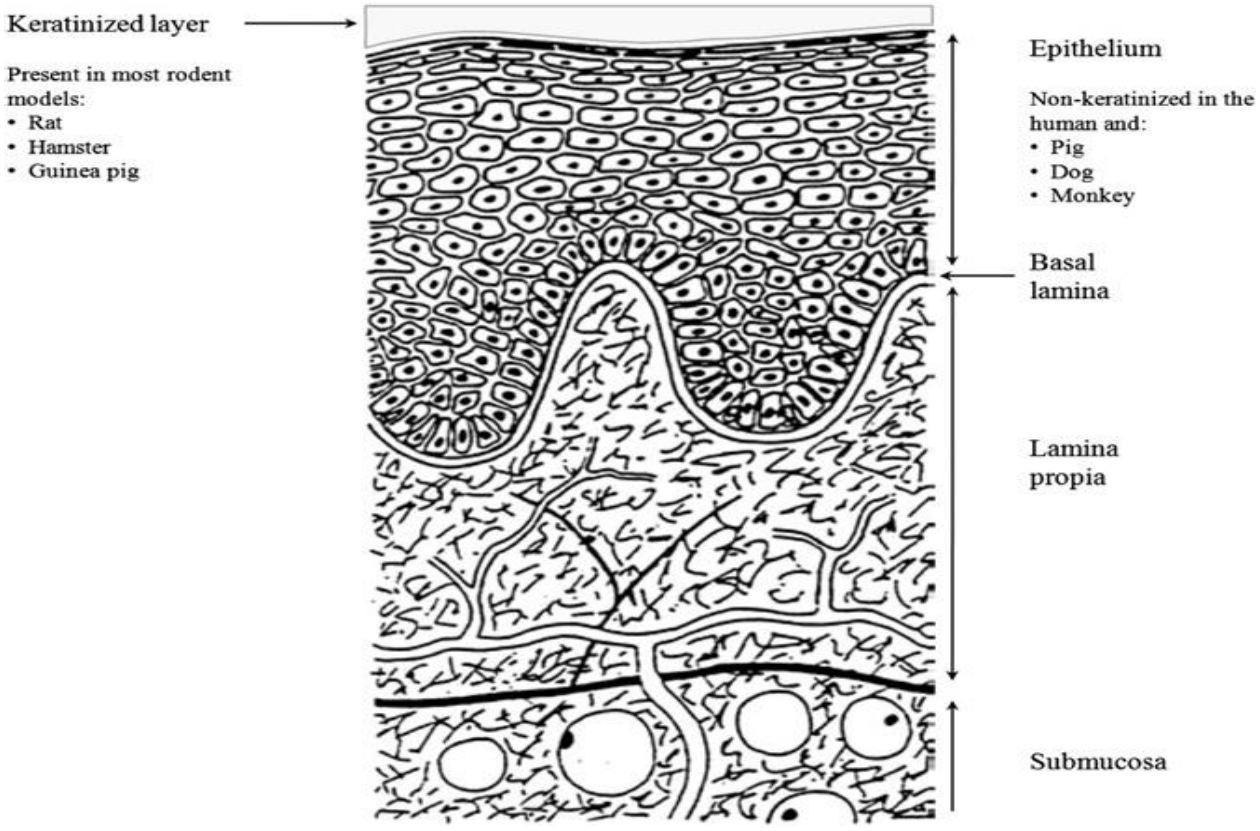

Figure 2: Schematic diagram of oral mucosa (Harris and Robinson, 1992; Collins and Dawes, 1987).

a) Lining mucosa:

- It is present in the buccal mucosa.
- It is stratified and surface is non-keratinized.
- It holds 60% percent of oral cavity.

b) Specialized mucosa:

- It holds 15% of the surface of the oral mucosa it is found in tongue.
- It is having both keratinized as well as non-keratinized structure.

c) Masticatory mucosa:

- It covers 25 % total surface of oral mucosa.
- It helps in grinding of food particles.
- It is found in hard palate and also in the gingival region.

Table-1: Characteristics of oral mucosa

Characteristics of tissue	Buccal tissue	Sublingual tissue	Gingival tissue	Palatal tissue
Structure	Non-keratinized	Non-keratinized	Keratinized	Keratinized
Thickness (µm)	500-600	100-200	200	250
Surface area (cm^2)	50.2±2.9	26.5±4.2	-	20.1±1.9
Permeability	Intermediate	Very good	Poor	Poor
Residence time	Intermediate	Poor	-	Very good
Blood flow**	20.3	12.2	19.5	7.0
Composed material	Cholesterol sulphate and glucosyl ceramides	Cholesterol sulphate and glucosyl ceramides	Ceramides and acyceramides	Ceramides and acyceramides

1.3.5. Salivary and salivary pH:

Saliva is the protective fluid or aqueous hypotonic solution which is essential for all tissues of the oral cavity. In general, saliva has a cleansing effect on the teeth. It safeguards the soft tissues from abrasion by rough materials and from chemicals (Jones et al. 2000) and also maintains oral microbial flora by maintaining the oral pH and enzyme activity (Herrera et al. 1988). Its activity increases as the viscosity of the saliva increases. Eating fibrous food and chewing vigorously increases salivation and this helps in digestion as well as improves cleansing of the teeth (Daniel, 1984). The total volume of saliva secretion is approximately 0.5-2 liters/day. This amount of fluid helps for hydration of oral mucosal drug delivery system (Slomiany et al. 1996). Saliva is an aqueous fluid (relatively less viscous) which consists of mucus (99% water), mucin (glycoprotein), 1% organic (sodium, potassium, calcium bicarbonate, phosphate etc.) and inorganic materials (urea, uric acid, glucose, amino acids, creatinine, lactate.etc.) (Ferguson 1987). Mucins are large molecules with molecular masses ranging from 0.5- 20 MDa and contain large amounts of carbohydrate.

Mucins are made up of basic units (approximately 400-500 kDa) linked together into linear arrays. These big molecules are able to join and act as lubricant allowing cells to move relative to one another, and may also contribute to cell-cell adhesion. (Slomiany et al, 1996). On physiological pH and due to the presence of sialic acid and sulfate residues, it forms a strongly cohesive gel structure that will bind to the epithelial cells surface as gelatinous layers (Bures et al. 2001). This gel layer is believed to have a significant role in mucoadhesion for drug delivery systems which work on the principle of the adhesion to the mucosal membrane and thus extend the dosage form retention time at the delivery site (Rathbone et al. 1994).

The vital factor of the salivary composition is flow rate, which in turn depends upon three factors: i) the time of day ii) the type of stimulus and iii) the degree of stimulation (Edgar, 1992). Depending on the flow of rate, the salivary pH ranges may vary from 5.5 to 7. When the sodium and bicarbonate concentrations are increased, they result in an increase of the fluid rate and the salivary pH (Shojaei, 1998). However, saliva flow decides the time duration of the released drug at the delivery site.

Many researchers have reported that there are some of the other intercellular materials, named membrane coating granules (MCGs) which act as permeability barrier materials for many compounds. MCGs are spherical or oval shaped organelles and are 200-300 nm in diameter and found in both keratinized and non-keratinized epithelia (Hayward, 1979). Physiological challenge related to the oral mucosa due to its unique structural and physiological properties, offers several opportunities for systemic drug delivery. Since the mucosa is vastly vascularized any drug diffusing across the oral mucosa membranes has direct access to the systemic circulation via capillaries and venous drainage. The rate of blood flow through the oral mucosa is

substantial, and is generally not considered to be the rate- limiting factor in the absorption of drugs by this route. Due to anatomic considerations, this includes capacity for direct visualization (which enables monitoring of therapy and direct placement of drug delivery system), oral mucosa is more amenable to the use of local drug delivery strategies than conventional dosage forms.

1.4.1 Oral mucoadhesive drug delivery system:

Oral mucoadhesive system is one of the best route of administration for transmucosal drug delivery due to its good accessibility for application and removal of a drug delivery device in emergencies, high permeability, high blood flow, and resistance to external stresses. All these features make the oral mucosa an alternative site for local disease treatment (oral cavity pathologies, such as gingivitis, periodontitis, stomatitis, dental diseases, oral ulcers, bacterial, and fungal infections), as well as systemic administration especially of hydrophilic macromolecular therapeutic agents (such as peptides, proteins, and polysaccharides (De Vries et al. 1991; Li et al. 1998).

There are many types of mucoadhesive dosage forms employed to prolong drug retention in the oral cavity, including tablets, ointments, gels, disks, patches and films Among these formulations, patches are more desirable over mucoadhesive disks and tablets, in terms of patient compliance and flexibility, and ensure more accurate drug dosing and longer residence time compared to gels and ointments. In general the drug classes used topically in the mouth are antimicrobials, anti-inflammatory and local anaesthetics, which are frequently administered in lozenges, mouthwashes and oral gels. Several methods and polymers are applied to formulate the oral mucoadhesive drug delivery system. (Collins and Deasy, 1990; Gandhi and Robinson, 1994).

1.4.2 Characteristics of an ideal mucoadhesive polymer:

The most important requirements of a good mucoadhesive polymer are as follows-

- It must be non-toxic with no undesirable physiological or pharmacological actions, and should not be expensive.
- Optimum concentration of polymeric adhesives.
- The polymer must not decompose on storage or during the shelf life of the dosage form.
- It should possess some site specificity.
- It should allow daily incorporation to the drug and offer no hindrance to its release.
- Spatial confirmation should be perfect.

1.4.3 Pressure sensitive adhesives:

Pressure sensitive adhesive (PSA) a material that adheres with no more than applied finger pressure, is aggressively and permanently tacky, exerts a strong holding force, and should be removable from a smooth surface without leaving a residue with little effort. For an adhesive bond to have measurable strength, elastic energy must be stored during the bond-breaking process. Therefore, pressure-sensitive adhesion is a characteristic of a visco-elastic material. The balance of viscous flow and the amount of stored elastic energy determine the usefulness of a PSA material (Mukherjee et al. 2006).

A balance of three properties, tack (initial adhesion), peel strength (mucoadhesion), and shear (cohesion) must be considered during the formulation of PSA based patches. Tack is the ability of a polymer to adhere to a substance with little contact pressure (Damodharan et al. 2010). Peel strength refers to the force required to remove the adhesive from a substrate once the bond has reached equilibrium. Shear strength is defined as the ability of adhesive to hold in position when shearing forces are exerted. Apart from the typical characteristics of PSAs (tack, peel, shear), some of the other properties such as UV-resistance, solvent resistance, humidity resistance, thermal resistance, color and costs are also considered while selecting the adhesives in a formulation.(Pfister, 1989).

1.4.4 Classification of pressure sensitive adhesives:

Generally, based on the source of raw materials, properties and characteristics, the pressure sensitive adhesives have been classified into various types (Figure 3 and Figure 4).

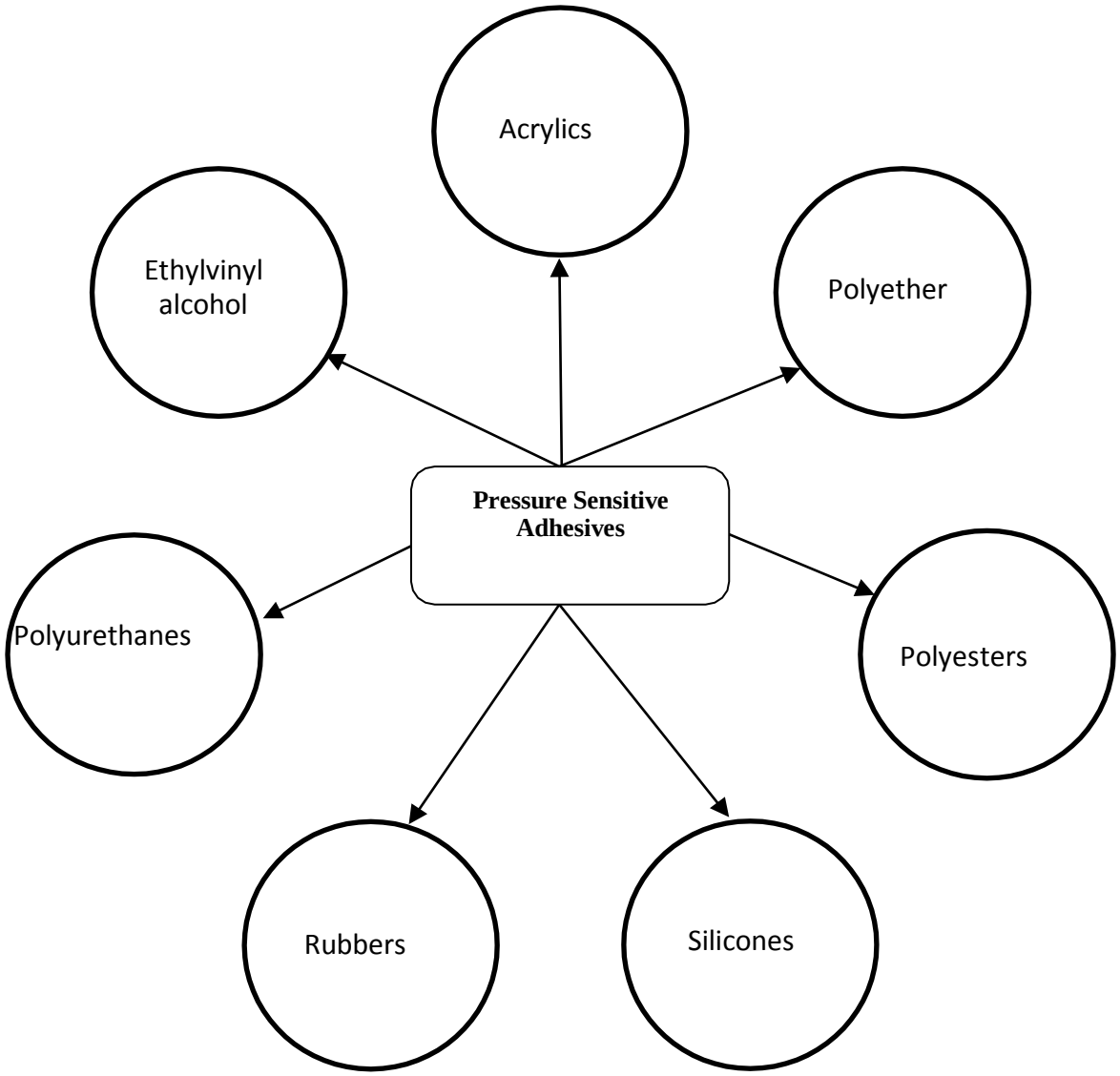

Figure3: Different classes of polymer classes as prospective resources for the manufacture of PSAs. (Czech and Kurzawa 2007).

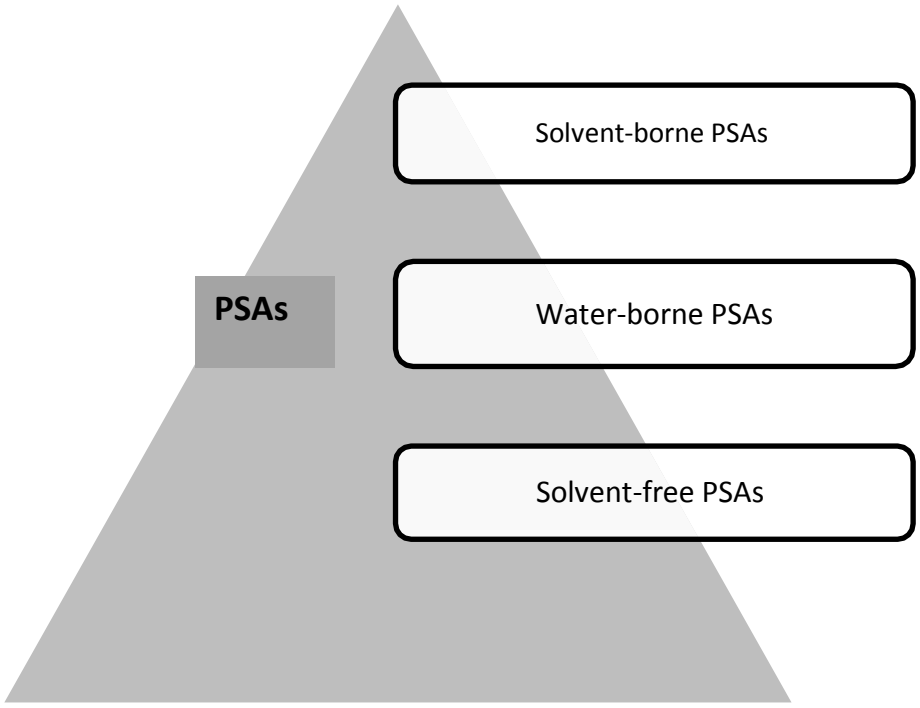

Figure 4: PSAs classification based on the properties and characteristics (Benedek, 2006).

1.5.1 Theories of mucoadhesion:

Mechanisms of polymer attachment to mucosal surface have not been understood yet. Nevertheless, certain hypothesis of mucoadhesion have suggested that it may occur due to physical entanglement (diffusion theory) and/or chemical interactions, such as electrostatic, hydrogen bonding, and Van der Waals' interactions (adsorption and electronic theories).

Five theories have been proposed to play a major role in mucoadhesion, namely, adsorption, diffusion, electronic, fracture, and wetting theories (Madsen et al. 1998). In the adsorption theory, primary and secondary chemical bonds of the covalent and non-covalent (electrostatic and van der Waals' forces, hydrogen, and hydrophobic bonds) types are formed upon initial contact between the mucus and the mucoadhesive polymer. Wetting theory describes the importance of contact angle and reduction of surface and interfacial energies to achieve good

mucoadhesion. In brief, it describes the spreadability coefficient. Usually this theory applies to liquid systems which present affinity to the surface in order to spread over it. For an appropriate spreadability the contact angle should be close to equal or zero.

Though the diffusion theory elucidates how the degree of penetration helps the adhesion force of mucin and polymer, the penetration rate based on the diffusion coefficient, flexibility and nature of the mucoadhesive chains, mobility and contact time. Fracture theory simply concerned the force required to separate two surfaces after adhesion is ascertained (Ahagon and Gent, 1975). Electronic theory defines the occurrence of adhesion by means of electron transfer between the mucus and the mucoadhesive system (Dodou et al. 2005).

1.5.2 List of mucoadhesive polymers: The various mucoadhesive polymers are mentioned below-

Table 2: List of mucoadhesive polymers:

Synthetic polymers	Natural polymers
Polyvinyl alcohol (PVA)	Tragacanth
Polyethylene oxide (PEA)	Sodium alginate
Polyvinyl pyrrolidone (PVP)	Pectin
Poly acrylic acid polymers (PAA)	Gelatin
Poly hydroxyethyl methacrylate (PHEMA)	Chitosan
Hydroxy propyl cellulose (HPC)	Lectin
Hydroxy propyl methylcellulose (HPMC)	Karaya gum

1.5.3 Design of mucoadhesive dosage form:

1.5.3.1 Types of mucoadhesive drug delivery systems:

Matrix type:

The buccal patch developed in a matrix configuration contains drug, adhesive, and additives which are mixed together. The structure of the matrix type design is basically a mixture of the drug with the mucoadhesive matrix.

Reservoir type:

The buccal patch developed in a reservoir system contains cavities for the drug and additives, separate from the adhesive. Impermeable backing is applied to control the direction of drug delivery; to reduce patch deformation and disintegration while in the mouth; and to prevent drug loss (Chien, 1987).

In general, mucoadhesive dosage forms are of three types based on their geometry.

- Single layer device with multidirectional release significant drug loss due to swallowing.
- Impermeable backing layer is superimposed that prevents drug loss in the oral cavity.
- Minimal drug loss is achieved by coating every face except contact face.

1.5.4 Film casting of oral mucoadhesive patches:

During the formulation development of films, particular focus should be 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 compound as well as the physical appearance of the films. Furthermore, the air bubbles in the casting solution may also lead uneven surfaces and heterogeneous thickness. So the removal of air bubbles is a vital step to maintain the homogeneity of the films. Another repetitive concern in the development of films for oral delivery is the use of organic solvents and the presence of solvent residuals which may cause hazards for the environment and the health (Morales and Mc Conville, 2011).

1.5.5 Factor affecting mucoadhesion of polymer:

A) Molecular weight:

- Generally, mucoadhesive strength of the polymer is directly proportional to its molecular weight, which means increasing the molecular weight increases the mucoadhesive strength in the linear polymers. For example, polyethylene glycol increases in the order: $2 \times 10^4 < 2 \times 10^5 < 4 \times 10^5$. At the same time, dextran with very high molecular weight, $\sim 2 \times 10^7$, shows mucoadhesion similar to that of PEG with molecular weight 2×10^5.
- The various hypothesis of polymers refer to as the optimum molecular weight for mucoadhesion should be between $< 4 \times 10^4$ and 4×10^6.

B) Ability to form hydrogen bond:

- The functional groups like (-COOH, -OH) have the capability of formation of hydrogen bonds.
- Polymers such as poly vinyl alcohol), hydroxylated methacrylate and poly (methacrylic acid), as well as all their copolymers, have acceptable hydrogen bond forming ability (Peppas and Buri, 1985).

C) Cross- linking density:

- The average pore-size, number and average molecular weight of the cross-linked polymers, and the density of cross-linking are three most important and inter-related structural parameters of a polymeric network.
- So, it is very clear that with increasing density of cross-linking, diffusion of water into the polymer network occurs at a very low rate which, in turn, causes an insufficient swelling of the polymer and a decreased rate of interpenetration between polymer and mucin (Gu et al. 1998).

D) Charge and pH:

- There is no notable literature about the influence of the charge of the membrane on the mucoadhesion. Some underlined theories about the charge of mucoadhesive polymers have been reported, where non-ionic polymers possess a smaller degree of adhesion compared to anionic polymers. However, strong anionic charge on the polymer is one of the required characteristics for mucoadhesion. Some cationic polymers are likely to demonstrate superior mucoadhesive properties, especially in a neutral or slightly alkaline medium.
- In some cases it has been observed that pH of the membrane is responsible for mucoadhesion as it can influence ionized or un-ionized forms of the polymers (Park et al. 1989).

E) Concentration:

- The significance of this factor depends on the development of 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 chains per unit volume of the mucus is small and the interaction between polymer and mucus is unstable

- 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 formation of coiled structure. As a result, the accessibility of the solvent to the polymer decreases, and chain penetration of the polymer is a longer penetrating chain length and better adhesion. However, for each polymer, there is a critical concentration, above which the

CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW:

Puratchikody et al., 2011 formulated novel mucoadhesive buccal patches of salbutamol sulfate with unidirectional drug delivery were first developed to overcome first-pass metabolism and subsequent low bioavailability of the salbutamol sulfate. The residence time of the tested patches ranged between 105 and 130 minutes. The *in vitro* study has shown that this is a potential drug de livery system f or salbutamol sulfate with a considerably good stability and release profile.

Cavallari et al., 2013 developed Mucoadhesive buccal patches, containing 8 m g/cm2 lidocaine ba se, w ere developed by solvent casting method technique, using a number of different bio-adhesive and film-forming semi-synthetic and synthetic polymers (Carbopol, Poloxamer, different type Methocel) and plasticizers (PEG 400,tririethyl citrate). *In vitro* drug release and permeation were conducted using a modified Franz diffusion cell. Lidocaine/Compritol solid dispersion in t he form of microspheres, embedded inside the patches, alone or together with free lidocaine, was also examined t o, prolong t he drug release. Simple changes of t he formulation consideration makes the effects in the drug release mechanisms (quick/ delay/ prolonged).

Obaidat et al., 2012 formulated Mucoadhesive bilayered patches consisting of ethyl cellulose as a backing layer and carbopol 934 a s a matrix-forming layer w as prepared. Combination of tetracycline and carvacrol showed excellent acttivity against *Candida albicans* and t he s elected bacterial s trains.

Evidence of synergism between the two components against *Pseudomonas aeruginosa* and *Bacillus cereus* was shown and it needs further investigation. The prepared patches showed a sustained release action for more than 6 h, acceptable bioadhesion, and stability. The patch consisted of 0.01 g tetracycline and 1.1 mg carvacrol per 10 cm^2 of film which contained the least effective concentrations of the two active components and the maximum delayed release and an acceptable mucoadhesion force

Nafee et al., 2003 formulated Mucoadhesive patches for delivery of cetylpyridinium chloride (CPC) and characterized. This study has pr oven t hat non-ionic polymer, polyvinyl alcohol (PVA), showed good mucoadhesive and swelling characteristics. Medicated PVA patches maintained a satisfactory residence time in the buccal cavity and ensured zero-order release of the drug over r elatively long periods (7 h) , which made them good candidates f or stability studies. Ageing did not affect the elastic properties of the PVA patches but affected the extent of drug release; this may be attributed to changes in the crystal habit of the drug as well as to slight agglomeration of the polymer particles.

Vamshi Vishnu et al., 2007 developed buccal patches systemic administration of carvedilol in the oral cavity has been developed using two different mucoadhesive polymers. An *in vitro* and *in vivo* condition for films of HPMC E15 LV shows the good results. The buccal delivery of carvedilol in healthy pigs showed a significant improvement in bioavailability of carvedilol from patches when compared to oral route. The bioavailability of carvedilol increased by about 2.29 times when compared to oral route. The results can be extrapolated to human beings as the structure and permeability of buccal membrane of pigs is similar to that of human beings.

Mukherjee et al., 2013 formulated pressure-sensitive adhesive based dental patches to ensure sustained release of drugs, to treat periodontal and other dental diseases locally. Polymers like duro-tak® 387-2516 and duro-tak® 387, ethylcellulose, polyvinylpyrrolidone, polyvinyl alcohol together with drugs amoxicillin trihydrate and diclofenac sodium were used to develop the experimental patches. The patches were further evaluated for mass variation, thickness, area, moisture-content and moisture-uptake, folding endurance, structural integrity in simulated saliva, bioadhesive strength, surface pH, scanning electron microscopy, cellular morphology, in vitro drug release and temperature-dependant stability study as per ICH guideline. Addition of release enhancers in the patches improved drug release. Drugs released in a sustained manner due to the generation of physical bonds between the polymers (as assessed by FTIR study) which might alter the length of drug diffusion pathways.

Ahmed et al., 2010 illustrated that gatifloxacin is a broad-spectrum antimicrobial agent, which is active against a number of aerobic, anaerobic, gram positive and gram negative periodontal pathogens. In the present investigation, chitosan strips containing Gatifloxacin (10%, 20% and 30% to the weight of polymer) were prepared by solution casting method using 1% v/v acetic acid in water. Further strips containing 30% gatifloxacin were cross-linked by exposing to the vapours of 2% v/v glutaraldehyde in water intended to extend the release. Macroscopical features revealed that drug was dissolved in the polymer matrix rather than dispersing. The prepared films were evaluated for their thickness, content uniformity, weight variation, tensile strength, hardness and in-vitro dissolution. The average weight and thickness of both the crosslinked and uncross-linked strips were uniform. There was a reduction in the tensile strength and increase in hardness when the films were cross-linked. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug and extended upto 19 days once the strips were cross-linked. Release kinetics of gatifloxacin from chitosan strips followed the higuchi's diffusional model and also showed zero order release profile.

Roy et al., 2009 illustrated that toothache is a serious problem worldwide. To give relief from this intolerable toothache, doctors prescribe painkillers along with antibiotics. Most of the painkillers, if not all, produce hyperacidity and gastric irritation upon oral administration. Oral antibiotics have slow onset of action and undergo hepatic "first-pass" effect. Moreover, available dental formulations are mostly liquid and last only few hours upon application, before being washed out by saliva. To overcome the

above-mentioned problems, a soft polymeric mold containing antibiotic and analgesic drugs and having an appropriate consistency to adhere to the tooth, was developed for sustained drug release to provide better relief in dental patients. Eudragit L 100-55, carbopol 971 P, gum karaya powder and ethyl cellulose were used to prepare the mold "Denticaps" containing Lidocaine hydrochloride and Amoxicillin trihydrate individually and in combination, by mixing and solvent evaporation technique. Different physicochemical characterization studies such as mucoadhesion test, water absorption capacity and swelling index were carried out. In vitro drug release studies showed sustained release of Lidocaine hydrochloride and Amoxicillin trihydrate in simulated saliva for 24 h. Further studies are warranted to succeed with these formulations in humans. Upon success, this type of dosage form may open up new avenues towards dentistry.

Ghosh et al., 2009 illustrated that oral administration of antibiotics to treat dental diseases are problematic because of slow onset of actions and hepatic first-pass metabolism Additionally, commonly used dental paints are generally washed out by saliva within few hours of application. To conquer the challenges, polymeric molds to be placed on an affected tooth (during carries and gum problems) were prepared and evaluated in vitro for sustained drug release for prolonged local action. Here, antibiotic and local anesthetic were used as model drugs. Dental molds were prepared using corn zein, carbopol 934 P, gum karaya powder, and poloxamer 407 by mixing and solvent evaporation technique. Different physicochemical evaluation studies such as tooth adhesion test, surface pH, swelling index, and drug-distribution pattern were carried out. Percentage swelling varied from 56% to 93%. Average tooth adhesion strength and mean initial surface pH of the formulations were 50 g and 6.5, respectively. As assessed by scanning electron microscopy, drug distribution was uniform throughout the matrix. Cumulative percentage release of local anesthetic and antibiotic in simulated saliva were 98% and 50%, respectively. In vitro drug-release studies revealed the sustained-release patterns of the drugs in simulated saliva at least for 24 h. The stability study shows that the drugs were stable in the formulations following the conditions as per ICH guideline. The formulation is a novel approach to deliver the drug(s) for a prolonged period for local action upon its application on an affected tooth.

Joshi.A.S. et al (2012) investigated on statistical optimization of Solid Lipid Nanoparticles (SLNs) of Ondansetron HCl for intranasal (i.n) delivery. SLNs were prepared using the solvent diffusion technique. The concentrations of lipid, surfactant and co-surfactant were independent variables in this design, whereas, particle size and entrapment efficiency (EE) were dependent variables. The particle size of the SLNs was found to be 320–498 nm, and the EE was between 32.89 and 56.56 %. The influence of the lipid, surfactant and co-surfactant on the particle size and EE was studied.

Daisy.Chella.Kumari.S.et al (2012) developed an optimized gastric floating drug delivery system (GFDDS) contains Ondansetron Hydrochloride as a model drug. Formulation developed by using various proportions of polymers such as HPMC K4M and Ethyl cellulose. This was employed to enhance the bioavailability and therapeutic efficacy of the drug. The sustained release GFDDS formulations of Ondansetron Hydrochloride using hydrophobic and hydrophilic polymers were prepared by wet granulation method. Optimization of formulation was done by studying effect of drug to polymer ratio on drug release.

Mahajan Y.Y. et al (2012) worked on formulation of Ondansetron HCl which is a serotonin receptor (5-HT3) antagonist used in the prevention of Chemotherapy and radiotherapy induced nausea and vomiting. The necessity for orally disintegrating tablet has been growing, especially for geriatric and paediatric patients because of their swallowing difficulties. In this study, the bitter taste of Ondansetron HCl was masked using Tulsion- 339 & flavour; also the disintegration time is reduced with using different super disintegrants. The FTIR studies showed drug and carrier were compatible. These were then compressed into tablet direct compression methods using different superdisintegrants like Shieffield ODT, Crosspovidone, Pharmaburst- 500, Polyplasdone XL-10, Ludiflash.

Malik. R. K. et al (2013) fabricated mucoadhesive beads and evaluated controlled release of Ondansetron HCl. Ondansetron hydrochloride is a serotonin 5-HT3 receptor antagonist mainly used for the treatment of emesis, which occurs as a side effect of chemotherapy. Mucoadhesive microbeads were fabricated by using chitosan as mucoadhesive and sustained release polymer. Sodium tripolyphosphate (Na-TPP) was used as a cross-linking agent. The microbeads were successfully prepared by ionotropic gelation technique. The particle size, entrapment efficiency, and mucoadhesive strength of drug-loaded formulations was measured by an optical microscope, direct crushing method, and *in vitro* wash-off method.

Sheba Rani. N. D et al (2015) illustrated the fabrication and evaluation design of self-adhesive matrix-type Ondansetron hydrochloride transdermal formulation in their work. Ondansetron HCl transdermal patches were prepared using solvent casting method. The matrix polymer composition was Eudragit E 100, polyvinyl pyrrolidone and either propylene glycol or dibutyl sebacate(DBS) as plasticizer. After fabrication, mean patch thickness, tensile strength, and moisture content, water absorption capacity and drug content of the patches were measured. In vitro release and permeation of the patches was determined using Franz diffusion cell. Mean patch thickness, moisture content, and water uptake increased with increased contents of polyvinyl pyrrolidone (PVP) and plasticiser. Higher levels of PVP and plasticiser increased drug release. Addition of release modifier such as Succinic acid (SA) and

Myristic acid (MA) to the patch formulations produced a significant increase in drug release from the patch.

Teodorescu. F. et al (2017) have established that, development of a skin- mounted patch capable of controlled transcutaneous delivery of therapeutics through thermal activation provides a unique solution for the controlled release of active principles for long time. Here, they report on a suitable transdermal patch for photothermal triggered release of Ondansetron a commonly used drug for the treatment of chemotherapy- induced nausea and vomiting. To achieve this, a dispersion of ODS-loaded reduced graphene oxide (rGO-ODS) nanosheets were deposited onto Kapton to produce a flexible polyimide-based patch. It is demonstrated that Ondansetron loaded Kapton/rGO patches have a high drug delivery performance upon irradiation with a continuous laser beam at 980 nm for 10 min due to an induced photothermal heating effect. The ability of Ondansetron impregnated Kapton/rGO patches as transdermal delivery scaffolds for Ondansetron across the skin is in addition investigated using porcine ear skin as a model. The actual superiority and interest of the proposed work is that the Kapton/rGO photoactivatable skin patch can be loaded with any drugs to make it versatile.

Sheba R. David et al (2018) they constructed their experiment on Peritonitis, which is a serious complication of peritoneal dialysis. *Staphylococcus aureus* infections could lead to peritonitis which causes reversal of peritoneal dialysis treatment back to hemodialysis. The aim of this study was to develop a controlled release silicone adhesive-based Mupirocin patch for prophylactic effect and analyze its antibacterial effects against *S. aureus*. Here, *in vitro* disk diffusion assay was performed on the Mueller–Hinton Agar plate to measure the zone of inhibition of the patches. The *in vivo* study was performed on 4 groups of rats with bacterial counts at three different time intervals, along with skin irritancy and histopathological studies.

Zaman. M. et al (2017) they prepared transdermal patches loaded with Ramipril and Repaglinide to develop matrix-type transdermal drug delivery system for enhanced permeability and improved bioavailability. Different formulations were designed by using different concentrations of HPMC-K4M as hydrophilic polymer and EC as hydrophobic polymer. Solvent casting method was used for the fabrication of transdermal patches. Oleic acid and propylene glycol were used to enhance permeability and polyethylene glycol 400 as plasticizer. *In vitro* and *ex vivo* permeation studies were executed using franz diffusion cell. The cumulative amount of drug permeated through skin was 55.22–112.72% for Repaglinide and 73.14–91.46% for Ramipril. The results showed that Korsmeyer–Peppas model was found to be dominating in most of the formulations and drugs followed diffusion mechanism. It could be concluded that HPMC-K4M and EC has great potential for Ramipril and Repaglinide as a vector for transdermal drug delivery effectively because of the formation of smooth surfaces of patches, high folding endurance, and entrapment efficiency with the ability to release the drugs in sustained manner.

CHAPTER 3

AIM OF THE RESEARCH WORK

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3. AIM OF THE REASEARCH WORK:

Mouth is full of microbial paradise; the aim of the study was to develop mucoadhesive dental patches of Amoxicillin trihydrate, Metronidazole & Lidocaine hydrochloride by solvent casting method using EC and HPMC as a polymer, DBP as a plasticizers to provide flexibility and durability in the formulation, PVP K-30 acts as a pressure-sensitive adhesives to provide desired tack effects.

Amoxicillin trihydrate is a β-lactum antibiotic widely used in the dental practice to treat both bacterial infection and periodontal disease. It is active against both Gram positive and Gram negative bacteria as reported in the literature.

Lidocaine hydrochloride is mainly a local anesthetic widely used in the dental procedures it blocks Na+ channel, widely used in the dental practices to relive pain or irritation caused by dental devices.

Metronidazole is an antimicrobial agent active against Gram negative anaerobes, wide range of bacteria and several protozoa thus it prevents bad smell in the oral mucosa.

CHAPTER 4

MATERIALS &EQUIPMENTS

4. MATERIALS & EQUIPMENTS:

4.1Chemicals used in the formulations:

Table 3 : List of chemicals used in the formulations

Chemical Name	Source
Polyvinyl alcohol	S.d Fine-Chem. Ltd., Mumbai, India
Polyvinylpyrrolidone K-30	Loba Chemico, Mumbai
Ethylcellulose	S.d Fine-Chem. Ltd., Mumbai, India
Dibutyl phthalate	E. Merck, Mumbai, India
Polyethylene glycol 400	E. Merck, Mumbai, India
Hydroxy Propyl β Cyclodextrin	Tokyo chemical Industry Co.ltd ; Tokyo, Japan
Sodium chloride	E. Merck, Mumbai, India
Disodium hydrogen phosphate	E. Merck, Mumbai, India
Potassium hydrogen phosphate	E. Merck, Mumbai, India
Ethanol	Qualigens Fine Chemicals, Mumbai, India

4.2 Equipments used in the formulations:

Table 4: List of equipments used in the formulation

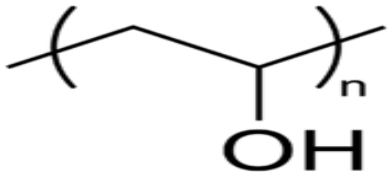
Equipment	Source
Electronic balance	Sartorius, GD-103, Goettingen, Germany
Magnetic stirrer	Remi Equiments Ltd, Kolkata, India
UV-Spectrophotometer	Cary 50 Bio, Varian, Australia
Water bath	Sicco, Kolkata, India
pH meter	Mettler Toledo, Gmblt, Switzerland
Digital calipers	Digmatic Massschieber, CD-6″CSX, Mitutoyo Corp., Japan
Millipore memberane filter	Whatman International Pvt. Ltd., England
FTIR Instrument	
Scanning electron microscope	EVO-18 Carl zeiss, Germany
Ultra sonicator (Bath type)	Trans-O sonic, Pvt. Ltd., Mumbai, India
Hot air oven	Barunboy, Kolkata, India
Light Microscope	Carl zeiss microscope GMBH 37081, Gottingen, Germany
Franz diffusion cell	Remco pvt. Ltd. India

4.3 Polymers used in the formulations:

4.3.1 Polyvinyl alcohol:

Synonyms: Poly (Ethenol), Ethenol, homopolymer, PVA; Polyviol, Vinol, Alvyl, Alkotex, Covol, Gelvatol, Lemol, Mowiol.

Structure:



Chemical Formula: $(C_2H_4O)_n$

Physicochemical properties

Description: It is an odourless, white to cream colored granular powder Molecular weight: It ranges from 30,000-2 00,000.

Density: 1.32 g/cm^3

Solubility: It is soluble on both hot and cold water and solubility decreases with increase in molecular weight. It is partially soluble in polyhydroxy compounds, amides, amines, etc. and practically insoluble in ketones, esters, chlorinated hydrocarbons, oil etc.

Melting point: It can be defined at two temperatures (i) 228°C (for fully hydrolyzed grades) and (ii) 180°C (for partially hydrolyzed grades).

Toxic effect: It is a non-toxic, non-irritant (concentration up to 10%) but 5% aqueous solution of PVA when injected subcutaneous in rats causes anemia and infiltrate various organs and tissues (Hall and Hall1993).

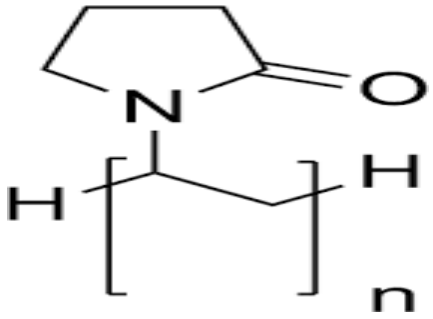
Uses:

- ❖ It is used as modifier and thickeners in polyvinyl acetate glues.
- ❖ Used as a lubricant in hard contact lens solution and also in eye drops.
- ❖ As a surfactant for the formation of polymer encapsulated nanobeads.
- ❖ Used in the protective chemical resistant gloves.
- ❖ Used as a fixative for specimen collection, especially stool samples
- ❖ As a embolization agent in medical procedures.
- ❖ It is also used in the treatment of dry eyes.
- ❖ Carotid phantoms for used as synthetic vessels in Doppler flow testing.

4.3.2 Polyvinyl pyrrolidone k-30:

Synonym : Plasdone K-32, Plasdone K29/32, Povidone K29/32 Povidone K29-32.

Structure:



Average Molecular weight-40000-80000

Molecular Formula- $(C_6H_9NO)_n$

Physicochemical Properties:

Description: PVP K-30 is a hygroscopic, amorphous, linear, non-ionic polymer. It is soluble in water, organic solvents, and pH stable. PVP K-30 is made from the monomer of N-vinylpyrolidone. Chemically it is- 1- ethenylpyrrolidin-2-one or 1-Ethenyl-2-pyrrolidon homopolymer. It should be stored in a tightly closed container and in a dry, cool place to avoid sunlight.

Density: 1.20g/cm^3

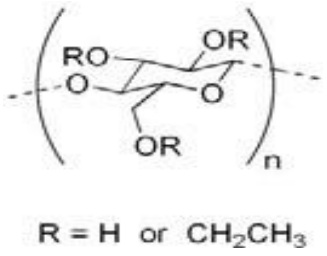
Uses :

- It is used as a thickening agent in tooth whitening gels
- It is used to make glossy transparent films.
- As an emulsifier and disintegrant for solution polymerization.
- For production of membranes, such as dialysis and water purification filters.
- as fuel, solvent in pharmaceuticals, treatment for poisoning by other alcohols.

4.3.3 Ethyl Cellulose :

Synonyms: Cellulose, ethyl ether, ethylated cellulose, ethylcellulose.

Structure:



R = H or CH_2CH_3

Physical properties:

Physical State: Solid

Appearance: white to light tan Odor: odorless

Melting point: 240°C (464°F) Density: 1.07-1.18 g/cm^3

Solubility: easily soluble in coldwater

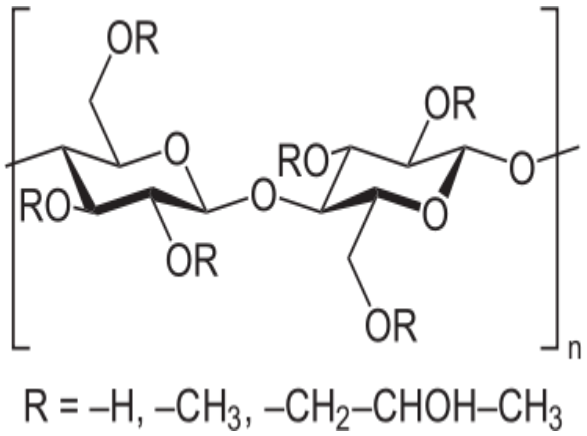
Toxic effects: Rat: LD50=5mg/kg, skin rabbit: LD50=5mg/kg

Uses: used as a food additive, as an emulsifier.

4.3.4 Carriers Used To Affect The Release Of Drug (s) From Dental Patch:

Hydroxy propyl methyl cellulose (HPMC)

HPMC E15 is a semi synthetic, inert, visco-elastic polymer. It is water soluble cellulose ether containing hydrocolloid with good film forming properties. The degree of substitution, types of functional groups substitution, and chain length of this polymer can affect permeability, mechanical properties and water solubility [Fernando A. Osorioa et al, 2011]. It can promote the drug release or permeability of the drug through skin.



R = –H, $–CH_3$, $–CH_2–CHOH–CH_3$

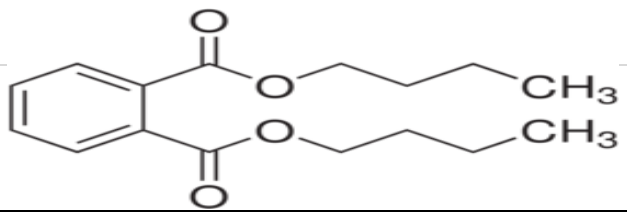
Molecular weight- 1261.45 gm/mol
Molecular formula- $C_{56}H_{108}O_{30}$
Melting point- 227-252°C

4.3.5 Plasticizers Used To Enhance The Mechanical Strength & Flexibility Of The Patches:

Dibutyl Phthlate :

Synonym: Di-n-butyl phthalate, Butyl phthalate, n-Butyl phthalate, 1,2-Benzenedicarboxylic acid dibutyl ester, o-Benzenedicarboxylic acid dibutyl ester, DBP, Palatinol C, Elaol.

Structure:



Chemical formula: $C_{16}H_{22}O_4$

Physicochemical properties:

Density: 1.05 g/ml

Molecular weight: 278.34 g/mol

Boiling point: 340°C

Melting point: -35°C

Soluble in: diethyl ether, acetone, very slightly soluble in cold water.

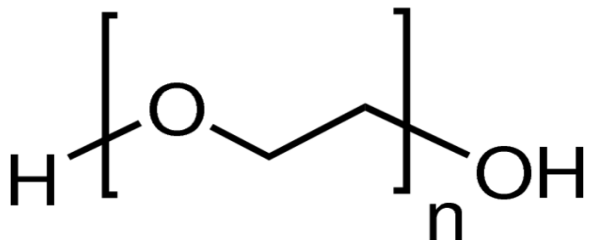
Uses:

DBP is an organic compound used as a lipophilic plasticizer. It is a color less liquid or sometimes having a yellowish color. Due to its wide liquid range and low toxicity it can be used as plasticizer. DBP has the ability to increase mechanical strength, flexibility and inter- molecular mobility in case of t patch. DBP is produced by the reaction of n- butanol and phthalic anhydride.

Polyethylene glycol-4000 (PEG-4000):

Polyethylene glycol (**PEG**) is a hydrophilic, polyether compound. PEG is also known as **polyethylene oxide** (**PEO**) or **polyoxyethylene** (**POE**), Depending on its molecular weight, the common structure of PEG is $H-(O-CH_2-CH_2)_n-OH$. Mainly PEG is the oligomer of Ethylene oxide. PEG is used as a plasticizer in patches to reduce brittleness, imrove flow ability and enhancing the resistance and tear strength.

Structure:

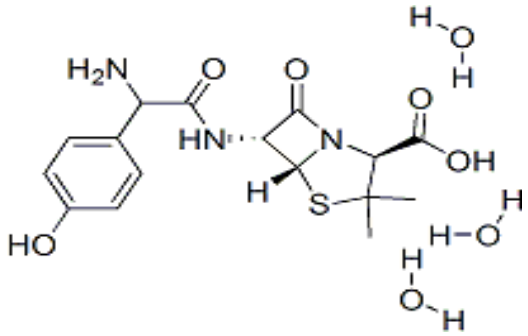


4.4.1 Drug Candidate

A) Amoxicillin Trihydrate : A broad-spectrum β-lactam group semisynthetic antibiotic similar to ampicillin except that its resistance to gastric acid permits higher serum levels with oral administration.

IUPAC Name: Amoxicillin; 4 -Thia-1- azabicyclo(3.2.0)heptane-2-carboxylic acid, 6 - ((amino(4-hydroxyphenyl)acetyl)amino)-3,3-dimethyl-7-oxo-,trihydrate;4-Thia-1- azabicyclo(3.2.0)heptane-2-carboxylic acid,6 -((amino(4- hydroxyphenyl)acetyl)amino)-3,3- dimethyl-7-oxo-, trihydrate, (2S-(2-alpha,5-alpha,6-beta(S*))).

Structure:



Chemical Formula: $C_{16}H_{25}N_3O_8S$

Physical Properties:

Appearance: solid (crystalline powder)

Molecular weight: 419.45 g/mole Color: off-white to white.

Melting point: 200°C **Refractive Index**: 302°C

Use: Semi-synthetic antibiotic related to penicillin. Antibacterial.

Indication: For the treatment of infections of the ear, nose, and throat, the genitourinary tract, the skin and skin structure, and the lower respiratory tract due to susceptible (only b- lactamase-negative) strains of *Streptococcus* spp. (a- and b-hemolytic strains only), *S. pneumoniae*, *Staphylococcus* spp., *H. influenzae*, *E. coli*, *P. mirabilis*, or *E. faecalis*. Also for the treatment of acute, uncomplicated gonorrhea (ano-genital and urethral infections) due to *N. gonorrhoeae* (males and females).

Pharmacodynamics:

Amoxicillin is a moderate-spectrum antibiotic active against a wide range of Gram-positive, and a limited range of Gram-negative organisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other beta-lactam antibiotics. Amoxicillin is susceptible to degradation by β-lactamase producing bacteria, and so may be given with clavulanic acid to decrease its susceptibility. The incidence of β- lactamase-producing resistant organisms, including *E. coli*, appears to be increasing.

Mechanism of action:

Amoxicillin binds to penicillin-binding protein 1A (PBP-1A) located inside the bacterial cell wall. Penicillin acylate the pe nicillin-sensitive t ranspeptidase C-terminal dom ain ope ns the lactam r ing. This i nactivation of t he enzyme prevents t he f ormation of a cr oss-link of two linear peptidoglycan strands, inhibiting the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that amoxicillin interferes with an autolysin inhibitor.

Absorption: Rapidly absorbed after oral administration.

Protein binding: In blood serum, amoxicillin is approximately 20% protein-bound.

Route of elimination:

Most of the amoxicillin is excreted unchanged in the urine; its excretion can be delayed by concurrent administration of probenecid.

Half life: 61.3 minutes

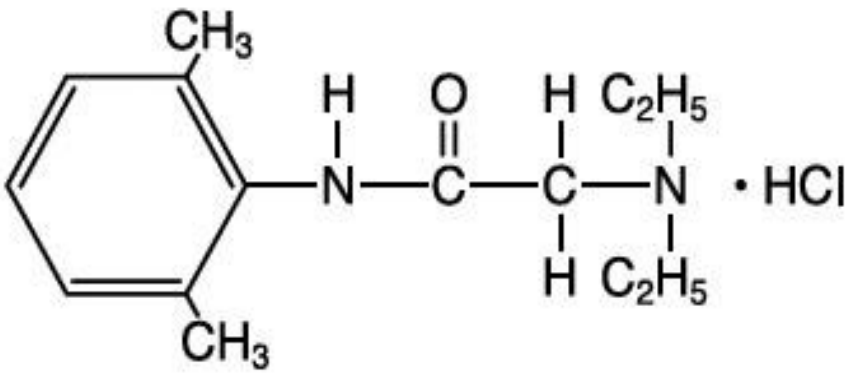
Toxicity:

Serious toxicity is unlikely following large doses of amoxicillin. Acute ingestion of large doses of amoxicillin may cause nausea, vomiting, diarrhea and abdominal pain. Acute oliguric renal failure and hematuria may occur following large doses.

B) Lignocaine hydrochloride : It is amide type of local anaesthetics it has been widely used. It has rapid onset of action, it has been also used in ventricular arrhythmias.

IUPAC Name : 2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide;hydrochloride.

Structure:



Chemical Formula: $C_{14}H_{22}N_2O$

Molecular weight: 270.801 g/mol.

Appearance: white crystalline powder.

Melting point: 270.801 g/mol

Usage: It is mainly used as local anaesthetics. A 2% Solution has been used for the application of mucous membrane.

Indication: Acute management of ventricular arrhythmias (cardiac surgery, acute MI)

Pharmacokinetics: Lignocaine upon i.v injection it is widely absorbed from mucous membrane and its duration of block block is about 1.5 hours when used with adrenaline. Lidocaine is metabolised in the liver by mixed function oxidases. It's one of the metabolites, xylide possesses local anaesthetic property.

Half life: 120minutes

Mechanism of action:

The site of action of lignocaine is cell membrane. Lignocaine block volatage gated NA^+channel .So no entry of NA^+ ions into the neurons.

No depolarization, no generation of action potential.

No generation of conduction of impulse to the C.N.S ,local anaesthesia.

Adr : Hypotension, bradycardia, agitation, convulsion may appear as side effects.

C) Metronidazole:

Metronidazole is a nitro imidazole derivative which is highly effective against most of the anaerobic bacteria, several protozoa such as *Entamoeba . histolytica*, Trochomonas *vaginalis.Giardia lamblia.*

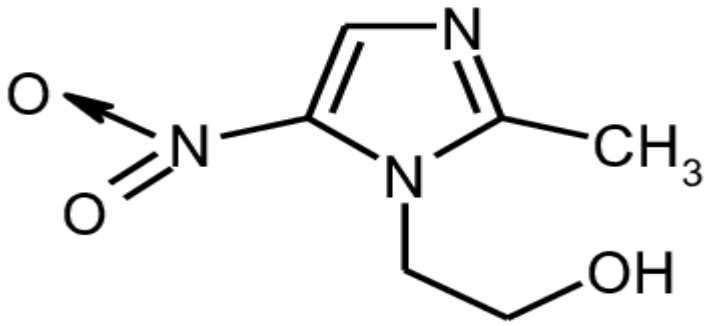
IUPAC NAME: 2-(2-methyl-5-nitroimidazol-1-yl)ethyl benzoate

Chemical Formula: $C_6H_9N_3O_3$

Physical Properties:

Appearance: White to yellowish white crystalline powder

Molecular weight: 171.156 g/mol



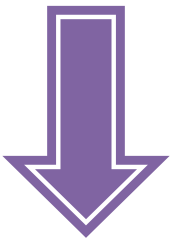
Structure :

Pharmacokinetics:

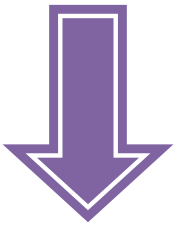
Metronidazole is avaliable for oral, i.v and topical administration.It is usually well absorbed after oral administration and poorly bound to plasma protein .It diffuses well into the tissues including brain ; therapeutic levels are achieved in various body fluids- saliva,semen,vaginaal secretion,bile breast milk and CSF. Metronidazole is metabolized in the liver and metabolites are excreted mainly in urine.

Mechanism of Action:

Metronidazole is a prodrug enters into the microorganism. So Nitro group is reduced to highly reactive radical.



Damages microbial DNA



Death of the organism (bactericidal effect)

ADR: Metallic taste, nausea, epigastric distress, skin rash, itching, flushing, dizziness, vertigo, headache.

Uses :

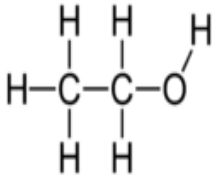
- Metronidazole is highly effective against most of the anaerobic infections, it is used in the treatment of abscess, pericoronitis, periodontitis etc .It has been often used in combination with penicillin and amoxicillin.
- It is widely used as antiamoebic drug as well as in giardiasis and guinea worm infestation.

4.5.1 Solvent used to dissolve the drug and polymers:

Ethanol :

Synonym: Absolute alcohol, Alcohol, Ethyl alcohol, Ethyl hydrate, Ethyl hydroxide, Ethylic alcohol, Ethylol, Grain alcohol, Hydroxyethane, Methylcarbinol.

Structure:



Chemical formula: C_2H_6O

Physicochemical properties

Molecular weight: 46.04 g/mol

Density: 0.790 g/ml

Boiling point: 78°C

Melting point: -114.1°C

Vapor pressure: 5.95 kPa

Uses: Antiseptic, as fuel, solvent in pharmaceuticals, treatment for poisoning by other alcohols

CHAPTER 5

METHODOLOGY

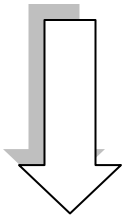
5. METHODOLOGY:

5.1 Drug-excipients interaction:

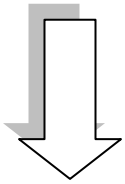
Any interaction which are, between the drug and polymers is always possibility in the films due to t he intact contact between them. Therefore, the prepared films are generally analyzed for their drug-excipients interaction study, to check whether they are stable or not. Instrumental techniques such as FTIR spectroscopy detects such interactions at the functional group level and checks the compatibility between drug and the excipients. In this experiment it was carried out using FTIR spectroscopy (Bruker optik, Germany). FTIR-spectra of original drug(s), excipients, physical mixture of drug(s) with excipients, formulation of patch without drug and formulation of patch with drug(s) were mixed separately with IR grade KBr and the pellets were prepared by applying pressure in a hydraulic press (Pressed pellet technique). The pellets were then analyzed over the region of 4000 cm^{-1} to 400 cm^{-1} by using FTIR instrument (Maji et al. 2014).

5.2.1 Preparation of backing membrane:

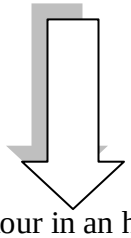
At first weighted quantity of polyvinyl alcohol (4% w /v) was mixed with warm glass of double distilled water



After that it is stirred until it arise more than 55°C and form a clear solution



The homogeneous solution (3 m L e ach) was then poured into the glass mold wrapped with aluminum foil so that one end is intact.



Mold was then stored at 60°C for 8 hour in an hot air oven for the formation of smooth uniform transparent backing membrane (Damodharan et al. 2010).

5.2.1. Casting of drug matrix over the backing membrane:

Mixture of ethyl cellulose and poly vinyl pyrrolidone k-30 (using combination of 1:3, 3:2, 2:1) was dissolved in ethanol (95%) by using magnetic stirrer. It has been showed that mixture of ethyl cellulose and polyvinyl pyrrolidone k-30 in the ratio of 2: 1 formed film and it is suitable in terms of plasticity, flexibility and .After that HPMC was dissolved in the above mixture. 1% v/v DBP (Gupta and Mukherjee 2003) is used as plasticizer in the above mixture to form a homogenous solution. Then PEG 400 (1% v/v) along with the required quantities of drugs were dissolve in the above mixture (Table-5). After that the entire dispersion containing the clear solution (1.5 mL each) was then poured in the backing membrane remained in glass mold which are prepared in first step. Then it was kept in a hot air oven and dried at 25°C and there was formation of flat uniform medicated matrix patch.

Figure 5 and 5a : Drug matrix with backing membrane

Table 5: Composition of various formulations:

Formulation NO.	Lidocaine (mg)	Metronidazole (mg)	Amoxicillin (mg)	Plasticizer	EC (mg)	HPMC (mg)	PVP K-30 (mg)	PEG 400 (mg)
F1	5	4	1	30%DBP	10	50	5	19.5
F2	5	4	1	40%DBP	10	50	5	19.5
F3	5	4	1	50%DBP	10	50	5	19.5
F4	5	4	1	40%DBP	10	50	5	18.5
F5	5	4	1	50%DBP	10	50	5	18.5
F6	5	4	1	50%DBP	10	50	5	17.5
F7	5	4	1	60%DBP	10	50	5	16.5

*DBP- Dibutyl phthalate, EC- Ethyl cellulose, HPMC- Hydroxy propyl methyl cellulose, PEG 400- Polyethylene glycol 400

In vitro evaluation of the formulations:

Among the various formulations, F4, F5 F6 and F7 were not chosen because of their highly adhesiveness and un-uniformity. Formulations F1, F2, F3 appeared to be best in terms of their morphology and uniformity so they are selected for further evaluation.

5.3.1 Average mass uniformity and thickness of for patches:

Twenty patches were chosen average mass was calculated by using a digital balance. The percentage variation of mass of each patches were determined from the mean value.

Thickness of the whole patches were determined taking more than four different randomly selected points of twenty patches using vernier scale and average thicknesses were measured from the mean value. (Mukherjee et al. 2006) (Table- 9).

5.3.2 Area of the patches:

Total diameter (D) of one patch was checked, using a millimeter scale and the area ($\pi [D/2]^2$) was measured and from there mean value was determined. (R.Mansadeepa et al. 2013).

5.3.3 Moisture content:

The availability of large amount of moisture affects the brittleness and friability of buccal films. Basically, the contents in the product regulate the degree of moisture in a particular film. The amount of moisture present in the film is generally quantified by using moisture con tent testing equipment, such as Karl fisher titration method or by weighing method. The prepared patches were marked after placing them in a zipper individually. Then each patch was weighed in weighing machine and then they were kept in a vaccum desiccator containing activated silica at 25°C. The mass was checked time to time until it became constant (Damodharan et al. 2010; Arora and Mukherjee 2002). Percent moisture content was determined by using following formula which is as follows.

$$\%\ moisture\ content = \frac{Initial\ mass - Final\ mass}{Final\ mass} \times 100$$

5.3.4 Moisture absorption:

Water absorption capacity of the prepared buccal film is of highest importance for two reasons. Basically, the absorption of water by the film is essential to provide better bioadhesion of the film with the buccal mucosa, which is dependent on the nature o f the polymer matrix. Secondly, it facilitates the drug release from the films, which is mainly by two mechanisms (diffusion and erosion). The percentage moisture absorption was determined from previously weighed patches exposed to 90% relative humidity (RH) in a desiccator until the constant mass of patches were obtained (Arora and Mukherjee 2002). The percentage of moisture uptake was as follows:

$$\%\ \text{Moisture uptake} = \frac{Final\ mass - Initial\ mass}{Final\ mass} \times 100$$

5.3.5 Folding endurance:

The flexibility and durability of mucoadhesive patches is an important physical character needed for easy application on the site of administration. The flexibility of the patches can be measured quantitatively by measuring of folding endurance. Folding endurance of patches was measured by folding one patch constantly at the same place till it broke from that part. Folding endurance is considered to be the main criteria that are to be fulfilled to observe excellent film properties (Khanna et al. 1997). The value of folding endurance can be determined by highest number of times a patch can be folded from a selected without breaking.

5.3.6 The Structural integrity in simulated saliva (pH 6.8):

Structural integrity of every patch was quantified using simulated saliva (Mukherjee et al. 2009). Every Patch was placed in separate petrdishes containing 10 mL of simulated saliva and they were placed in an incubator at 37 ± 0.2° C for 8 h as reported in the literature (Ghosh et al. 2009). Different time points of every 15 minutes, the patches were quantified for changes such as stickiness, color, texture and shape as depicted earlier.

5.3.7 Bioadhesive strength:

Bioadhesive strength was quantified to determine the maximum force required for detaching the applied patches from the mucous membrane (Nair et al, 2013). This is will change time to time and depends upon the choice of polymer and its binding ability in the mucosal surface.

At first mucous membrane of the animal was collected from the slaughterhouse. The mucous membrane was then cleaned with distilled water and immersed in simulated saliva (at 37 ± 0.5° C for 3 min). After that the mucous membrane was fixed with a double-sided adhesive tape on the top surface of a cub (each side 3 cm) made up of plaster of paris base (figure-6). Each experimental formulation was then attached to the mucous layer. A physical balance with two circular pans, hanged from a rod which was then balanced with a fulcrum on a stand was used as a modified bioadhesion test assembly (Gupta et al. 1993). Lower surface of a pan was attached to the mucoadhesive patch by both-side adhesive tape. Weights were given on the other pan until the adhesive patch was detached from the mucous membrane. The maximum force required for complete displacement of the patch from the mucous membrane was recorded (Mukherjee et al. 2009).

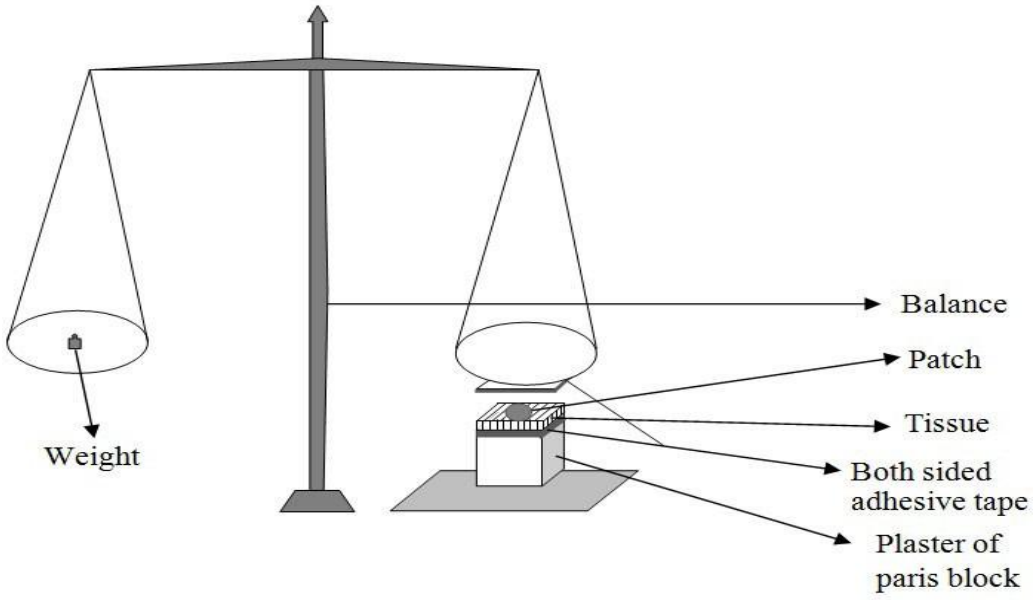


Figure 6: Bioadhesion test assembly

5.3.8 Surface pH study:

A film with too much acidic or basic pH affects the area of application and causes damages to oral mucosal membrane because there is chance of buccal secretion to neutralize the pH, it may cause patient discomfort and damage of buccal region. In general, the salivary pH range is 5.5-7.0.For the determination of surface pH of the prepared formulation, each patches were placed separately in 1mL distilled water for two minutes at room temperature (Obaidat et al. 2011), and the pH was checked by bringing the electrode in touch with the surface of the patch. Then it was allowed to equilibrate (Priya et al. 2011; Chopparapu et al. 2012).

5.3.9 Scanning electron microscopy study (SEM):

Scanning electron microscope (Carl Zeiss Evo-18,Germany) is used to check the drug distribution pattern in the formulated dental patches .SEM study was done before and after *in vitro* drug release study (Mukherjee et al. 2009) by placing the samples onto stubs and sputtering platinum under vacuum before analysis. They were visualized at an acceleration voltage of 9 KV.

Figure 7: Platinum coating of stubs during Scanning electron microscopic study

Figure8: Carl Zeiss Scanning Electron Microscope

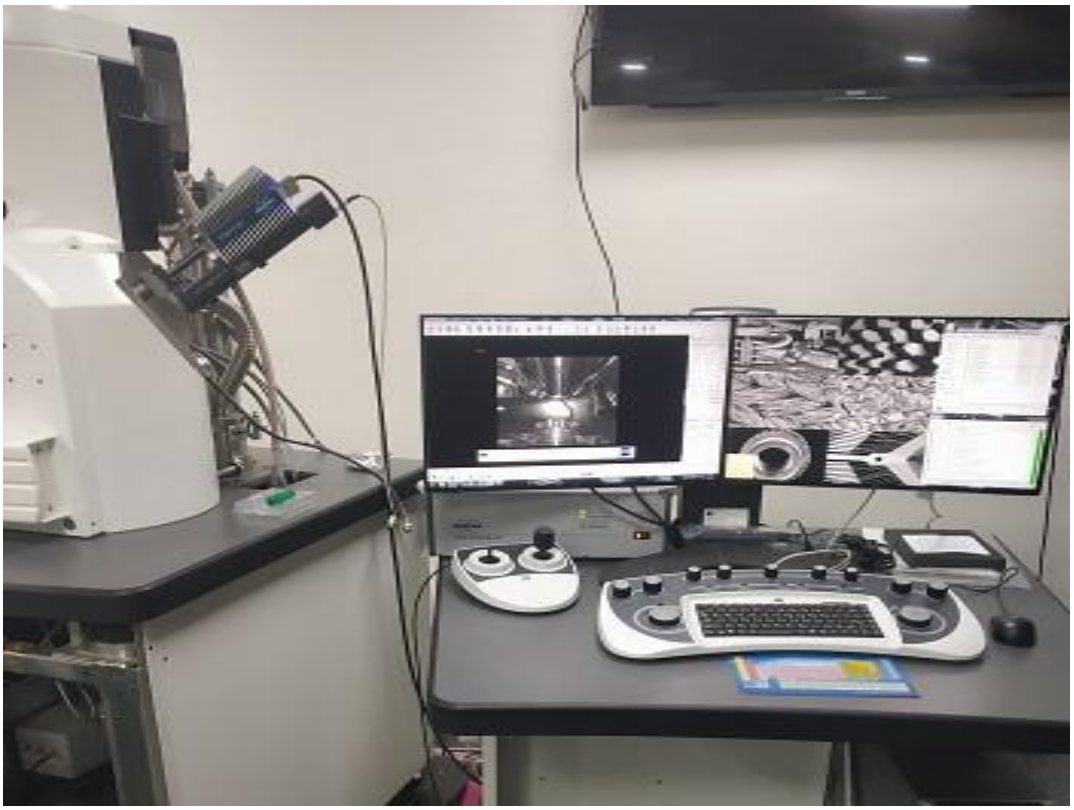


Figure 9: Scanning Electron Microscope (SEM) assembly

5.3.10 The effect of patch on the cellular morphology of mucous membrane:

Head of a goat was collected and the skin was removed in slaughter house. After that experimental patches with or without drug(s) were applied to the mucosal membrane of goat for a time period upto 8h. Then, control and patch treated portions of the mucosal membrane were taken. After that they were washed separately and fixed with 10 % v/ v formalin solution. Then, tissue portions were blocked using paraffin. Then they were sectioned by using microtome .After that it was stained by Haematoxylin-Eosin solution and visualized under compound microscope.

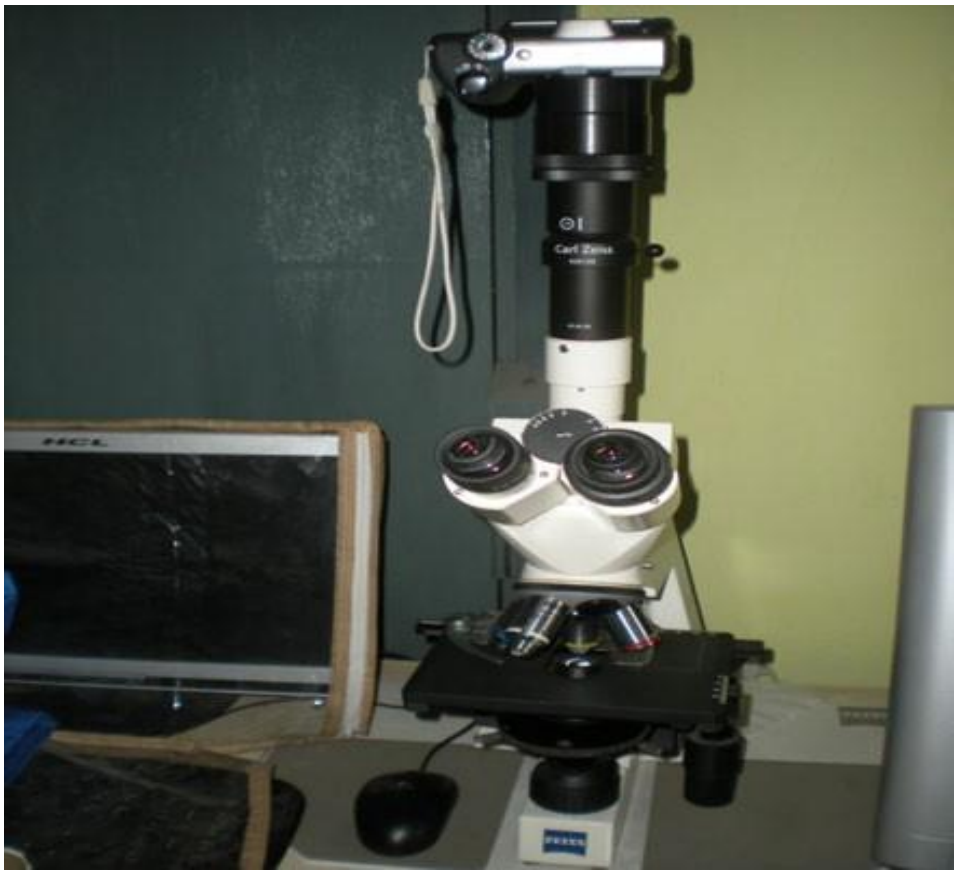


Fig 10: Carl Zeiss Microscope

5.3.11 *In Vitro* release kinetics study:

Release of drug from the formulated patches is required for diffusion through the buccal epithelium. Drug release studies were performed to determine the cumulative drug release from the formulation for a given period of time. No specific *in vitro* method has yet been reported in the literature for the drug release studies of buccal films. Standard or modified dissolution apparatus with certain modifications or Franz diffusion cells (FDC) were used by different researchers, for the inspection of the drug release from buccal films. Further, the choice of media for the dissolution studies also varied among the different research groups. However, to obtain meaningful results it is necessary to use a dissolution medium that is simulates saliva. There is no exact composition described and given in the USP to

prepare simulated saliva. *In vitro* drug release from mucoadhesive patches was performed in modified Franz diffusion cells.

The Franz diffusion cells have receptor compartment of volume 50 mL and effective surface area of diffusion or permeation was 3.935cm^2 considering the outer diameter of donor cell. One drug loaded patch was mounted between donor and receptor cell in such a way that the film is in touch with receptor fluid. Franz diffusion cells were placed in a multi-cell magnetic stirrer. The receptor cell was filled up with media simulated saliva pH 6.8 and its content was stirred by a magnetic bead placed inside the cell. Temperature of receptor compartment was maintained by circulating water bath at 37±0.5˚C by using external jacketed path. Circulating water was supplied by a constant temperature water bath. Drug diffuses from patch to receptor fluid. Samples (5mL) were taken out from different time period and replaced by equal amount of media to maintain the sink condition.

Corresponding absorbance of the samples was measured by UV-visible spectrophotometer at their respective λ_{max} values i.e., 273 nm for amoxicillin, 318 nm for metronidazole and 269 nm for lidocaine. The amount of drug release results were plotted as cumulative percentage drug (s) release against time for different formulations (Mukherjee et a l. 2009).After that data obtained from *in vitro* drug release study were plotted in various kinetic models such as zero order, first order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell kinetic model.

Then coefficients o f de termination (R2) value and rate constants f or z ero-order (K_0), first order (K_1), Higuchi model (KH), Korsmeyer-Peppas model, Hixson-Crowell model (K_{HC}) were determined (Rudra et al. 2010).

5.3.12 Stability study:

Stability generally refer to as how the drug substance and drug product varies with time under the influence of variety of environmental factors like temperature, humidity and light

The effect of temperature and humidity on the drugs and polymers in the patches were quantified by accelerated stability study (Figure-24, 25). According to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, the samples of formulation F3 were kept in Zone-III temperature conditions (40° C, 75% R H), withdrawn a t different time period (3rd month) was analyzed by FTIR-spectroscopy.(ICH ,2003).

5.3.13 Statistical analysis:

One way ANOVA followed by Turkey's multiple comparison experiment was performed to compare data to identify statistical significance. p values < 0.05 were considered satisfactory. All calculations were performed using the graph pad prism software (version 5).

5.3.14 Determination of drug content in Dental patches:

Drug content of sample patch was analyzed by cutting a patch of 1cm ^{2} area from fabricated dental patches. This small patch ($1cm^2$) was then put into volumetric flask containing 100mL of media (simulated saliva pH 6.8) and stirred by using a magnetic stirrer for 24 h. Content was shaken and filtered. Drug content of the filtrate was measured by observing absorbance by UV-Visible spectrophotometer at a wavelength of 318nm for Metronidazole,269 nm for lidocaine and 273 nm for Amoxicillin using Simulated saliva as blank . Finally drug content was expressed as $\mu g/cm^2$ of patch area. Blank solution was made up by the blank patch without drug.

CHAPTER 6

RESULTS

6. Results:

6.1 The UV absorption spectra of metronidazole, lidocaine & amoxicillin:

For determination of maximum absorption spectra of metronidazole, a dilute solution of metronidazole in simulated saliva (pH 6.8) was prepared and scanned between 190-600 nm in Uv-Vis spectrophotometer using simulated saliva as blank reference. The spectrum showed that distinct peak (absorption maxima $\lambda_{max)}$ at 318nm for metronidazole, at 269 nm for lidocaine and at 273 nm for amoxicillin.

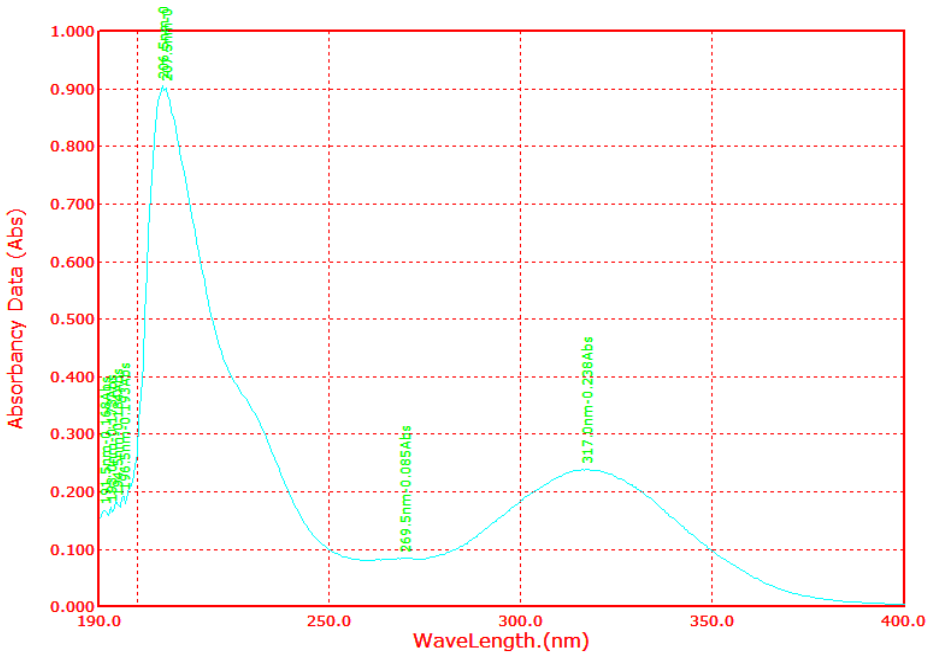


FIGURE 11: The UV absorption spectra of metronidazole, amoxicillin trihydrate, lidocaine hydrochloride in simulated saliva (pH 6.8)

6.1.1 The calibration curve of metronidazole-

Calibration curve was prepared for studying the in vitro drug release and for the determination of drug loading in the dental patches. .The concentrations prepared for prepared calibration curve were 20µg/ml, 40 µg/ml, 60µg/ml, 80µg/ml and 100µg/ml. The calibration curve for metronidazole was prepared in simulated saliva (pH 6.8). Figure 12 depicted the calibration curve in metronidazole in simulated saliva. Corresponding data of absorbance of Metronidazole sample against various concentrations of drug are given in the Table 6.

Table 6: The mean absorbance spectra of metronidazole sample against the various concentrations of drugs in simulated saliva pH 6.8

Concentration (µg/ml)	Mean absorbance
20	0.158±0.065
40	0.301±0.140
60	0.440±0.207
80	0.573±0.262
100	0.773±0.365

* Data show mean ± SD (n=6)

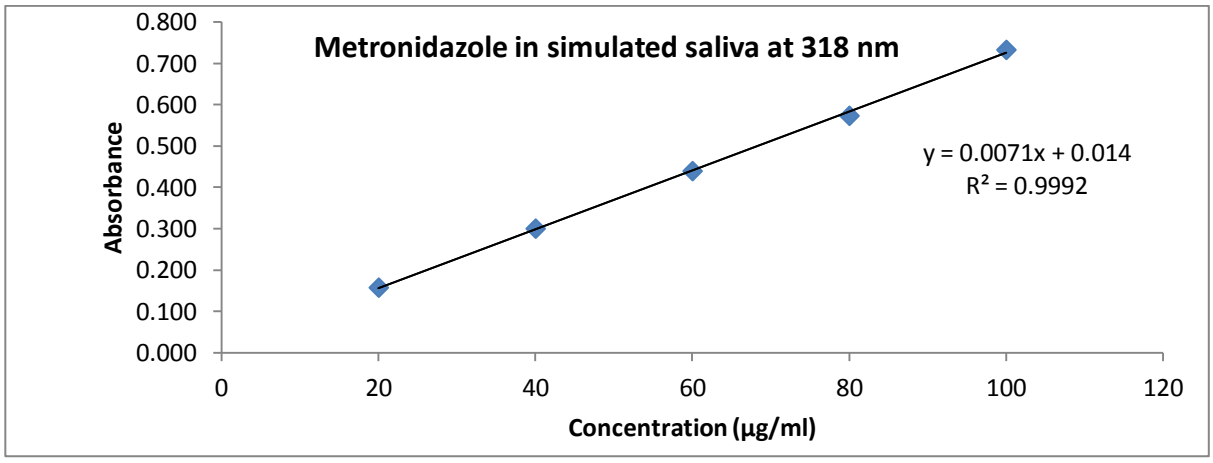


Figure: 12 Calibration curve of metronidazole in simulated saliva pH 6.8

6.1.2 The UV absorption spectrum of lidocaine:

Calibration curve were prepared for studying the *in vitro* drug release and for the determination of drug loading in the dental patches. The drug concentrations prepared for calibration curve were 2µg/ml, 4 µg/ml, 6µg/ml, 8µg/ml and 10µg/ml. Figure 13 depicted the calibration curve of lidocaine in simulated saliva (pH 6.8). Corresponding data of absorbance of lidocaine sample against various concentrations of drug has been given in the Table 7.

Table 7: The mean absorbance spectrum of lidocaine sample against the various concentrations of drugs in simulated saliva pH 6.8

Concentration (µg/ml)	Mean Absorbance
2	0.099±0.058
4	0.176±0.090
6	0.259±0.126
8	0.349±0.177
10	0.431±0.209

* Data show mean ± SD (n=6)

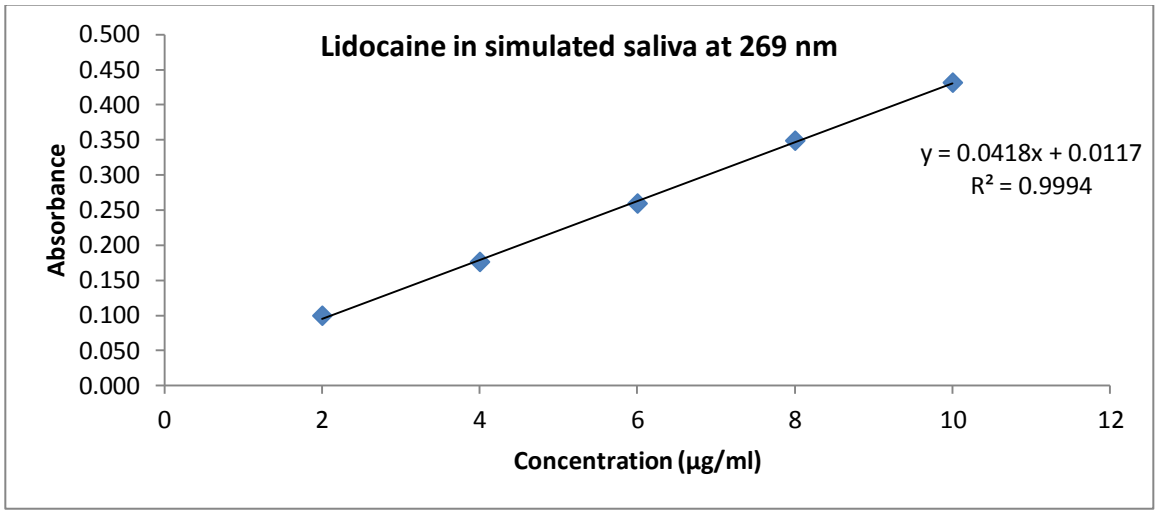


Figure: 13 Calibration curve of lidocaine in simulated saliva pH 6.8

6.1.3 The UV absorption spectrum of amoxicillin:

As stated earlier calibration curve were prepared for studying *in vitro* drug release and for the determination of drug loading in the dental patches. The drug concentrations prepared for calibration curve were 2µg/ml, 4 µg/ml, 6µg/ml and 8µg/ml, 10µg/ml. The

calibration curve for amoxicillin was prepared in simulated saliva (pH 6.8). Figure 14 depicted the calibration curve of amoxicillin in simulated saliva. Corresponding data of absorbance of amoxicillin sample against various concentrations of drugs are given in the Table 8.

Table 8: The mean absorbance spectrum of amoxicillin sample against the various concentrations of drugs in simulated saliva pH 6.8

Concentration (µg/ml)	Mean Absorbance
2	0.106±0.027
4	0.176±0.035
6	0.254±0.048
8	0.349±0.053
10	0.431±0.082

n=6 is represented as mean absorbance where as± refer to as S.D

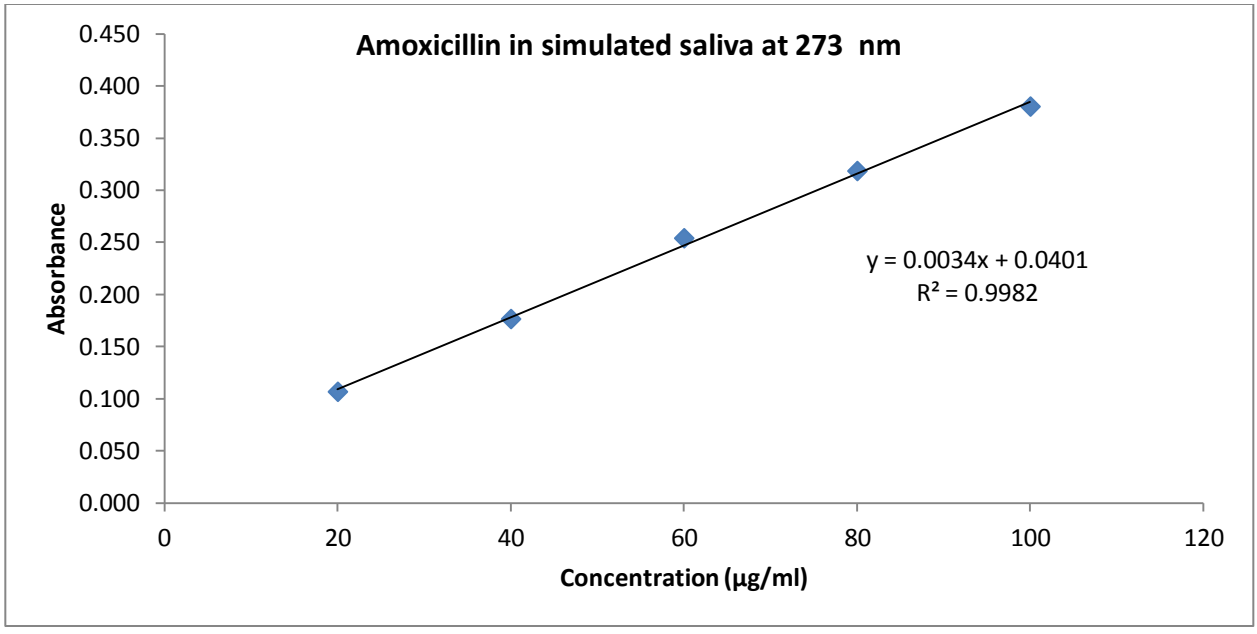


Figure: 14 Calibration curve of amoxicillin in simulated saliva pH 6.8

6.2 Drug-excipients interaction study:

In the experimental study, FTIR spectroscopy was performed to analyze the interactions if any, between the drug(s) and the excipients, at the level of functional groups. The drugs amoxicillin, metronidazole, lidocaine and different excipients (HPMC, PVP K 30) and physical mixtures of each of the drug and the excipients , formulation F 0, formulation F3, and formulation (F3) after the storage at 40° C; 75% RH up to three months, were characterized in the FTIR figures.

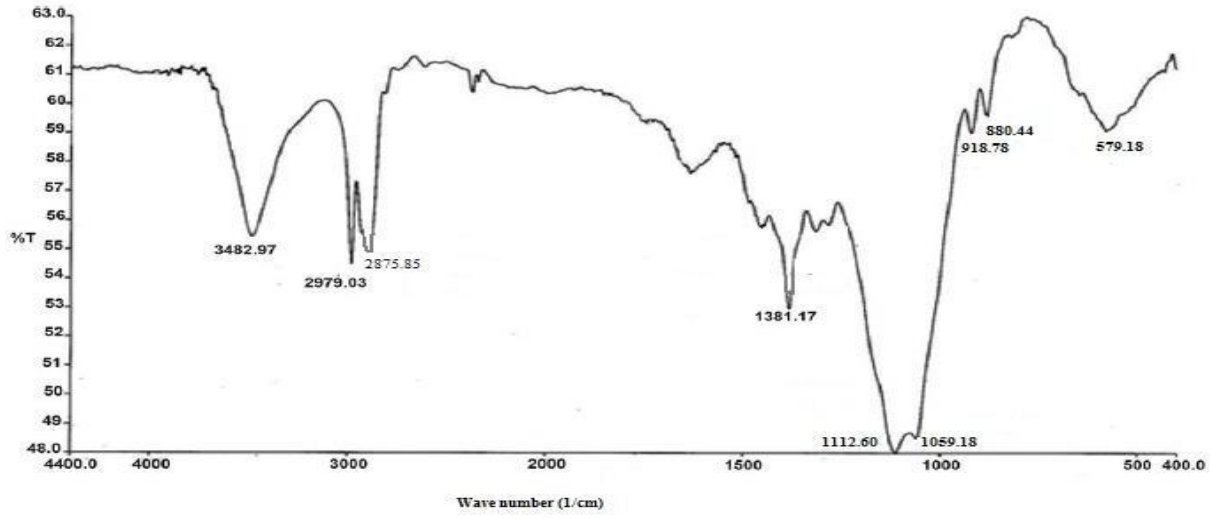


Figure 15: FTIR spectra of EC

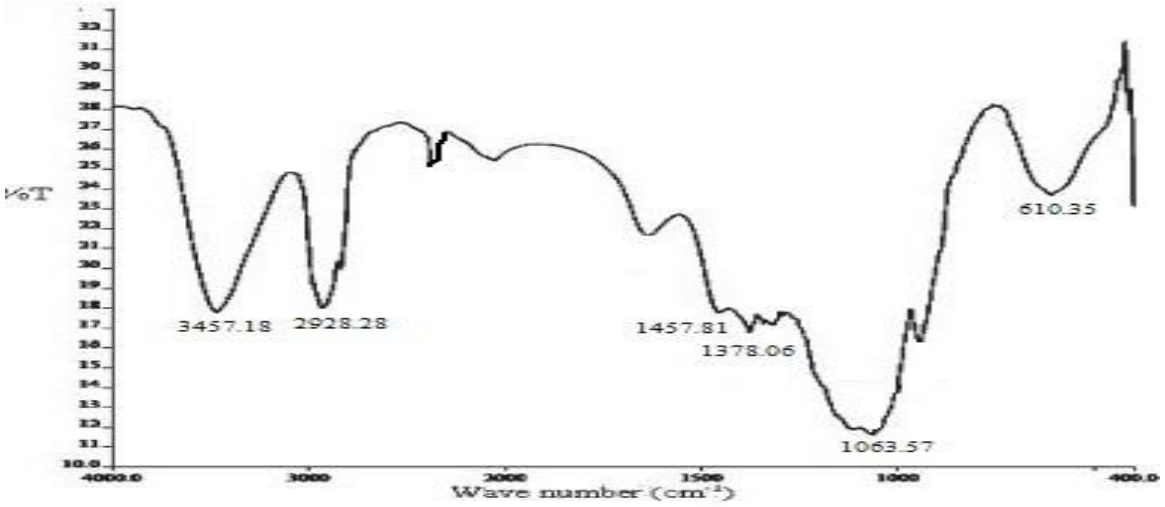


Figure16: FTIR spectra of HPMC

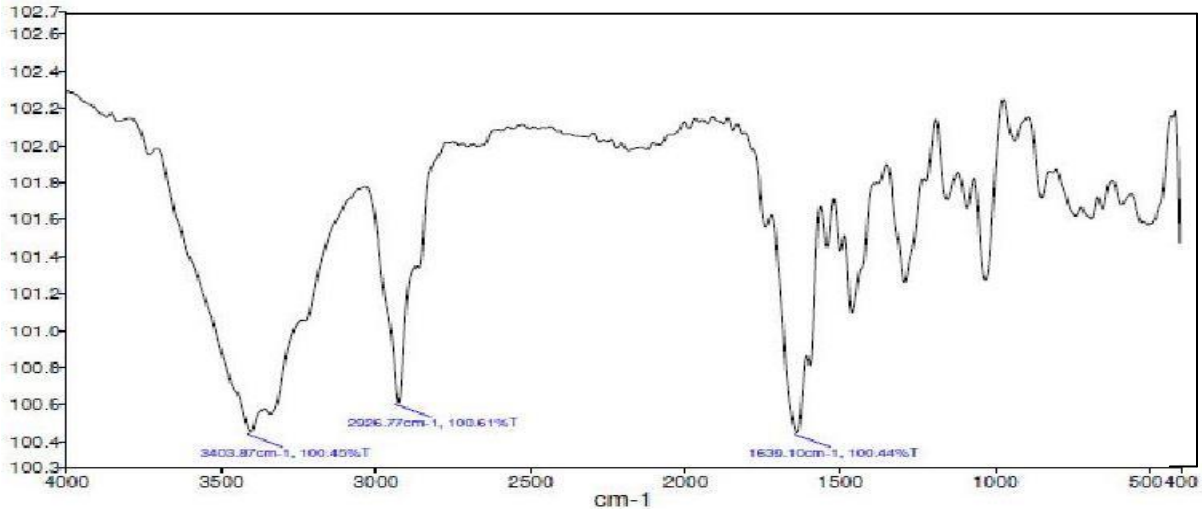


Figure17 : FTIR spectra of PVP K-30

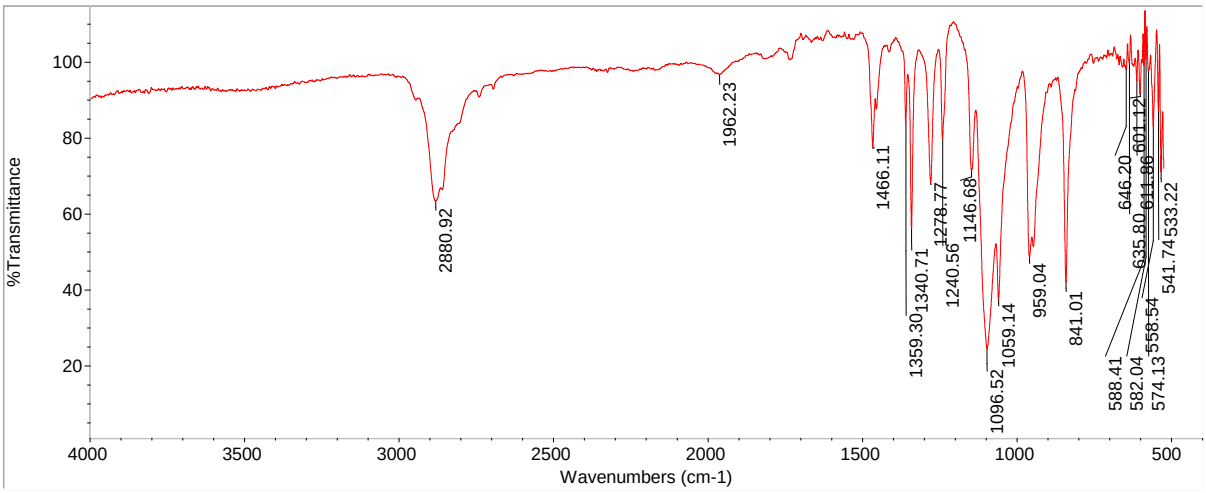


Figure18: FTIR spectra of PEG 400

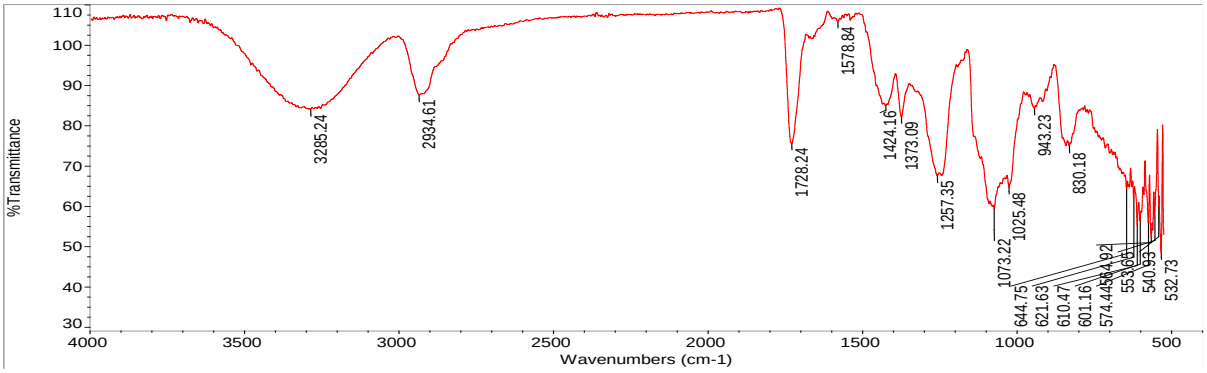


Figure19: FTIR spectra of formulation F3 containing amoxicillin, Metronidazole, lidocaine

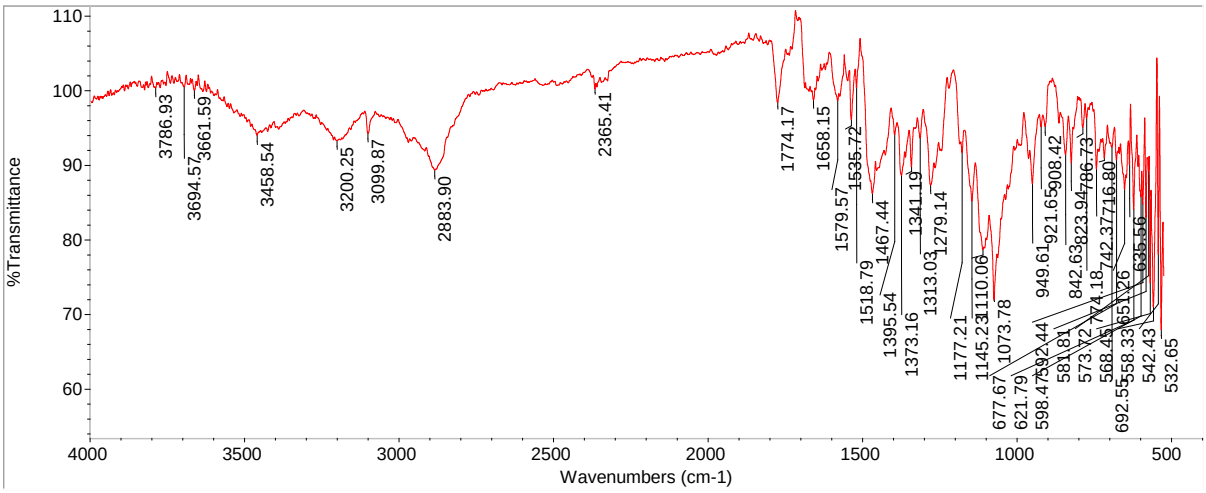


Figure 20: FTIR spectra amoxicillin trihydrate, metronidazole and lidocaine excipients mixture

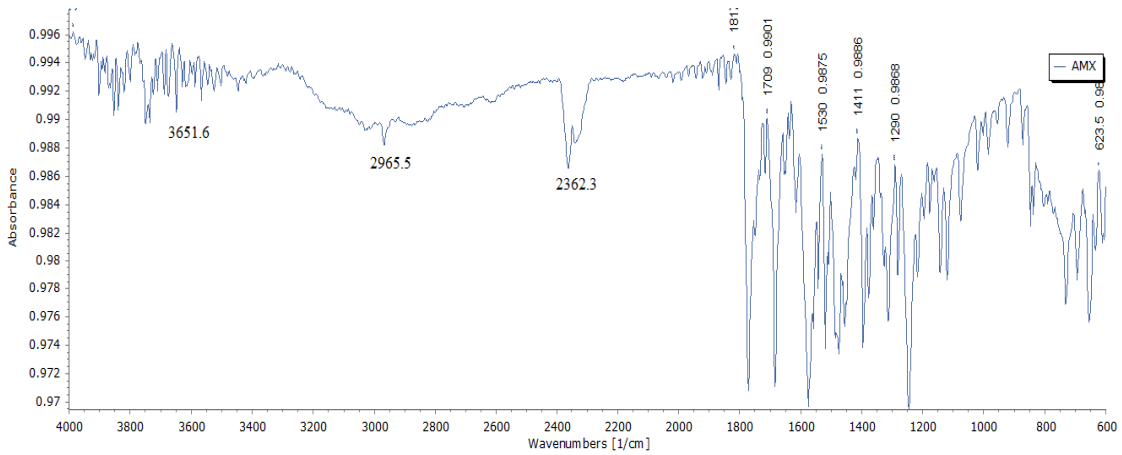


Figure 21: FTIR spectra of Amoxicillin

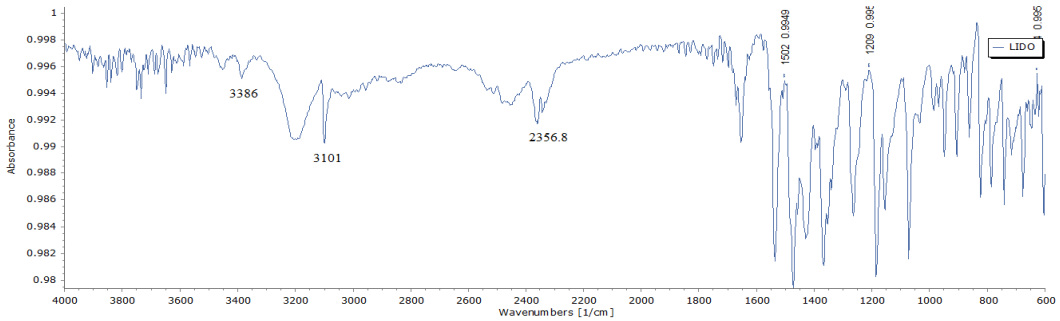


Figure 22: FTIR spectra of Lidocaine

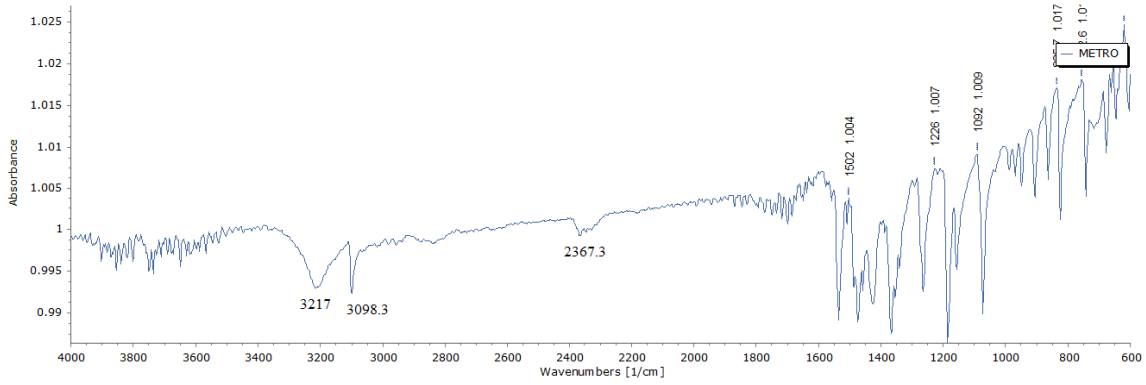


Figure 23: FTIR spectra of Metronidazole

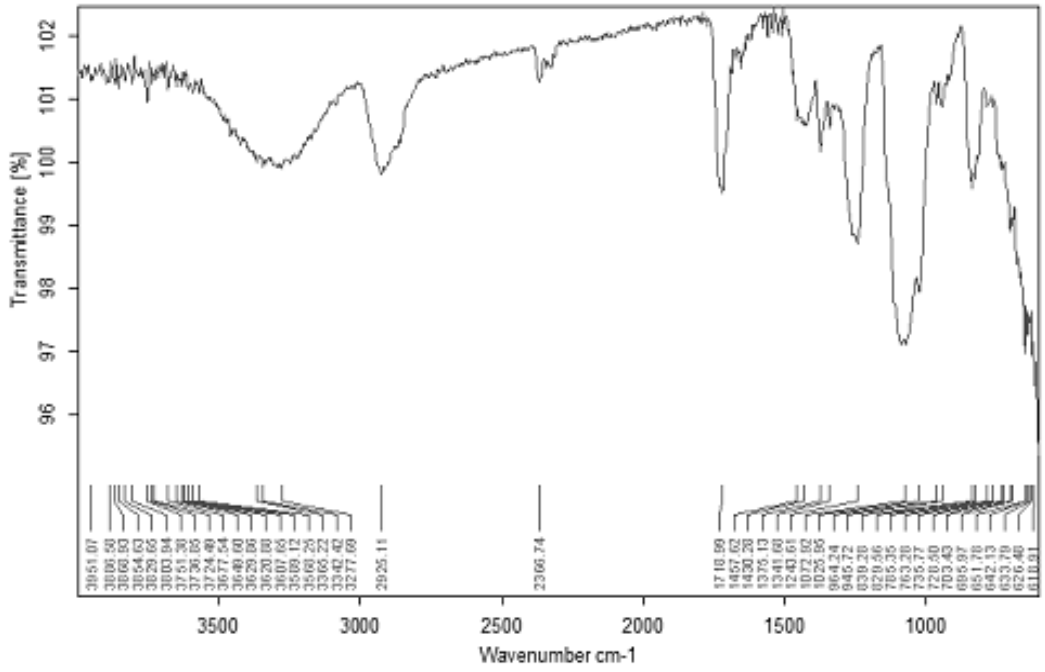


Figure 24: FTIR spectroscopy of F3 formulation after the storage at

40° C; 75% RH up to three months

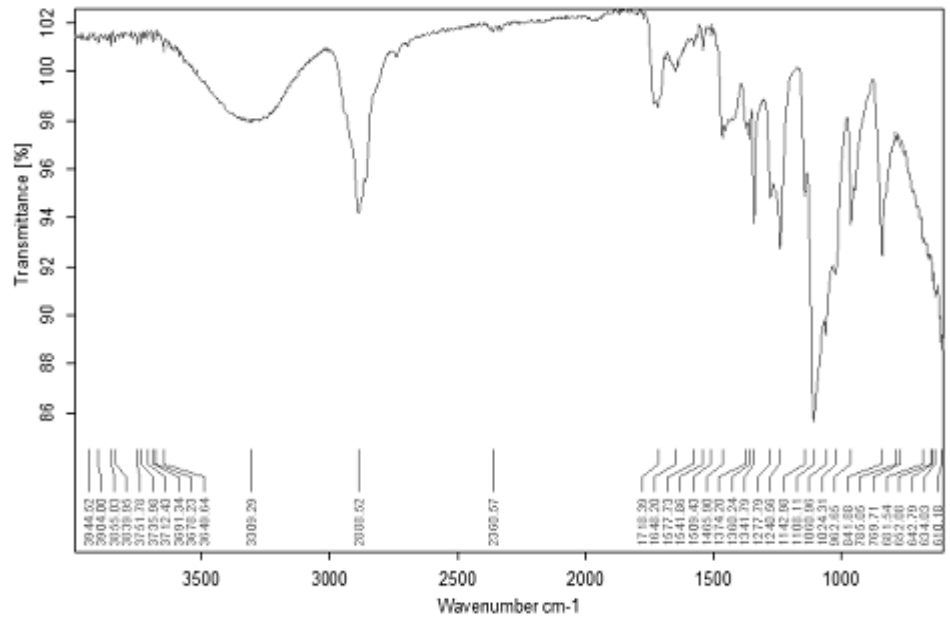


Figure 25: FTIR spectra of F0 formulation

Observation-

- The spectrum of EC (figure-15) revealed that, at 579.18 cm^{-1}, 880.44 cm^{-1} and 918.78 cm^{-1} a strong bonding of phenyl molecule, 1059.18 and 1112.60 cm^{-1} showed C-OH stretching, at 1381.17 cm^{-1} a medium CH_3 bend observed, 2875. 85 cm^{-1} and 2979.03cm^{-1} a weak aldehyde stretching present, at 3484.97cm^{-1} alcohol stretch, C=O stretch and N-H stretch present.

- FTIR spectrum of HPMC (figure-16), showed aromatic C-H bending at 610.35 cm^{-1}, aromatic C=C bending at 1378.06 and 1457.81 cm^{-1}, at 2928.28 cm^{-1} presence of alkyl C-H stretch and at 3457.18 cm^{-1} there was a presence of broad alcohol stretch as well as amine N- H stretching.

- Spectrum of PVPK-30 (figure-17) showed important bands at 2926.77 cm^{-1}, 1639.1 cm^{-1} denoting stretching vibrations for C-H, C=O, respectively. However, a broad band was also observed at 3403.87 cm^{-1}, which was due to presence of water (Paradkar et al., 2004).

- Spectrum of PEG 400 (figure-18) showed spectrum at 2880.92 cm^{-1} denoting C-H Stretching, 1466.11 cm^{-1} showed aromatic C=C bending, 841.01 showed aromatic C-H bending.

- The formulation F3 (figure- 19) showed spectrum at 3285 cm^{-1} denoting C-H stretching of alkynes, 2934.61 cm^{-1} denotes stretching of alkane, 1728.54 cm^{-1} denotes C=O stretching due the presence of carboxy molecule, 1728.24 cm^{-1} denotes C=O stretching of aldehyde molecule, 1578.84 cm^{-1} denotes C=C of aromatic molecule, 830.18 cm^{-1} showed aromatic C-H bending.

- The spectrum of drug excipients mixture (figure 20)showed spectrum at spectrum at 2883.90 cm^{-1} denoting C-H stretching of alkane , 3200 cm^{-1} and 3099 cm^{-1} showed C-H stretching for aromatic rings,1658.15 cm^{-1} denotes C=O stretching of carboxy molecule.

- Spectrum of Amoxicillin (figure-21) showed spectrum at 2965.5 cm^{-1} denoting C-H stretching of alkane , 3651.6 denotes OH stretching of alcohol, 1530 cm^{-1} denoting C=C stretching of aromatic molecule, 1290 cm^{-1} denoting C-O stretching of alcohols.

- Spectrum of Lidocaine (figure-22) showed spectra at 3101 cm^{-1} C-H stretching of aromatic rings, 3386 cm^{-1} denoting N-H stretching,1209 denoting C-N vibrations.

- Spectrum of Metronidazole (figure-23) showed spectra at 1092 cm^{-1} denoting C=S stretching, 3212 cm^{-1} denoting C-H stretching of aromatic rings, 3098 cm^{-1}denoting C-H stretching of alkene.

- Spectrum of formulation F3 after stability study(figure-24) showed spectra at 3283 cm^{-1} denoting C-H stretching of alkynes, 2932.61 cm^{-1} denotes stretching of alkane,
  1725 cm^{-1} denotes C=O stretching due the presence of carboxy molecule, 1729 cm^{-1} denotes C=O stretching of aldehyde molecule, 1575.84 cm^{-1}denotes C=C of aromatic molecule, 832.28 cm^{-1} showed aromatic C-H bending.

6.3.1 Average mass variation, thickness, diameter and area of the patches:

The average mass, whole patch thickness, area, diameter of the mucoadhesive patches have been given in Table-9. It was observed that patches were very thin in size.

The whole patch thickness in the formulation F1 was found to be 0.57 ± 0.03 mm and in formulation F3 the whole patch thickness was 0.59 ± 0.02 mm.

In formulation F1 average mass range was 0.40 ± 0.09 g , formulation F2 average mass was found to be in the range 0.55 ± 0.03 g and in formulation F3 average mass variation was in the range 0.57 ± 0.03g.

The average diameter of whole patch in formulation F1 was found to be in the range of 3.41 ± 0.02 cm, formulation F2 average diameter was 3.35 ± 0.04 cm, and in the formulation F3 average diameter was 3.42 ± 0.04.

The area of the patch in formulation F1 was 4.37 ± 0.14 cm^2, formulation F2 it was found to be in the range of 4.54 ± 0.10 cm^2 and in the formulation F3 area of the patch was 4.66 ± 0.12 cm^2.

6.3.2 Moisture content and moisture absorption:

The average percent moisture content of the formulations F1 was found to be in the range 1.16 ± 0.58 weight% , formulation F3 it was 0.78 ± 0.23 weight% and in the formulation F3 average moisture content was 0.78 ± 0.23 weight%

average percentage moisture absorption of those formulations were found to vary between 102.8 ± 3.20weight% and 11.20 ± 3.08 weight% in formulation F1 and formulation F3 respectively. The given results proves that in presence of moisture content and moisture absorption in the patches would not be dried up and there is no unnecessarily bulkiness and brittleness.(Arora and Mukherjee 2002).

6.3.3 Folding endurance and structural integrity of the formulations in simulated saliva:

The folding endurance experiment was performed for specific patches. The results of folding endurance in the formulation F1 was in the range 93 ± 4.07 and in the formulation F3 folding endurance was 93.5 ± 5.47. (Table- 9). The data proves that the patches would retain their integrity for sufficient time period after they are applied on the mucosal area (Priya et al. 2011). Structural integrity of the formulations in simulated saliva proves that the formulations will maintain its texture, shape and there is no distortion of the patches in the presence of saliva for a long time (8h). It was observed that there is not any notable swelling of the formulations in presence simulated saliva.

6.3.4 Bioadhesive strength:

Table -9, gives a idea about the bioadhesive strength of the dental patches containing drug(s). The bioadhesive strength of the formulation F1 was found to be in the range 13.27 ± 0.12 kg/ mm^2 and in the formulation F3 was found to be in the range of 12.52 ± 0.38 kg/ mm^2.The data shows that the prepared patches will attach in the mucosa, and taken out from the place of application with very little effort.

6.3.5 Surface pH:

The surface pH was measured for the selected patches were shown (Table 9). The pH of t he formulation F1 was found to be in the range 6.08 ± 0.14 and in the the formulation F3 it was found to be in the range of 6.43 ± 0.23 .These data proves that the surface pH of the prepared patches were within the range of normal buccal pH as mentioned in the literature.(Bruschi and Freitas 2005). So, the patches would not cause any irritation in the oral mucosa upon application.

Table 9. Physical characterization of different experimental formulations.

Physical characteristics (unit)	Formulation F1 (Avg* ± SD**)	Formulation F2 (Avg ± SD)	Formulation F3 (Avg ± SD)
Mass variation[†] (g)	0.40 ± 0.09	0.55 ± 0.03	**0.57 ± 0.03**
Whole patch thickness[†] (mm)	0.57 ± 0.03	0.55 ± 0.02	**0.59 ± 0.02**
Diameter of the patch[†] (cm)	3.41± 0.02	3.35 ± 0.04	**3.42 ± 0.04**
Area of the patch[†] (cm^2)	4.37 ± 0.14	4.54 ± 0.10	**4.66 ± 0.12**
Moisture content[§] (weight %)	1.16 ± 0.58	1.12 ± 0.22	**0.78 ± 0.23**
Moisture absorption [§] (weight %)	102.8 ± 3.20	11.47 ± 2.32	**11.20 ± 3.08**
Folding Endurance	93 ± 4.07	116 ± 6.28	**93.5 ± 5.47**
Bioadhesive strength[#] (kg/ mm^2)	13.27 ± 0.12	12.65 ± 0.49	**12.52 ± 0.38**
Surface pH[#]	**6.08 ± 0.14**	**6.59 ± 0.27**	**6.43 ± 0.23**

[*]Average; [**]standard deviation; [#]mean ± SD (n = 3); [§]mean ± SD (n = 6); [†]mean ± SD (n = 20).

6.3.6 Scanning Electron Microscopy study:

SEM study is performed in the patches to evaluate the surface morphology and the drug distribution pattern in the patches (Damodharan e t a l. 20 10).In experimental patch SEM study was shown before drug release and also after the drug release (figure 28 and 30) In SEM, a tiny electron beam scanned across the surface of the sample molecule (drug) to inspect the topographies of samples at very high magnifications. It analyzes the surface of drug particles so that any fine details about surface morphology can be observed. The SEM picture were given below-

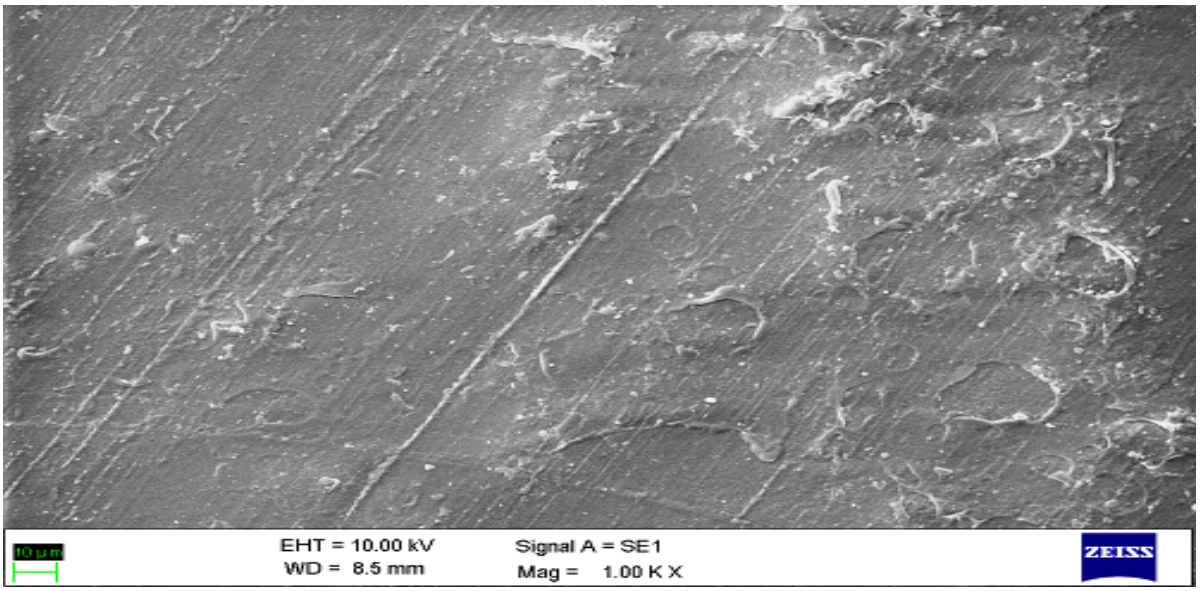


Figure : 26 The formulation F0 (blank patch)

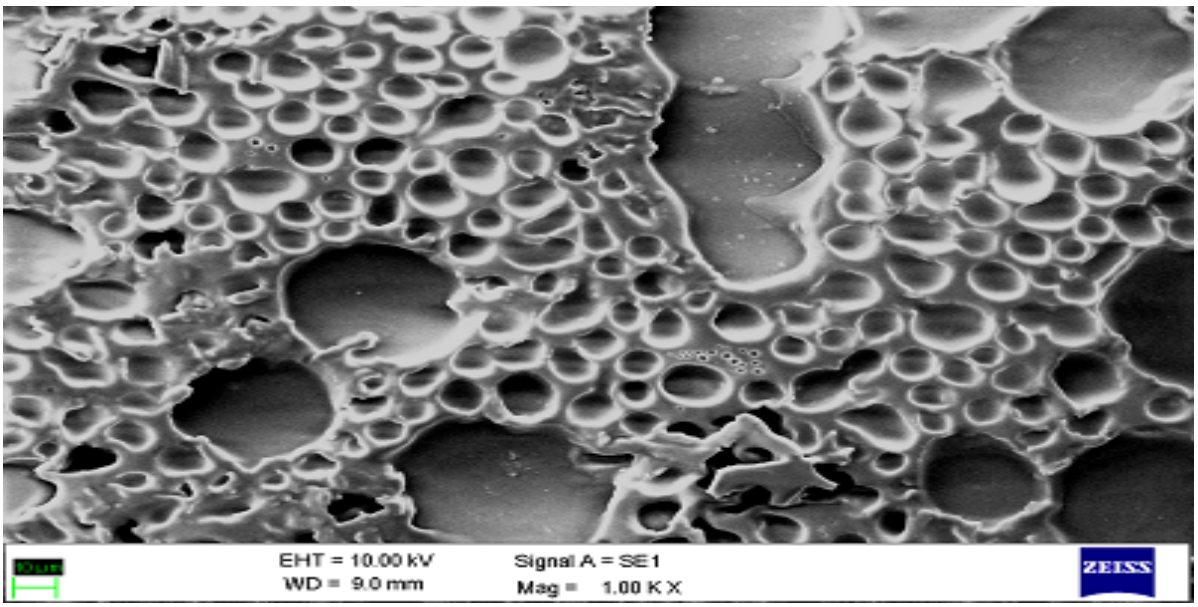


Figure: 27 Formulated patch F1

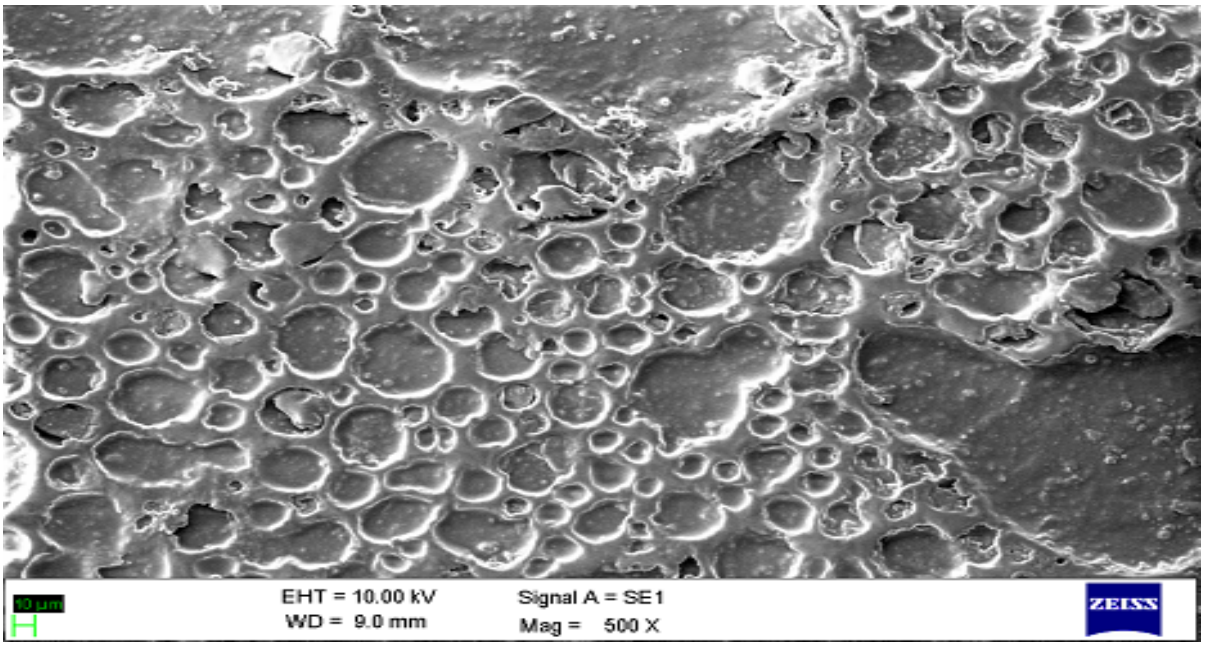


Figure: 28 Formulated patch F2

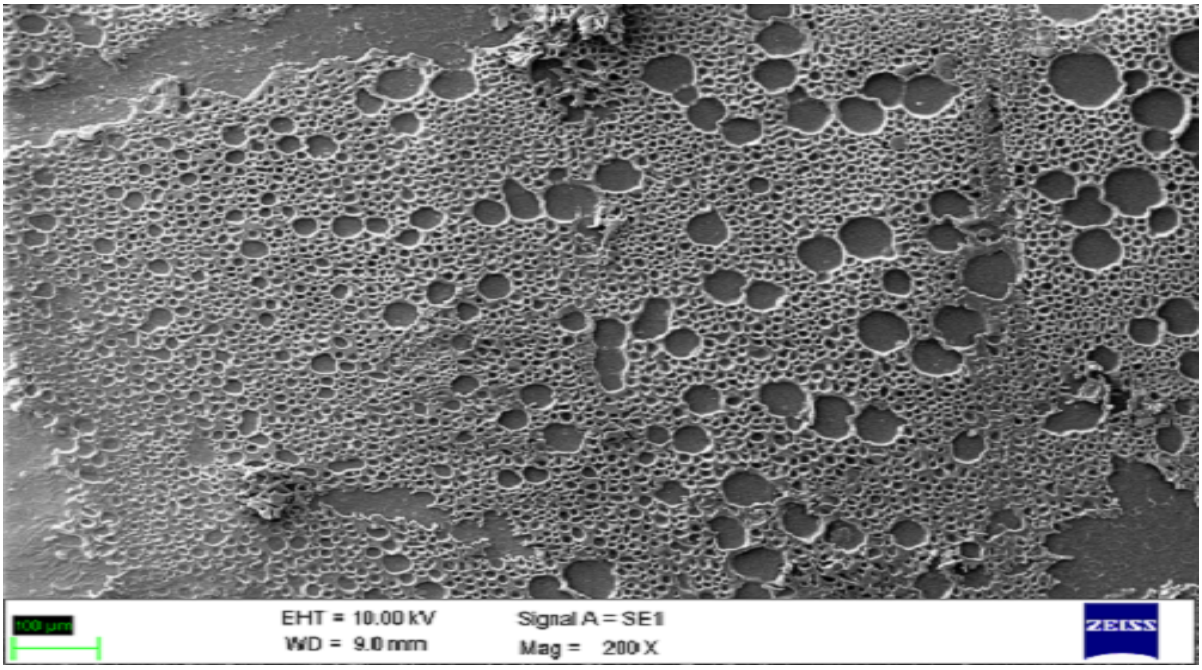


Figure: 29Formulated patch F3

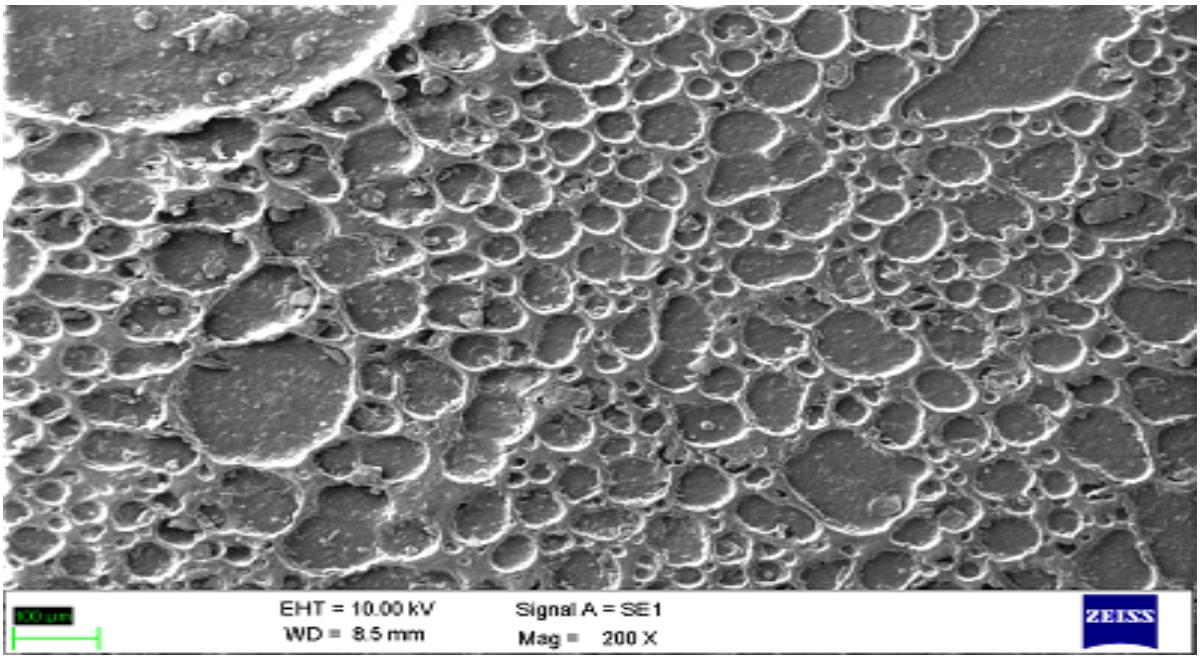


Figure: 30 Formulated patch F4

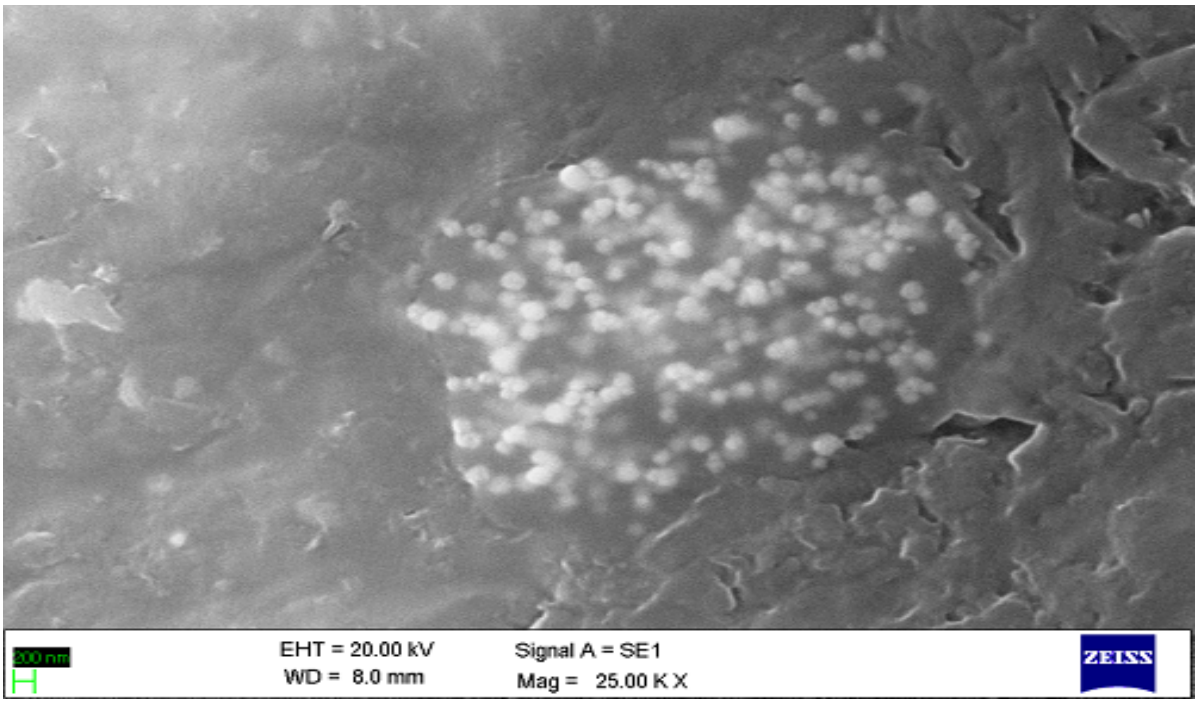


Figure: 31 Formulation F3 after release study.

The SEM study suggests that the drugs are uniformly distributed in the surface of polymeric matrix (Figure-27-30). Figure 30 suggest that most of the drug particles are

found to diffuse out from the formulation. Figure 26 suggest blank patch without drug shows smooth surface of the polymeric matrix.

6.3.7 The effect of patch on the cellular morphology of mucous membrane:

The relative investigational study that has been performed taking buccal mucosa and submucosa with and without the application of patches exhibited that there was not significant morphological changes in mucosal and sub mucosal area (Figure -31), after 8h of administration of the patches on the buccal tissue.

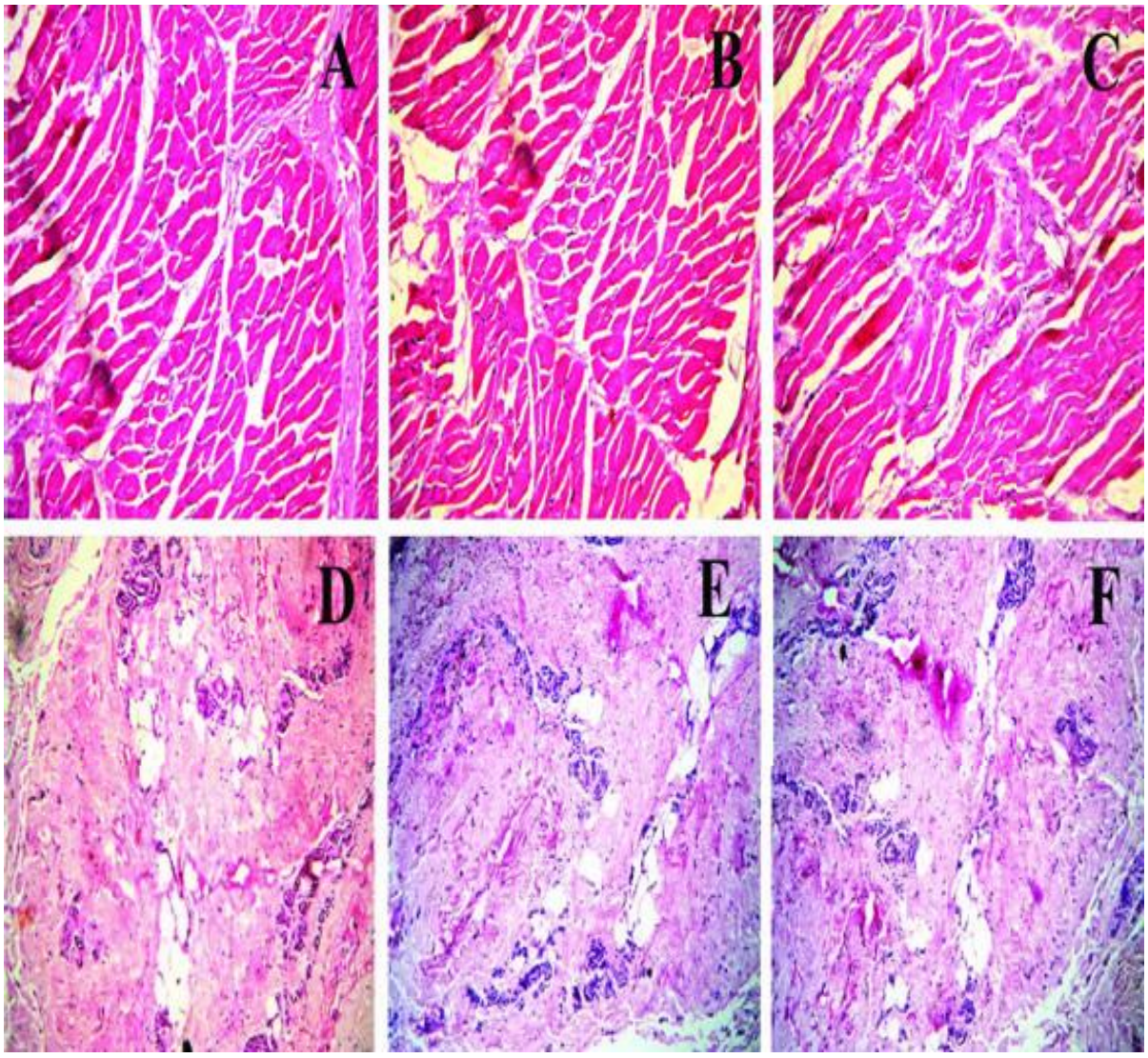


Figure 32 Picture of histopathology of goat mucosa and submucosa when the experimental patches were applied and without the application of developed patches.

(figure- A) normal histopathology mucosa, B) treated (with patch without drug) mucosa, C) treated (with patch containing drug) mucosa, D) normal (without patch) submucosa, E) treated (with patch without drug) submucosa, F) treated (with patch containing drug) submucosa.

6.3.8 The stability study:

Formulation (F3) was selected the best, on the basis of its further characterizations and drug release pattern, it was stored at 40°C; 75% RH for 3 months as mentioned in the ICH guidelines.. After 3 month-storage period, when the FTIR spectra of the formulations were compared with the freshly prepared formulation (F3), no significant change of peak was observed (Figure 19& 24). This proves that drug (s) and had not interacted in the mentioned temperature and formulation remains stable on storage.

6.3.9 *In vitro* drug release and release kinetics study:

Release of drug(s) from dental patch mainly involves diffusion factors. The excipients such as-PVP K-30 and HPMC are hydrophilic in nature and facilitates diffusion of drug whereas EC is hydrophobic in nature and it also controls rate of diffusion. Dissolution of PVP K-30 molecules can create pores in diffusion medium, which increases the rate of permeation as well as diffusion.

It was shown in all the cases that an initial bursting effect appeared in all the patches with the plasticizers DBP and release enhancer PEG. Bursting effect from the patches with PEG was much greater in comparison with that of patches without PEG.

Figure 33: Franz Diffusion Cells during drug release study

Diffusion studies were carried out by using various patches containing pure drug (10 mg), plasticizer (30 %, 40 %) and other patch carriers, such as- HPMC, EC, and PVP K-30. Amount of drug diffused out and amount of drug permeated periodically were determined and data of cumulative amount of drug released, Q (µg/cm^2) was plotted against time. The cumulative amount of drug release for lidocaine, amoxicillin and metronidazole are 134.79 µg/cm^2, 712.57 µg/cm^2, 509.77 µg/cm^2, respectively.

In order to determine the drug-release kinetic patterns, the drug release data were analyzed using various kinetic models that are Zero Order, First Order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell kinetic models. R^2 values for the studied kinetics have been included as Table 10. The release of lidocaine hydrochloride from the dental patches showed to follow first order kinetics, while amoxicillin trihydrate and metronidazole release were found to obey apparent zero-order kinetics.

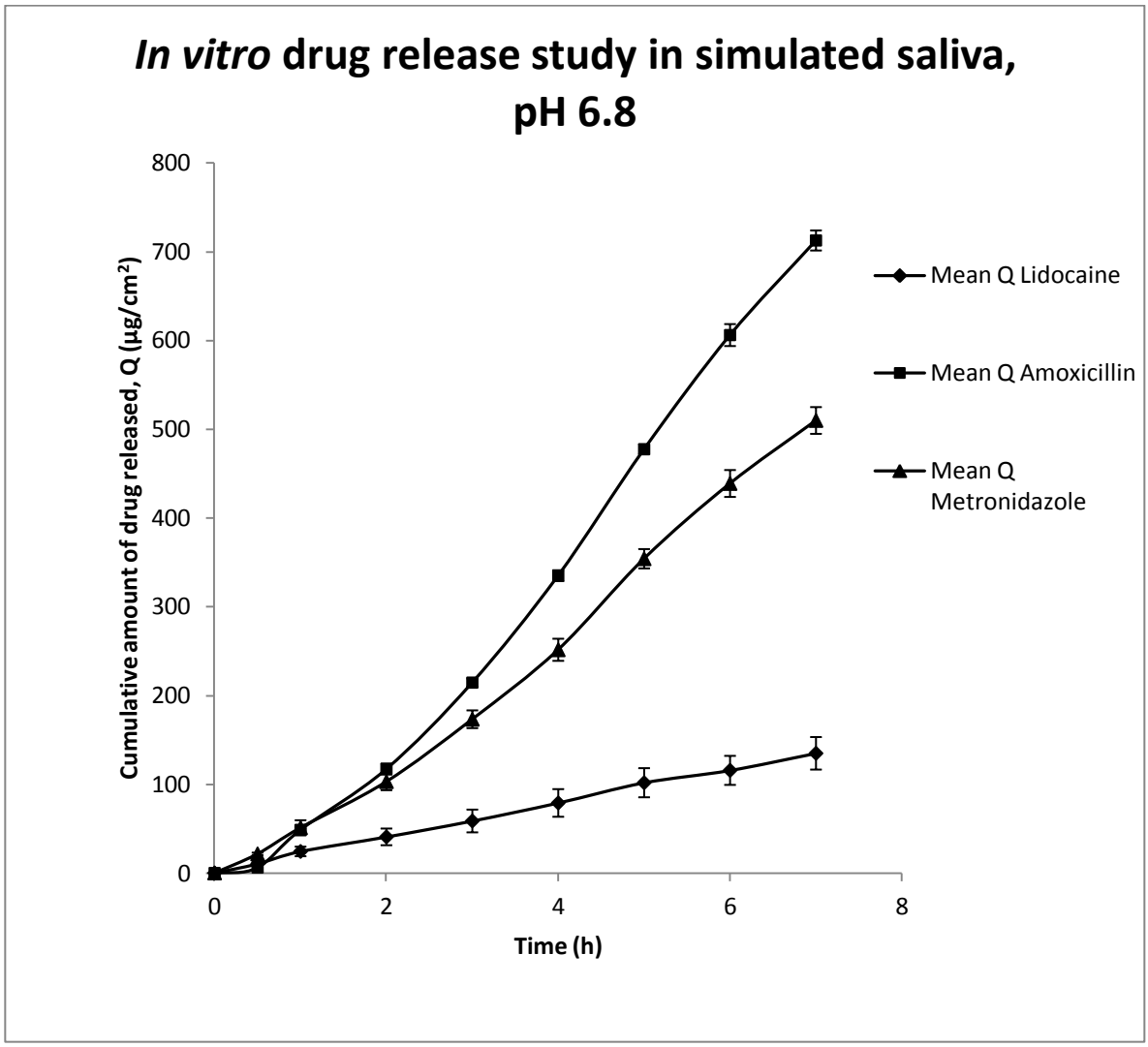


Figure 34: Cumulative amount of drug(s) released versus time (h)

Table10. Coefficients of determination (R^2) of drug release patterns tested on various kinetic models.

S.No.	*In vitro* kinetic model on which drug release data were assessed	Corresponding R^2 values (Lidocaine)	Corresponding R^2 values (Amoxicillin)	Corresponding R^2 values (Metronidazole)
1.	Zero order	0.9981	0.9835	0.9902
2.	First order	0.9983	0.9796	0.9898
3.	Korsemeyer peppas	0.9345	0.8497	0.8735
4.	Higuchi	0.9982	0.981	0.9899
5.	Hixson-Crowell	0.9531	0.9627	0.7826

CHAPTER 7

DISCUSSIONS

7. Discussions:

7.1 The drug excipients interaction study by FTIR Spectroscopy:

Drug excipients interaction is one of the most essential as well as significant pre-formulation experiment that gives a idea if there is any interaction between drug (s) and excipients .It also gives a idea about drug stability. This experiment can be performed by various methods such as Infra Red Spectroscopy (IR) Differential Scanning Colorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR) etc.

In our experimental study we have chosen FTIR spectroscopy to determine any interaction between drug and excipients. When the physical mixture of amoxicillin, metronidazole, and lidocaine and excipients were compared, data shows that there has not been any shifting of peaks of the drug and excipients. It suggests that there is no chemical interaction between drug and excipients. But there is chance of formation of physical interaction such as formation of weak hydrogen bond or formation of bonds due to vander Walls force of attraction or dipole dipole interaction (Maji et al., 2014). The results of the FTIR spectroscopy clearly proves that the solid adhesive patches have desired from the PVP K-30 polymer which acts as a pressure sensitive adhesives. PVP K-30 also helps to sustained release of drug by modification of drug diffusion pathways.

7.2 Evaluation of prepared Dental Patches:

7.2.1 Average mass variation, thickness, diameter and area of the patches-

The results of various characterizations like mean mass variation, thickness; diameter and area of the patches were performed. The prepared patches were found to be very thin in size, glossy and smooth in appearance. The average mass of patch was found to be in the range of 0.57 ± 0.03g for the formulation F3 the low average mass suggest that the formulation was less in weight and very comfortable for use. The whole patch thickness and diameter in the formulation F3 was found to be 0.59 ± 0.02 mm 3.42 ± 0.04 respectively. It also support that prepared formulation was very thin.

7.2.2 Moisture content and moisture absorption:

Moisture content study generally gives idea that how much moisture is present in the prepared formulation. The average percent moisture content of the formulations F3 was found to be in the range 0.78 ± 0.23 weight %.

Moisture absorption study clearly replicates the capability of the formulation to hold maximum amount of moisture when the formulation is taken in hot and humid climatic condition. The average moisture absorption for the formulation F3 was found to be range of 11.20 ± 3.08 weight %. Data shows that the value of moisture content and moisture absorption is very low it might prevent the dryness and brittleness of the developed formulations. The prepared patches will provide patients compliance because prepared patches will not become bulky due to excessive salivary secretion.

7.2.3 Folding endurance and structural integrity of the formulations in simulated saliva-

The results of folding endurance in the formulation F1 was found to be in the range 93 ± 4.07 .The result clearly replicates the patch would not break during transportation, it is very tough because PVA is used as a backing membrane. When the patches were exposed in the simulated saliva for a time period of 8h there has not been any decomposition has been observed in the formulation. The results indicate that the prepared patches perpetuate their structural integrity in simulated saliva pH 6.8.

7.2.4 Bioadhesive strength-

Bioadhesive strength generally denotes ability of patches to keep attach to a surface of a mucous membrane by forming strong bond. The mucoadhesive polymer having functional groups with H-bond like -COOH, -OH, gives good mucoadhesion property. PVP K-30 is a water soluble polymer made up of N-vinylpyrrolidone and having functional groups -OH, and -COOH to satisfactory adhesive property, that would helps remain attached to the oral cavity for a longer period of time. The bioadhesive strength of the formulation F3 was found to be in the range of 12.52 ± 0.38 kg/ mm^2. The bioadhesive strength varies on the basis of bond formation. If it is weak the formulated patch will not be capable to be intact in the oral mucosa even a very short time period on the other hand if there is very strong bond the formulated patches cannot be separated in the oral mucosa. The results of the bioadhesive strength gives a idea that patch will remain intact in the oral mucosa for a longer time period ,they will not cause any harm in the oral mucosa and it can be easily removable and replicable.

7.2.5 Surface pH study-

The surface pH of the formulated patch F3 was found to be in the range 6.43 ± 0.23.The resulting pH of the prepared formulation was in the range of oral cavity pH as reported in the literature. The data proves that the prepared patch will not create any hazard in the oral cavity; it would not cause excessive buccal secretion to neutralize the pH.

7.2.6 Scanning Electron Microscopy study -

The SEM study gives a clear idea about the drug distribution pattern in the matrix. If we compare formulation FO (figure-26) and formulation F3 (figure-29) we could see that in the formulation FO (blank patch without drug) there is a smooth matrix and in the formulation F3 (formulation with drug) drug particles are widely scattered and distributed in the matrix.

7.3.7 The effect of patch on the cellular morphology of mucous membrane:

The time period when formulations were attached on the buccal tissue of goat for 8 h, it was observed that there was not significant changes of mucosal and sub mucosal area at the site of application of patches and in other areas. This gives a clear idea that formulated patch does not cause any damage in the above mentioned areas.

7.3.8 *In vitro* drug release and release kinetics study:

PEG 400 is used as release enhancer in the mucoadhesive patches were the main cause of improved cumulative percent drug(s) release *in vitro* during a time period of 8 h of the drug release study. Addition of drug release enhancers in the patches might increase high amount of water uptake and/or polymer hydration (Escobar-Chavez et al. 2012) than those formulation that has been prepared without drug release enhancer. Results of the *in-vitro* release were put into different kinetic models to know and evaluate the release kinetics of d rug(s) from patches. Drug release kinetic patterns as assessed by various kinetic models show that more linearity (by assessing R^2 values) of the drug release kinetic plots was towards first order model followed by Higuchi model. Further, the data gives a clear idea that addition of release enhancer increase and affect the release behaviour. (Siegel et al 1981).

7.3.9 The stability study:

In the stability study, formulations were preserved at 40°C with 75% RH as per ICH guidelines. The formulation was then taken out form the stability chambers after 3rd month of stability study. After that FTIR spectra was determined .Then the FTIR spectra was compared with the FTIR spectra of newly developed dental patch. The data shows that there is no significant shift in the FTIR spectra of the formulation which are stored in the stability chamber when it is compared with newly prepared formulation. Hence, there is no chance of formation of any chemical interaction between the drugs and the excipients. So, the formulations were stable at least up to 3 months at 40°C a; 75% RH.

CHAPTER 8

CONCLUSION

8. Conclusion:

We are now in the 21st century, science is developing and the new technologies are coming, so why should we lag behind. Beautification and maintenance of personal hygiene is a important criteria in this era. So, dental formulations will take important part to fulfill these criteria.

The developed dental patches were found to control drug release for a longer period of time *in vitro* and it will be very useful to combat common dental diseases. The result of various characterizations gives a perfect picture that the prepared patches will be stable after storage, would not cause any hazard in the mucosal area and it would be intact during transportation.

It is mainly a *in vitro* study, so there is a necessity to develop *in vivo* toxicity study by using a worthy animal model to identify any reaction that might take place and maintain suitable *in vitro* and invivo correlation.

CHAPTER 9

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