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Preparation of Tinidazole Gel and Studying its Permeation Behavior

تحضير جل من مادة التينيدازول ودراسة سلوك النفاذية

By

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Fo, Dr. Feras

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Declaration

I declare that this dissertation submitted for my master's degree and titled " Preparation of Tinidazole Gel and Studying its Permeation Behavior" was supervised by me in the Department of Pharmacy at Birzeit University. Any information taken from the literature was acknowledged throughout the text and cited in the reference list. This science thesis or any part of it has not been submitted for any other degree or professional qualification.

Signed: .....

Walaa Y. Talhami

Date: / /2023

Table of Contents

List of Abbreviations and Acronyms

Abbreviation	Definition
A	Surface area of the membrane
Abs	Absorbance
API	Active pharmaceutical ingredient
AU	Absorbance unit
Av.	Average
BCS	Biopharmaceutics classification system
BP	British pharmacopeia
cp	Centipoise
C	Concentration
C_0	Initial concentration of Tinidazole solubility
Cn	Initial concentration in the receptor compartment
Ci	Final concentration of the sample
C_o	Concentration in donor compartment
°C	Celsius
CMC	Carboxymethyl cellulose
cm	Centimeter
cm^2	Centimeter square
COA	Certificate of analysis
D	Diffusion coefficient

DDS	Drug delivery system
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EDA	Effective diffusion area
EMA	European medicines agency
Eq.	Equation
FDC	Franz diffusion cell
g	Gram
g/mole	Gram per mole
GI	Gastrointestinal
GIT	Gastrointestinal tract
h	Membrane thickness
hr.	Hour
hrs.	Hours
HPLC	High-performance liquid chromatography
HPMC	Hydroxy propyl methyl cellulose
ICH	International conference harmonization
IS	Internal standard
J	Flux (slope)
K	Membrane vehicle partition coefficient
TL (L)	Lag time
M	Molar
m	Cumulative amount per unit area
mg/ cm^2	Cumulative amount per unit area
m AU	Milli-absorbance unit
ml	Milliliter
µm	Micrometer
mm	Millimeter
mol	Mole
MT	More than
ND	Not detected

nm	Nanometer
P	Permeability coefficient
PAA	Polyacrylic acid
PB	Phosphate buffer
PBS	Phosphate buffer saline
PG	Propylene glycol
PE	Penetration enhancer
PEG	Polyethylene glycol
PPG 15 SE (Arlamol)	Propylene glycol 15 stearyl ether
PVP	Polyvinyl pyrrolidone
Q	Cumulative amount
Qty	Quantity
RH	Relative humidity
Rpm	Round per minute
RSD %	Relative standard deviation %
SA	Sample absorbance
SD	Standard deviation
T	Time
TNZ	Tinidazole
µg	Microgram
USP	United States pharmacopeia
UV	Ultraviolet
Vi	Volume sample
VR	Volume of receptor compartment
λ	Wavelength

List of Figures

List of Tables

Abstract

Tinidazole is an antiprotozoal that belongs to BCS Class II drugs and it is practically insoluble in water, which is why in the experiments we used the concept of mixed solvency to solubilize Tinidazole in water and enhance its solubility.

The aim of this study is to develop and evaluate Tinidazole mucoadhesive gel as buccal drug delivery due to the characteristics of the buccal mucosa which is smooth and relatively immobile surface and accessibility so that makes it very suitable for bio-adhesion system. The promising dosage form can increase the residence time in epithelium tissue to achieve the desired pharmacological effects due to high drug flux at the absorbing tissues. Tinidazole, when taken by oral administration does not give the required competency because it is affected by enzymatic degradation in the stomach. So, this reason is considered as the main limitation for decreasing the drug bioavailability.

The buccal mucoadhesive gel is composed of the active pharmaceutical ingredient (Tinidazole), the mucoadhesive agents, the penetration enhancers, the co-solvents and the vehicle. The excipients were selected upon compatibility study done at 25 ºC/60% RH and 40 ºC/75% RH for one-month period. The purpose is to achieve the highest possible permeability of the active ingredient in order to give the highest pharmacological effects and the least side effects from Tinidazole drug product as mucoadhesive buccal gel.

In this study we worked out the solubility profile in different media (0.1N HCl, Acetate Buffer pH 4.5, Phosphate Buffer pH 6.8 and Phosphate Buffer pH 7.4) by means of shake-flask method. For further study we selected the phosphate buffer

pH 7.4 based on the physiological characteristics of the oral cavity and Tinidazole solubility (0.104 g/100ml). The permeation study of Tinidazole gel was carried out by using Franz diffusion cells. The buffer in the receptor compartment of Franz diffusion cell was selected based on the results of solubility of Tinidazole (g/100 ml) in Phosphate buffer pH 7.4 containing different concentrations of Tween 80 at 37 ºC in order to fulfill the sink conditions. It was found that Phosphate buffer pH 7.4 containing 0.3% Tween 80 had the highest solubility value (3.184 g/100 ml) that fulfill the sink conditions. The optimized formulation was selected based on the in vitro residence time of gel on buccal mucosa that was determined by using rabbit buccal mucosa and disintegration test apparatus at 37°C. Two formulations containing Carbopol, Povidone, mixture of HPMC, and without (F4) or with (F5) 1% Xanthan gum as mucoadhesive polymers, recorded the highest residence times (4.0 and 4.5 hrs.) respectively.

The in-vitro release and permeation studies were done by using FDC equipped with artificial membranes that mimic the periodontal membrane, such as nylon 66 (polyamide), semi-permeable dialysis tubing cellulose, Chicken eggshell and Permeapad® Biomimetic Barrier. The receptor compartment was filled with 20 ml of phosphate buffer pH 7.4 containing 0.3% of Tween 80, operated at 600 rpm and the samples were withdrawn up to 6 hours in each study to determine by spectrophotometric mean the amount of Tinidazole released / permeated by time. The cumulative amount released per unit area over periods of time was calculated. Three trials were prepared from each selected formulation by the same method, but by adding different potential penetration enhancers (PEs) with 0.3% concentrations

to each trial. The penetration enhancers selected were Tween 80, Cremophor RH40 and Arlamol (sum 6 trials). All trials were tested for the in-vitro release / permeation by using the same methodology of Franz diffusion cells under physiological conditions phosphate buffer pH 7.4 containing 0.3% of Tween 80 for the same period of testing.

For the release study profiles by using polyamide and dialysis membranes, the results had showed F4 with Arlamol as penetration enhancers represented the highest release values observed between all formulations where the cumulative amount per unit area versus time periods about six hours was equal (2.630865626 mg/cm^2) and (%) drug released was (41.30%).

The trials were evaluated for cumulative amount (mg), cumulative amount per membrane unit area (mg/cm^2) and flux ($mg/cm^2/h$) The results of permeation profiles when Chicken eggshell membrane was used showed less permeation in the formulations without penetration enhancers than the formulations containing, Arlamol as penetration enhancer and the highest value for F4 with Arlamol which recorded the highest value of the flux (Jss) which was equal (0.3605 $mg/cm^2/h$) and permeability coefficient (0.0058 cm/hr.). When Permeapad® membrane was used, the results of both formulations (F4, F5) when used penetration enhancers showed more better results than formulations without penetration enhancers, where F4 with Arlamol Permeapad® had the most values of the flux (Jss) which was equal (0.403 $mg/cm^2/h$) and permeability coefficient (0.0064cm/hr.).

Chapter Ⅰ: Introduction

1. Introduction

The oral cavity is an individual component ecosystem colonized with a vast number of microorganism classes which are more than 700 microorganisms. Many factors might alter the component of oral microbiota, so leading to infectious disease. Local therapies and procedures represented by dental hygiene are the major types of treatment. Thus, the emerging modern applications of drug delivery system for handling these oral disorders including periodontitis, dental caries, candidiasis and peri-implantitis. The oral cavity is one of the parts of the digestive system which consists of many anatomical structures such as teeth ,oral mucosa, periodontal tissues, maxillary and mandibular bones, as well as other soft and hard tissue (Liang et al. 2020). The oral cavity is considered the attractive position for the delivery of drug with ease administration and would bypass the enzymatic degradation and avoidance of first pass metabolism in the liver. Buccal drug delivery, specifically, refers to the delivery of drugs within buccal mucosa to affect local and systemic pharmacological actions (Hao and Heng 2003).

The drug delivery system may react to special conditions of oral cavity such as pH, temperature, enzymes, and may provide more accurate drug delivery. The characteristic of oral environment especially in the physiological environment, saliva has a normal pH range of 6.2–7.6, the average pH is 6.7 and the temperature is around 37 °C (Liang et al. 2020).

Periodontal diseases are highly epidemic and a great public health problem. It can affect up to 90% of the worldwide population. Gingivitis, the

mildest form of periodontal disease, is categorized as a nondestructive periodontal disease, caused by the bacterial biofilm (dental plaque) that accumulates on teeth relevant to the gingival (gums). Some of the most common symptoms associated with gingivitis are bad breath, and bleeding, bright, tender or swollen gums, who claims that 10–15% of the population worldwide has this disease, it is a local inflammation in the periodontal pockets, and is caused by dental plaque. This disease includes the destruction of periodontal ligaments, formation of periodontal pocket and resorption of alveolar bone, resulting of the degeneration of the teeth. Also, periodontitis is result from various types of microflorae (mostly anaerobes), figure (1). Diverse local or systemic approaches were used for the effective treatment of periodontitis. Recently, controlled local drug delivery approach is much better in comparison with systemic approach because it mainly focuses on developing the therapeutic results by realizing factors like site-specific delivery, low dose requirement, bypass of first pass metabolism, reduction in gastrointestinal side effects and decrease in dosing frequency. Generally, it provides a safe and effective method of treatment, the disorder which improves patient compliance (Nasra et al. 2017) (Nguyen and Hiorth 2015) (Joshi et al. 2016)

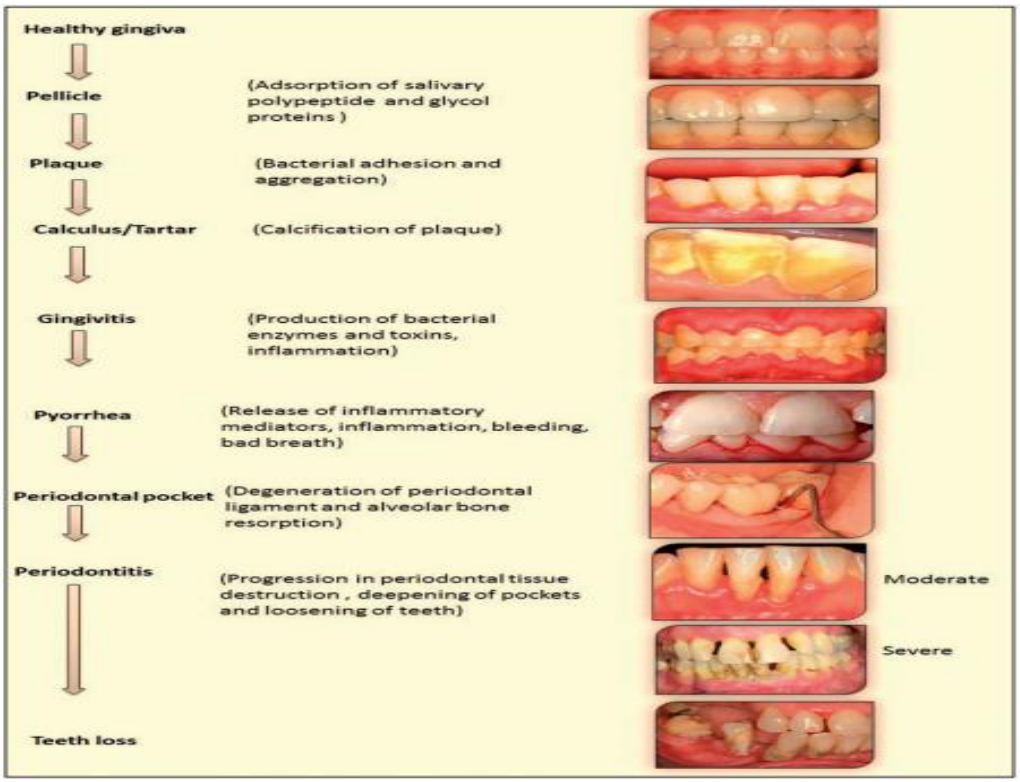


Figure 1:Various Phases of Periodontal Disease (Joshi et al. 2016).

Chronic periodontitis predominantly affects adults, but aggressive periodontitis may sometimes happen in children. This pathophysiological situation persists through periods of activity and dormancy, until the affected tooth is extracted or the microbial biofilm is therapeutically removed and the inflammation subsides. The gravity of the periodontal disease hangs on environmental and host risk factors, both modifiable (for example, smoking) and non-modifiable (for example, genetic susceptibility). Avoidance of forming this will be achieved with daily self-performed oral hygiene and professional removal of the microbial biofilm on a quarterly or bi-annual basis. New treatment modalities that are actively explored include, host modulation therapy laser therapy and tissue engineering for tissue repair and regeneration.

Antimicrobial therapy, as shown in this study includes preparing Tinidazole buccal mucoadhesive gel (Kinane, Stathopoulos, and Pappano 2017). The advanced therapeutic drug delivery allows to use techniques that faster releaser rate and are easier in administration compared with conventional ways. So, the advanced methods are more biocompatible and bio-adhesive. Thus they easily adhere to the mucosa in the dental pocket with less irritation at the site of application and the easy removal by normal catabolic pathway (Joshi et al. 2016).

Periodontal diseases are highly prevalent and can affect up to 90% of the worldwide population. Gingivitis, the mildest form of periodontal disease, is classified as a nondestructive periodontal disease, caused by the bacterial biofilm (dental plaque) that accumulates on teeth adjacent to the gingiva (gums). Some of the most common symptoms associated with gingivitis are bad breath, and bleeding, bright, tender or swollen gums.

Tinidazole, a structural analogue of metronidazole, is an antiprotozoal agent that has been extensively used in developing countries for many years with established efficacy and acceptable tolerability. It has been recently approved by the US Food and Drug Administration for the therapy of trichomoniasis, giardiasis, amebiasis, and amebic liver abscess and periodontitis (Fung and Doan 2005). (TNZ), [1-(2-(ethyl sulfonyl) ethyl)-2-methyl-5-nitroimidazole], is an effective antiprotozoal and antibacterial agent. It is used for the treatment of amoebiasis, giardiasis and trichomoniasis (Praveen et al. 2014). It is also used for the treatment of trichomoniasis (a sexually transmitted disease that can

influence men and women), giardiasis (an infection of the intestine that can cause diarrhea, gas, and stomach cramps), and amebiasis (an infection of the intestine that can cause diarrhea, gas, and stomach cramps and can expand to other organs such as the liver). Tinidazole is also used to treat bacterial vaginosis (an infection caused by an overgrowth of harmful bacteria in the vagina) in women. Tinidazole is in a class of medications called nitroimidazole antimicrobials. It works by killing the organisms that can cause infection. Tinidazole has molecular mass of (247.273 g/mol) and chemical formula (C8H13N3O4S) (PubChem 2023b). It is completely absorbed into the bloodstream, with a mean bioavailability of 99% and the plasma half-life of Tinidazole is approximately 12-14 hours. Tinidazole is excreted by the liver and the kidneys. Tinidazole is excreted in the urine mainly as unchanged drug (approximately 20-25% of the administered dose). Approximately 12% of the drug is excreted in the feces (Chang et al. 2013) (Abou-Taleb et al. 2011)

It also has the potential for reducing the systemic side-effects from injected or ingested treatment where an oral mucosal disease is the target of therapy.

1.1 . Mucoadhesive Drug Delivery System

Mucoadhesive drug delivery systems interact with the mucus layer covering the mucosal epithelial surface and mucin molecules, thus prolonging the residence time of the drug at the absorption area. The drugs which have local action or those which have maximum absorption in gastrointestinal tract (GIT) require increased duration of stay in GIT. Consequently, the advantage

of mucoadhesive dosage forms is to enhance the concentration of plasma and thus to increase the therapeutic activity.

Drug absorption is almost restricted at the area of absorption by the residence time. In oral delivery, for instance, a drug is affected by gastric enzymes, so the activity is less; therefore, mucoadhesion is an important strategy for prolonging the mucosal residence time of drug delivery systems. Both systemic and local delivery can be optimized and enhanced with mucoadhesive dosage forms by retaining in intimate contact with the absorption site or the site of action, which results in high local drug concentrations, thus a high flux through the absorbing tissue, figure (2) below was shown the mucoadhesive buccal DDS diagram (Boddupalli, et al. 2010).

Figure 2: Mucoadhesive Buccal DDS (Sharma et al. 2020)

1.1.1 Anatomy and Physiology of Mucus Membrane:

Mucous membranes are the moist cover that lining the cavity of gastrointestinal and respiratory tracts which contains the connective tissue (the lamina propria) above of which epithelia tissues covering the surface and featured with moist structure because the presence of a mucus layer. The layers of epithelial could be either single layered (e.g., the stomach, small and large intestine and bronchi) or multilayered/stratified (e.g., in the esophagus, vagina

and cornea). Also, there is a salivary gland that secrets the mucus and saliva which is about 99% water with organic and inorganic compounds. Also, the secretions of saliva is high during working hours .The thickness of this mucus layer varies on different mucosal surfaces, from 50 to 450μm in the stomach to less than 1μm in the oral cavity .The main functions of mucus are protection and lubrication (Khairnar and Sayyad 2010).

1.1.2 Anatomical Physiology and Nature of Oral Cavity:

The mouth could be separated into two segments: the outer layer vestibule that is enclosed by the cheeks and the lips. The second segment is the oral cavity itself which comprises the hardened soft paten as well as the bottom of the mouth and tonsils.

Physical description of the oral cavity:

Classification of the oral cavity underlines the mucosa into three sorts according to their function.

1. Masticatory mucosa that involves the mucosa surrounding the teeth and the hard palate. The features of these areas of the epithelium are keratinized.
2. Lining mucosa which covers the lips, cheeks, base of the oral cavity, lower part of tongue, buccal mucosa and the soft palate. These areas of the epithelium are non- keratinized.
3. Specialized mucosa which is lining the dorsum of the tongue with high amount of keratinization (Mahajan et al. 2013).

- **Review of the Oral Mucosa:**

1. Structure

The constitution of the oral mucosa is the squamous stratified (layered) epithelium, basement membrane, the lamina propria and submucosa. In addition, it consists of a lot of sensory receptors involving the tongue taste receptors. The epithelium of the buccal mucosa is around 40-50 cell layers thick, while that thickness of epithelium of sublingual includes somewhat fewer cells (Mahajan et al. 2013). In addition, the oral cavity contains salivary glands that located below the mucosa which produce the mucin, a major component of the mucus layer on the mucosal level and the function of mucus is promoting and production of saliva which turn moisten and lubricate the mucosa layer so assist the masticatory process and binding the foods bolus previous of swallowing. Furthermore, the secretion of saliva protects the oral cavity from harmful materials promotes the constituents of microbial flora in the mouth and maintains the pH range between (5.5 and 7.0). As well for buccal cheek administration, another animal models such as rabbits, dogs and pigs are more suitable therefore they include non-keratinized buccal mucosae. Also, the buccal mucosa thickness in these animal models (rabbit, 600 μm; dog and pig, 770 μm) is comparable to that of humans buccal (500–800 μm) as in figure (3) (Kraan et al. 2014).

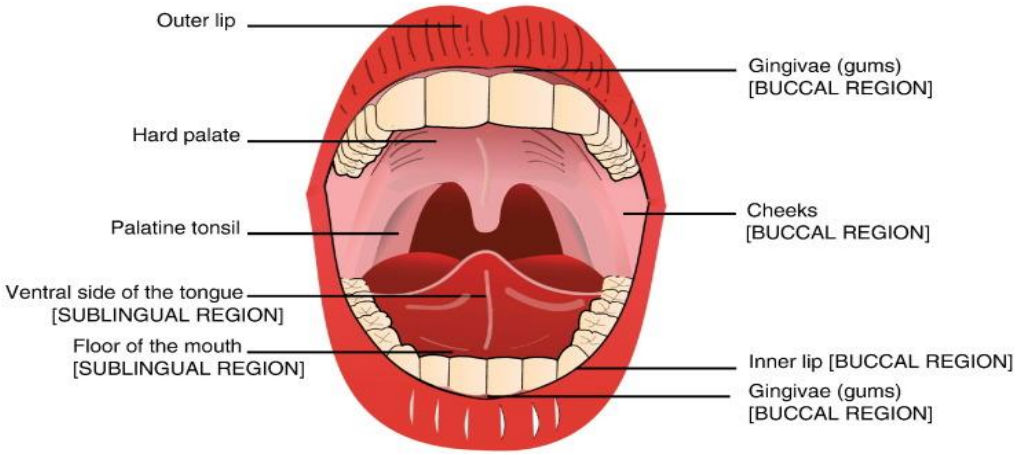


Figure 3: The Anatomy of the Oral Cavity (Kraan et al. 2014).

2. Permeability

The epithelia intermediate across epidermis and intestinal mucosa is almost somewhat leaky. It is estimated that the mucosa of the buccal permeability is 4-4000 times greater than that of the skin. Generally, in the oral mucosa, the permeability order depends on the degree of keratinization and the thickness of the layers. Thus, the order of permeability decreases with an increase of these factors (Mahajan et al. 2013). The buccal mucosa thickness in the human has been predicted to be in the range of 500–800 μm, while the sublingual mucosal thickness is about 100–200 μm (Kraan et al. 2014) . So, the degree of permeability of the sublingual region is higher than the buccal one, and the buccal is greater than the palatal. The features of these tissues are that the sublingual is non-keratinized and thin, while the buccal is non-keratinized and thicker, and the palatal is keratinized and intermediate in thickness (Mahajan et al. 2013).

3. Environment

The oral epithelial cells are enclosed by an intercellular matrix substance and mucus. The principal constituents of these substances are complexes composed of carbohydrates and proteins. These complexes substances are free associations with the tissues or may be attached to certain regions in these areas (Mahajan et al. 2013).All this form plays an important role in adhesion and lubrication. So the mucus has a remarkable place in buccal mucoadhesion drug delivery system (Madhavi et al. 2013).

4. Composition of Mucus Layer:

Features of mucus are viscid secretion and translucent that set up get gel that is contentious and thin. The variety of the thickness in human is about 50-450 µm. The goblet cell secretes the mucus that covers the epithelia. The overall percentage formulae of the components are (Mahajan et al. 2013) :

- Water: 95%
- Glycoprotein and lipids: 0.5 - 3.00%
- Mineral salts: 1%
- Free proteins: 0.5-1.0%

5. Functions of Saliva and Mucus Layer:

The functions of saliva and mucus layer are shown in table (1)

Table 1: Functions of Saliva and Mucus Layer (Mahajan et al. 2013) (Banya , joshi2021).

Role of Saliva	Role of Mucus
1. Protection of all the tissues fluid in the oral cavity	1. Protective especially as a result of hydrophobicity feature
2. Demineralization of the tooth enamel / continuous mineralization	2. Barrier: The function of the mucus layer as a barrier of the drugs in tissue absorption and the effects of the bioavailability
3. The oral cavity is wet	3. Cell adhesion: Mucus has strong adhesion and attachment properties
4. Hydration of the dosage forms in the oral mucosa	4. Lubrication: It is to save the mucus from the goblet cell that is substantial to compensate for the removal of the mucus layer due to digestion, bacterial degradation and solubilization of mucin molecules.
5. Helps in chewing, tasting and swallowing	5. Made up of proteins and carbohydrates

6. Mechanisms of Mucoadhesion

The procedure of mucoadhesion mechanism is wetting and swelling of polymer then interpenetration between polymer and mucus which covers the epithelial membrane and finally, formation bonds between them show figure no. (4).

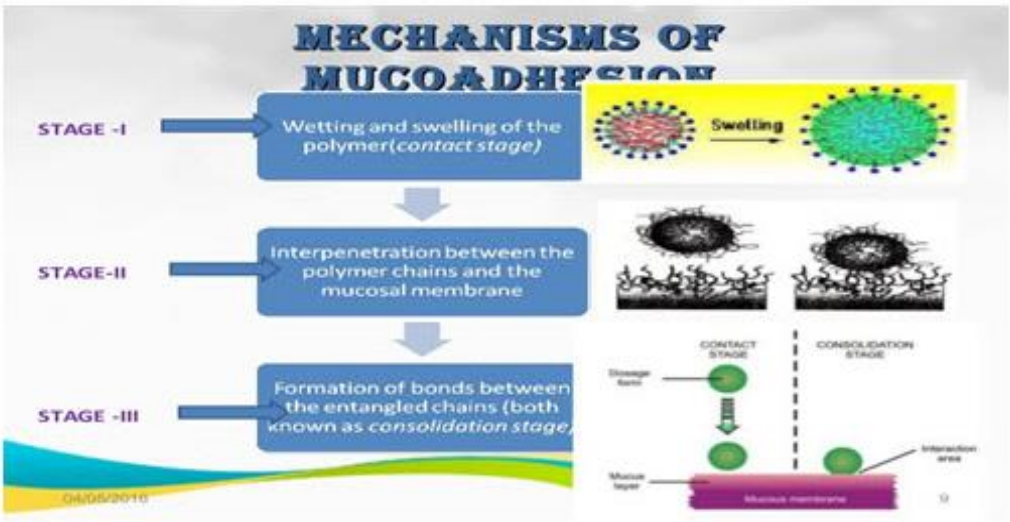


Figure 4: Mechanism of Mucoadhesion (Banya, joshi2021).

7. Stages of Mucoadhesion

Stages of Mucoadhesion as Figure (5)

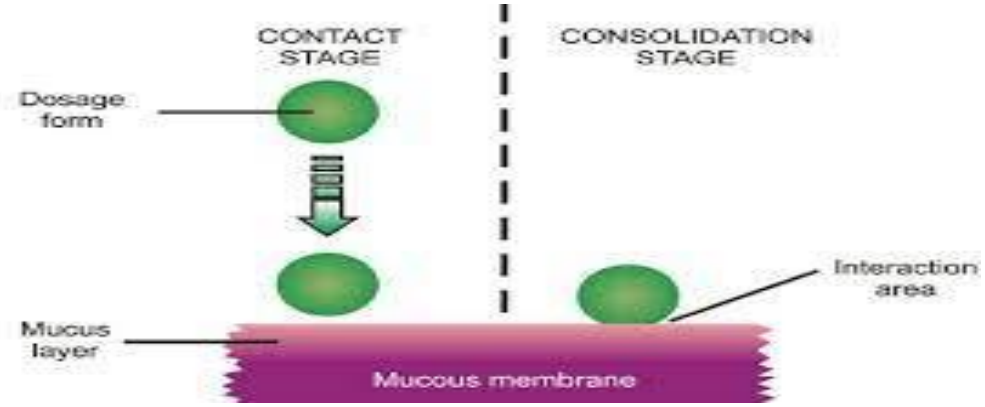


Figure 5: Stages of Mucoadhesion (Pawar et al. 2013)

Bioadhesion may lead to shortness of attachment between the drug and the site of absorption, thus the time is short between them, so we improve the contact time by adding mucoadhesive polymer like polycarbophil (Carbopol 934P). This occurs by utilizing mechanisms represented by two stages which are contact stage represented by intimate contact deeply with the mucus layer. The other stage is

consolidation which is resulting from taking water and good wetting of the bioadhesion surface so the presence of moisture is essential due to plasticize the systems. Then the bioadhesive polymer swells, and penetrates into the tissue surface, or leads to interpenetration of bioadhesive chains with those of the mucus. Finally, the chemical bonds become low so the links are broken (Ducheˇne, Touchard, and Peppas 1988) (Mahajan et al. 2013).

Essentially, there are two theories describing the concept of consolidation step:

1.The diffusion theory

2.The dehydration theory

The diffusion theory as regards, the mucoadhesive materials interact with the glycoprotein of mucus which results interpenetration of their own chains and formation the secondary bonds. In addition, the mucoadhesive molecules take place in this theory if preferred the chemical and mechanical interactions. According to the dehydration theory , the materials in an aqueous environment that can be jellify so able to contact with mucus so the dehydration happened due to the variance of the osmotic pressure (Mahajan et al. 2013).

7. Factors Affecting Mucoadhesion

The factors are summarized according to adhesion theory in table no. (2)

Table 2 :Factors Affecting Mucoadhesion (Mahajan et al. 2013) (Shaikh et al. 2011).

Factors	**Properties**	**Comments**
Polymer related factors	1. Molecular weight	The molecular weight of polymer is increased by mucoadhesive the force and reach up to 10000.
	2. Active polymers concentration	In dosage forms the higher the concentration of polymers leads to increasing the mucoadhesive strength.
	3. Polymer chain flexibility	Flexibility of polymer chain is a main factor for interpenetration and enlargement.
Environment related factors	1. pH	pH affected the surface charge for both the polymers and the mucus. If pH is higher than the p*K* of the polymer, it will be mainly ionized; if the pH is lower the p*K* of the polymer, it will be mainly unionized.
	2. Applied strength	When placed the mucoadhesive system it is important to defined the strength.
	3. Initial contact time	The initial contact time rises due to the mucoadhesive strength.
	4. Swelling	The presence of water and concentration of the polymers are essential in swelling.
Physiological variables	1. Mucin turn over	The residence time is restricted when the mucin turned over in the system.
	2. Disease state	The disease state changes mucus turn over (e.g., bacterial and fungal infections, cystic fibrosis, gastric ulceration, ulcerative colitis, inflammation the eyes).

Factors affecting mucoadhesion are summarized in figure (6)

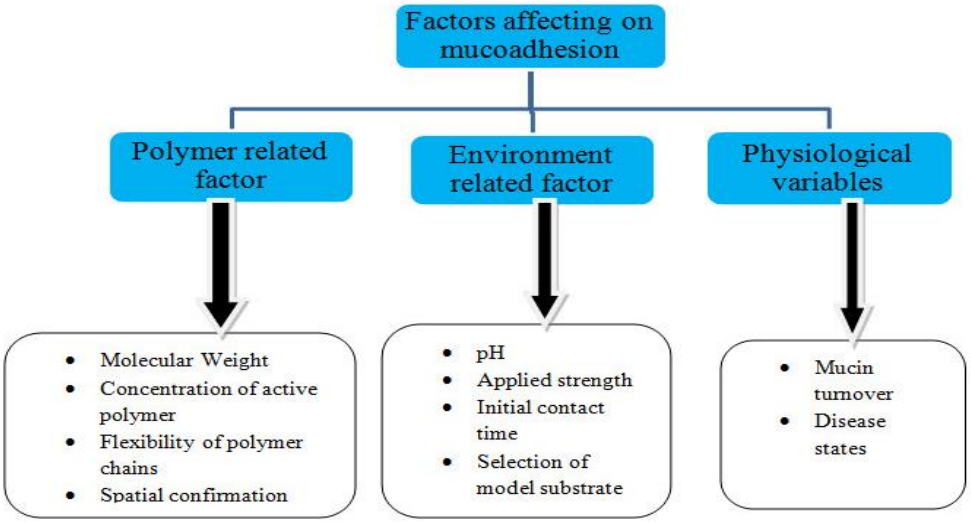


Figure 6: Factors Affecting Mucoadhesion (Banya, Joshi,2021)

2. Ideal Characteristics of Mucoadhesive Polymers

The presence of the polymers is important in order to promote the adherence between the active pharmaceutical ingredient to the mucus membrane, thus the agents should have these features as swelling to control later the disintegration in the saliva.

1. The adhesiveness between the polymer and the mucus surface need high molecular weight up to 10000 or more.
2. Long chain polymer to assist the interpenetration and enlargement but shouldn't be as too long because that disserve the diffusion.
3. Viscosity is high.
4. Cross linking degree and this will impact on the mobility of the chain and the resistance to dissolution. In general swelling need the existence of water and favored the controlled release dosage forms.

5. Conformation of spatial.
6. Polymer chain flexibility which plays role in interpenetration for both the agent and the mucus surface.
7. Polymer concentration which is correlates proportional with mucoadhesive strength also it depends on the dosage form.
8. Degree of ionization and charge will influence on the charge of the mucoadhesion. Hence the mucoadhesion strength can be allocated as anion>cation>non-ionic.
9. The hydration is optimum because the excessive hydration reducing the mucoadhesive strength.
10. Optimum pH.
11. The polymers should be biocompatible, nonirritant, nontoxic, nonabsorbable and favored be biodegradable.
12. The cost shouldn't be high to remain in competitive so choose the economic polymer(Mahajan et al. 2013) (Shaikh et al. 2011).

3. Mucoadhesive Polymers Types

Mucoadhesive Polymers Can be Broadly be Classified as Follow:

Natural polymers: Tragacanthin, Sodium alginate, Guar gum, Xanthan gum, soluble starch, Gelatin, Chitosan.

Synthetic Polymers:

1. Cellulose derivatives (Methylcellulose, Ethyl cellulose, Hydroxyl Ethyl cellulose, Hydroxyl propyl cellulose, Hydroxy propyl methylcellulose, Sodium carboxy methylcellulose)
2. Poly (Acrylic acid) polymers (Carbomers, Polycarbophil).
3. Poly hydroxyl ethyl methyl-acrylate.
4. Poly ethylene oxide.
5. Poly vinyl pyrrolidone.
6. Poly vinyl alcohol(Mahajan et al. 2013).

Mucoadhesive has been considered to be used as different polymers classes. A good example of a mucoadhesive is Polyacrylic acid. PAA is co-polymerized with poly (vinyl pyrrolidone) (PVP) or polyethylene glycol (PEG) to enhance these characteristics. So, when these polymers are used, they enhance the delivery of drugs through the buccal mucoadhesive system.

Devices

A lot of laminated devices have been expanded to accomplish sustained drug release.

Devices Classification:

• **Monolithic:** It is the system that dissolves or disperses a drug in a polymer diffusion system from a polymer-drug mixture to determine the overall drug flow rate from the device.

• **Reservoir or (membrane):** is a system which controls the rate of general drug release by diffusional resistance via a polymeric membrane (Mahajan et al. 2013). This improves mucoadhesive characteristics of the thiolate polymers (e.g., tensile strength enhancement, highly cohesive properties and fast wetting and swelling by taking up water. So new generation of bio-adhesive polymers is formed (Khairnar and Sayyad 2010).

1.1.3. Classification of Drug Delivery Via the Membranes of the Oral Cavity:

1. Sublingual drug delivery system is what supplies us with the drug at the end of the mucosal membrane that covers the bottom layer of the mouth to the blood circulation
2. Buccal drug delivery system is what supplies us with the drug at the end of the mucosal membrane to the blood circulation by setting the drug in the middle of the cheek and gums.
3. Local drug delivery system transfers the drug in the mouth (Madhavi et al. 2013).

The schematic diagram of sublingual and buccal region as figure no. (7) is showed the comparison between two regions.

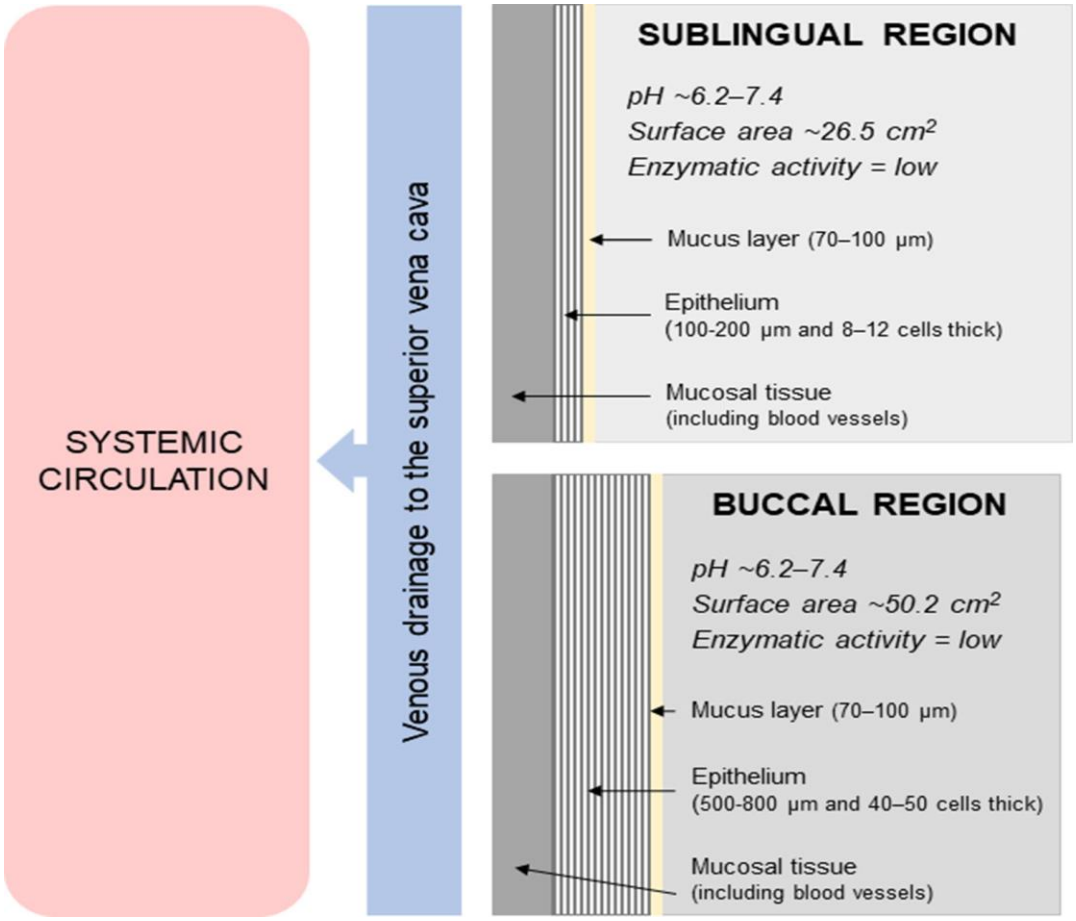


Figure 7: Schematic Diagram of Sublingual and Buccal Region (Hua 2019).

1.1.4. Advantages of Mucoadhesive Drug Delivery Systems

Mucoadhesive delivery systems offer many advantages over other oral controlled release systems by virtue of the prolongation of residence time of drug in gastrointestinal tract (GIT) and at local drug therapy.

- Targeting and localization at a specific site of the dosage form, moreover, excellent approach so onset of action is rapid.
- Thus, the mucoadhesive systems are known to provide intimate contact between dosage forms and the absorptive mucosa region, resulting in high drug flux at

the area of an absorption tissues (Shaikh et al. 2011) (Boddupalli, et al. 2010) , also enhance the release of drug at the site of action so improve the local and systemic effects ,thus increase the bioavailability (Mahajan et al. 2013).

- Improvement of patient compliance
- Protection the drug from degradation in the acidic environment in gastrointestinal tract.
- Enormous blood supply that leads to good blood flow rates and enhancement of absorption (Mahajan et al. 2013).

1.1.5. Disadvantages of Mucoadhesive Drug Delivery Systems

- Ulcerous effects due to prolonged contact time from drugs which have ulcerogenic properties.
- Prevent drinking and eating,
- Accept the patient in terms of the irritancy and tasty.
- One of the main limitation in the development of the oral mucosal drug delivery is the lack the in vitro model screening to know which drugs are suitable for administration (Mahajan et al. 2013).

1.2. Buccal Drug Delivery System

Relevant anatomical features of buccal mucus membrane represented by surface area around 30 cm^2 contains three distinct layers, epithelium, basement membrane, and connective tissues. Also, some of these layers are soft like sublingual and others are hard plates keratinized like gingival and the thickness of

the epithelium in the range of 500–800μm and 40–50 cells thick. Thus, the mucus is secreted from salivary glands that form the saliva forming a (0.1–0.7) mm thick layer. The time of turnover is about 5-6 days. Oral mucosa permeability barrier property in the intercellular materials is derived from membrane-coating granules (Shaikh et al. 2011)

Buccal drug delivery can have two different therapeutic goals either local therapy in the oral mucosa (e.g., antimycotics, antiviral agents, local anesthetics or corticosteroids) or systemic treatment (e.g., normally large proteins, unsteady proteins, peptides polysaccharides or oligonucleotides, as accurately as normal small drug molecules). Buccal mucosa gives a number of advantages in comparison with other routes of drug administration. It has a rich blood supply that flows directly into the jugular vein and therapy, sparing the drug from first-past metabolism of the liver and GI degradation tract by enzymatic degradation. The schematic diagram of buccal mucosa is obtained as figure no. (8)

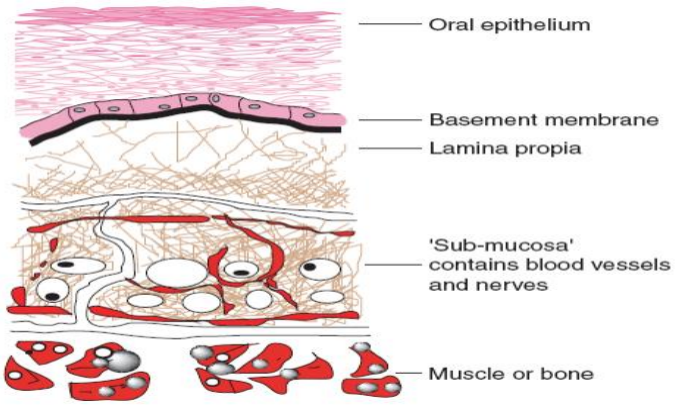


Figure 8: Schematic Diagram of Buccal Mucosa (Patel, Liu, and Brown 2011).

For prolonging the residence time, we can alternate the conventional dosage forms like oral gels, oral liquids or lozenges with buccal mucoadhesive such as patch. So, that can be made by put polymers and excipients that improve solubility and overcome the low permeability of buccal mucosa. Additionally, it is often laminated by a water impermeable backing layer to inhibit release of drug and release of the saliva (when required) and the convenience of patient.

Through the buccal drug delivery system, the medicine immediately passes through blood circulation so give faster onset of action when compared with conventional oral drug formulation, easily administered without pain, short enzymatic activity (e.g., salivary amylase) due to non -adherent saliva and less mucin in buccal region, little hepatic metabolism and vigorous bioavailability. The buccal mucosa covers the internal cheeks and buccal dosage form which is set in the oral cavity between the superior gums and the cheeks to heal systemic and local situations. In general, bucco-adhesive DDS is very useful in drug having short half-lives, poor permeability and for solubility of drugs that are influenced with the decomposition from enzymatic degradation. Moreover, slow disintegrating or non- disintegrating allow to sustained and prolonged effects for these types of drugs also these could be more stable in the oral cavity which owning pH neutral in contrast to other parts of gastrointestinal tract. All of these advantages give safety and ease of administration, and in case of emergency (Gupta et al. 2021) (Hua 2019).

The buccal region within the oral mucosal cavity has an attractive site for drug administration. The buccal region is less permeable than the sublingual but it is the preferred route for transmucosal drug delivery system because it is immobile

mucosa and smooth muscle, thus it considered the suitable for sustained of drug delivery system (Mahajan et al. 2013).

1.2.1. Fundamental Components of Buccal Drug Delivery System:

a. Medication

b. Bio-adhesive polymer

c. Permeation enhancers

1.2.2. Advantages of Buccal Drug Delivery System

1. Administration of the drug without effort and with ease in emergency cases.
2. Release of the drug for long as possible.
3. In cases of injury, unconsciousness and uncooperative patient.
4. Drug has excessive bioavailability because it bypasses hepatic metabolism thus high pharmacological effects.
5. Some drugs are unstable in acidic environment of stomach, so this route can be used.
6. Passive diffusion is the technique of drug absorption.
7. The high rate of absorption occurs as a result of direct contact with the surface of the absorbing membrane.
8. Quick onset of action.
9. Quick cohesion and adhesion to buccal mucosa tissues.
10. Flexibility in size , shape , surface and physical state (Pawar et al. 2013) (Hanif, Zaman, and Chaurasiya 2015).

1.2.3. Disadvantages of Buccal Drug Delivery System:

1. Inconvenient for patients have technical procedure to keep the drug in buccal region without swallow it.

2. Not all the drug types can be delivered by this route of administration but only small quantity can be provided.

3. Buccal administration not suitable with extended release that take long time so this uncomfortable in case of drinking or eating.

4. Drug may bitter, unpalatable or may cause irritation so make swallowing and adhering to gastrointestinal parts (e.g., esophagus) or may cause expulsion of medication.

5. The risk of aspiration of medication is low although there is a chance to cause this problem so preferred to set the patient as upright position (Hua 2019).

1.2.4. Ideal Drug Candidates for Buccal Drug Delivery System:

1. Molecular size – 75-600 Daltons.
2. Molecular weight between 200-500 Daltons.
3. Drug should be lipophilic or hydrophilic in nature.
4. Stable at buccal pH.
5. Taste.
6. Drug should be odorless.
7. Drugs which are absorbed only by passive diffusion should be used (Pawar et al. 2013).

1.2.5. Physiological Factors Influencing Buccal Drug Delivery

There are several physiological factors must be considered when design and development the buccal route of administration and these factors may affect drug bioavailability, stability, efficacy and safety.

- Residence time of the formulation: The residence time influence highly on the absorption in the buccal regions. These could range according to the patient and the formulation of drug. In terms of need to the disintegration and dissolution is vary between the formulations previous the drug absorption. Additionally, patients must prohibit drinking, chewing, eating or swallowing until absorption of the drug is done. The effectiveness of the medication will decrease due to swallowing and this especially affects hard to some patients including younger children.
- Drug absorption: For achieving efficacious absorption, the drug needs the balance between hydrophilic and lipophilic properties. This includes the drug soluble in aqueous fluids of buccal area and has high solubility across epithelium membrane which is occurred by passive diffusion. Moreover, drugs with low to medium molecular weights are suitable to this route. As well many factors influence on drug absorption such as open sores or exist of inflammation, also smoking conversely decrease drug absorption because the blood vessels vasoconstriction.
- Saliva pH: Drug absorption depends on pH of the saliva by influencing the ionization state. Depending on physiochemical properties of drugs and the molecules submit passive absorption via transcellular (through the cells) or

paracellular diffusion (between cells). The most common pathway is transcellular diffusion and this proportional to the lipid solubility drug which is in the non-ionized form so absorption is better than the ionized form. The drugs with high pka values have relatively neutral pH of the saliva so this is favored for absorption in buccal administration. In contrast, the paracellular pathway the drugs with hydrophilic and ionized form molecules are preferred. It should notice that the buccal absorption is affected by pH of the saliva which is altered provisionally in case such as personal factors (e.g., oral disease) or environmental (e.g., drinks and foods).

- Saliva flow: Adjusting the disintegration rate and dissolution of the medication by flow of the saliva so influence on buccal administration such as in the case dry of mouth so this gives negative effects. Conversely, if flow of the saliva is significant this leads to swallowed before absorption occurred. There are factors can be affected on the saliva flow ,for example , age , medications (e.g., anticholinergic drugs) and medical conditions (e.g., glossodynia, Sjogren's syndrome, dysphagia ,cheilosis, dehydration and problems with mastication)(Hua 2019).

1.2.6. Buccal Bioadhesive Semisolid Dosage Forms

These pharmaceutical forms include polymers, either natural or synthetic, in the form of a powder, which is disperse in polyethylene or an aqueous solution such as Arabase.

Buccal delivery of the desired drug uses polymers by receiving the anatomy and physiology, in addition to the characteristics and specifications of the required drug and the mechanism of permeation (Salamanca et al. 2018). All of these factors prolong contact time in the site of administration; so they improve the performance of the desired drug (Lee, Park, and Robinson 2000).

1.2.7. Evaluation of Buccal Delivery Systems

The tests that are made for buccal adhesive depend on the types of dosage form, for example the tablets evaluation tests like hardness friability, dissolution, etc. Each patch and film need tensile strength and hygroscopicity, etc. As for ointments and gels we need viscosity, in vitro release and permeation ,etc. (Reddy, Chaitanya, and Rao 2011).

1.2.8. Buccal Drug Delivery and Mucoadhesivity

For the development of buccal DDS, we use recent devices called mucoadhesive which are used for connecting the mucin biological membrane with materials to achieve suitable route of administration. So the polymers of mucoadhesive have some features such as: Hydrogen bond groups with anionic hydrophilicity, appropriate mucosal surface for wetting and swelling and flexibility to penetration into tissues (KV et al. 2010).So the recent devices by using polymers with these properties can give us development in various dosage forms either solid or semisolid dosage forms in treating some diseases like oro-mucosa disorder ,etc. (M. Sharma et al. 2020).

The dosage forms that reached the advanced clinical trials from buccal drug delivery system is few. The alternative strategies to conventional dosage forms occurred by using the mucoadhesive polymers or permeation enhancers. Often physiological factors as saliva and swallowing influence the conventional dosage forms so reduce the contact time of adhesion so unpredictable of absorption. So, the improvement of mucosal retention time and/or permeability thus increase the absorption for these traditional dosage forms. For example, of these enhancement materials permeation enhancers (e.g., surfactants, chelator agents, fatty acids, bile salts, cyclodextrins) all that happened by: (i) changing the rheology of mucus; (ii) increasing the lipid bilayer membrane fluidity; (iii) achieving the ingredients at tight junctions; (iv) reducing the enzymes of mucosal; and (v) elevating the drugs thermodynamic activity. Additionally, the integration of mucoadhesive polymers also improvement the formulation so increasing the adhesion time in this area. Essentially, this has been done for solid dosage form and semisolid dosage forms which includes natural polymers (e.g., hyaluronic acid, various gums and agarose) or synthetic polymers (e.g., cellulose derivatives). For solid dosage forms as (tablets, patches, films) place impermeable baking layer to permit unidirectional drug delivery (Hua 2019)

1.2.8.1. Mucoadhesive Dosage Forms for Buccal Administration

General Consideration in Dosage Form Design

- **Physiological Aspects**

  The amount of saliva is delivered across the oral cavity and the short residence time that equals<5-10 minutes. To overcome these problems, we need

to design buccal dosage form by preparing a formulation containing an adhesive material to adhere on buccal areas in the oral cavity and to prolong it in the site of administration (Salamat-Miller, Chittchang, Johnston 2005).

- **Pathological Aspects**

Many diseases that occur in the oral cavity alter the thickness of epithelium tissues. So, this would change the properties of the barrier. Some diseases and treatments may change the amount of mucus secretion and the surface mucosa alteration. All of these reasons help the researcher to creation design buccal adhesive dosage form. In addition, the drugs that potentially change the physiological conditions in the oral cavity are not suitable for buccal delivery system (Salamat-Miller, Chittchang, Johnston 2005).

- **Pharmacological Aspects**

Buccal dosage form may be designed to enter systemic circulation or to go to local treatment. This design is determined by several factors, for example, characteristics and specifications of the drug, selection of the suitable dosage form intended for application, base site of action and site to be treated like (periodontal pocket, gingival, teeth buccal mucosa or systemic) (Salamat-Miller, Chittchang, Johnston 2005).

- **Pharmaceutical Aspects**

Regardless of the types of dosage form, drug should be released from the delivery system. Therefore, the drug is taken up by oral mucosa. Some polymers are used for enhancing the solubilization of poorly soluble drugs in water like Tinidazole. Other factors affect drug release and penetration across the buccal

mucosa which should be regard in designing the formulation. The permeability characteristics of buccal mucosa is continually changed by the rapid turnover (3-8 days compared to 30 days for skin) (Salamat-Miller, Chittchang, Johnston 2005). The permeability of the buccal mucosa is predicted to be 4–4000 times higher than the skin (Hanif, Zaman, and Chaurasiya 2015). The permeability in buccal oral mucosa is less, thus less absorption and poor bioavailability. When adding permeation enhancers to formula, we can improve the permeation and absorption, giving good bioavailability and pharmacological effects.

Beside considering physiochemical properties of the drug, the organoleptic properties should be considered since the buccal delivery systems are to be risky to highly advanced sensory organ (Salamat-Miller, Chittchang, and Johnston 2005).

1.3. Semi-Solid Dosage Forms

1. 3.1. Gel

Gel is a soft, solid or solid-like material consisting of two or more components, one of which is a liquid, present in substantial quantity. Thus the gel includes the cross-links polyacrylic acid that attaches to mucosa surface for extended periods of time to get controlled release characteristic (Pawar et al. 2013). By using the semisolid materials, like gel, scientific researchers widely use this dosage form in antibiotic industries because it gives targeted delivery (Paulsson 2001). When bio-adhesive polymers are in semisolid dosage forms like gel, they give synergistic effects by affecting on drug itself, polymer properties and the biological environment (Phanindra, Moorthy, and Muthukumaran 2013).

1.3.2. Adhesive Gels

Diverse adhesive gels are utilized in sustained release in buccal mucosal membranes. The most important one is acrylic acid which provides cohesive action at the site of application. The novel designed promise prolongs time release, and the system formulation: a poly (hydroxyethyl methacrylate) layer as barrier, poly (methacrylic acid-g-ethylene glycol) as a biosensor and poly (ethylene oxide) to promote muco-adhesion. The limitation of this gel is the inability to deliver measured dose of drug, so it isn't suitable for drugs having narrow therapeutic window (Reddy, Chaitanya, and Rao 2011).

1.3.3. Formulation Aspects of Buccal Gel:

1) Drug Substance
2) Excipients
3) Penetration enhancers
4) Flavoring and coloring agents

1.4. Tinidazole

Tinidazole (1-(2-ethyl sulfonyl ethyl)-2-methyl-5-nitroimidazole) is a nitroimidazole antiprotozoal agent effective against trichomonas vaginalis, entamoeba histolytica and giardia lamia infections. The reduction of nitro group of Tinidazole occurs by cell extracts of trichomonas. The free nitro radical of Tinidazole generated from the reduction is responsible for antiprotozoal activity. A

literature survey found that estimation of Tinidazole by HPLC (Ahmed, Abdelaziz, and Saeed 2019).

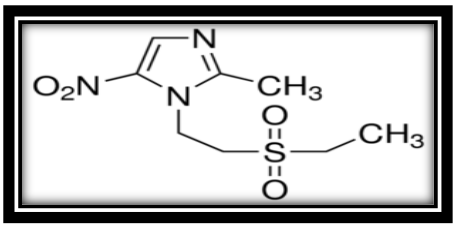


Figure 9: Structure of Tinidazole

Tinidazole has higher bioavailability and higher half-life than metronidazole. Therefore, it is a promising achievement of treatment for periodontitis. On the other hand, preparing oral dosage forms from Tinidazole leads to significant adverse effects in GI tract (Tian, Shen, and Jv 2016).

1.4.1. Tinidazole Specification

The British Pharmacopeia (BP) recommends non-aqueous titration for the estimation of Tinidazole, while USP pharmacopoeia recommends HPLC method and the validation of analytical methods and procedures associated to International Conference Harmonization. Also the characteristics of performance meet the request of analytical applications (Ahmed, Abdelaziz, and Saeed 2019).

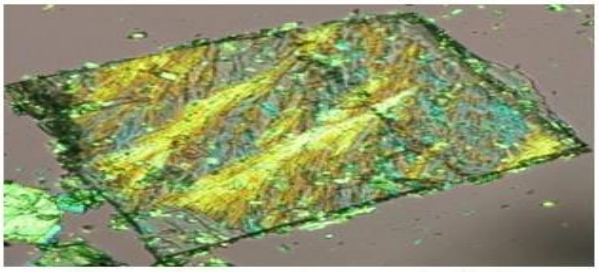


Figure 10 :Microphotograph of Tinidazole (Patnala et al. 2017).

In pharmaceutical preparation and biological fluids there are several analytical methods for the quantitative determination of tinidazole ,for example, high performance liquid chromatography , high performance thin layer chromatography, UV-visible spectrophotometry, derivative UV-spectrophotometry, gas liquid chromatography ,packed column supercritical fluid chromatography, flow injection analysis, voltammetry ,polarography and capillary electrophoresis (Ahmed, Abdelaziz, and Saeed 2019).

Table 3:Physiochemical Properties of TNZ (PubChem 2023b)

Physiochemical properties of TNZ	
Molecular Formula	$C_8H_{13}N_3O_4S$
Molecular Weight	247.27g/mole
Physical Description	Solid
Color/Form	Colorless crystal from benzene
Melting Point	127-128 °C
LogP	-0.35
Log Kow	-0.35

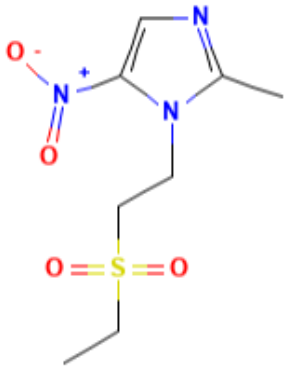


Figure 11: Chemical Structure of TNZ (PubChem 2023b)

1.4.2. Stability of Tinidazole

Tinidazole is considered stable drug substance. When Tinidazole is subjected to forced and extensive degradation was found to occur in alkaline medium, under oxidative stress and in the photolytic conditions. Mild degradation was observed in acidic and neutral conditions. The drug was stable to thermal stress (Bakshi, Monika, and Saranjit Singh. 2004).

1.4.3. Solubility of Tinidazole

The solubility of Tinidazole is practically insoluble in water, soluble in acetone and in methylene chloride, sparingly soluble in methanol. In general Tinidazole class (II) as categorized in BCS which is (low solubility, high permeability) and the solubility is about 0.37 µg /ml in aqueous media (Patnala et al. 2017).

Tinidazole has approximate solubility of 0.2 mg/ml in ethanol, 10 mg/ml in DMSO, 20 mg/ml in DMF and 0.11mg/ml in 1:8 solutions of DMF: PBS (pH 7.2).

1.4.4. Measurement of Dissolution and Drug Release from Bioadhesive Dosage Forms

1.4.4.1. Franz Diffusion Cell

Franz diffusion cell was used for estimating vitro drug permeation and having several advantages, for example, little remediation of tissues, no continuous sample collection and the drug required for analysis is low amount (Salamanca et al. 2018).

Franz diffusion cell is considered one of the most commonly used static designs for studying in vitro permeation. FDC composed from the donor chamber which is called upper chamber and the tested sample is placed through the cell on the membrane sac, the flat ground glass joint is linked with the chamber of the cell and the membrane. The membrane is located horizontally between the donor and the receptor chamber also this semi-permeable membrane may be substituted with animal membrane or any other cellular membrane furthermore all of these parts easy to assemble and handle. The lower part is called receptor chamber which contains the medium that lets the drug to diffuse through it by the magnetic stirrer inside the chamber and the heating plate for keeping the medium at a constant temperature. See figure no (12) (Yadav et al. 2018)

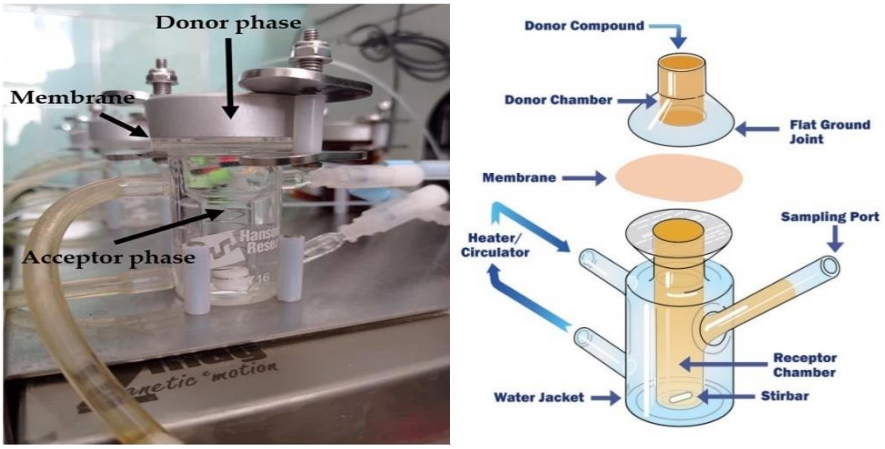


Figure 12: Franz Diffusion Cell Parts (Bartos et al. 2021)

The diffusion cells used are those made of glass, static type, and they include three separate parts. These cells in diffusion are termed static as the receiver compartment is periodically renewed through the tests by sampling liquid and standing a new fluid (Mustapha et al. 2011).

1.5. Excipients

1.5.1. Polymers

1.5.1.1. Carbomer /Carbopol 934P

Carbopol 934P manufactured by crossing linkage of alkyl sucrose or pentaerythritol, it has molecular weight 1x106 - 4x106 and it is viscosity around 29.400-39.400 cp at 25 C^0 also the neutralized aqueous solution is 0.5%. It has pH 2.5-3 and is soluble in water, glycerin and alcohol.

Carbopol 934P is white, fluffy, acidic, hygroscopic powder with a slight featured odor. It is used as mucoadhesive material and excellent thickening, suspending, gelling agent. It is unaltered by temperature and also resistance to bacterial growth moreover may mask the unpleasant taste of the formulation (Mahajan et al. 2013).

1.5.1.2. PVP-K25

PVP-K25 is derived from *N*-vinyl-pyrrolidone which has molecular weight (23,000–32,000 Daltons), it is biocompatible, biodegradable, inert and nontoxic. Moreover, it is hydrophilic polymer and display complex affinity for both hydrophilic and lipophilic drugs. PVP is pH stable and temperature resistant also it is soluble in different solvent polarities and it has good binding effects (Franco and De Marco 2020).

1.5.1.3. Xanthan gum

Xanthan gum is an anionic poly-saccharide arises from fermentation of bacterial plant called Xanthomonas campestris. It has tendency to dissolve in hot glycerin, it is pseudoplastic specially shear-thinning also it has good viscosity in the existence of salt and stable at raised temperature also the viscosity of the solutions

is 1500 to 2500 cp range at 1%. Xanthan gum than other organic gums can handled of electrolytes, acids and bases (Mahajan et al. 2013).

1.5.1.4. Hydroxy Propyl Methyl Cellulose HPMC grades

1.5.1.4.1. Methocel E5

Hydroxypropyl methylcellulose (HPMC) E5 is a cellulose ether that can consider semisynthetic, viscoelastic polymer also the raw material from cotton and wood so it safe for human. It is soluble in cold water and some organic solvents. The characteristic of this compound is tasteless, odorless, white or creamy white fibrous or granular powder. E5 used for increasing viscosity in the formulations (Mahajan et al. 2013).

1.5.1.4.2. Methocel K100M

Hydroxypropyl methyl cellulose (HPMC) K100M is semisynthetic polymer, soluble in cold water and insoluble in alcohol, chloroform and ether, it is stable at pH 3-11 but incompatible in extreme pH conditions and oxidizing substances. It is tasteless, odorless, white or creamy white fibrous or granular powder. The uses in pharmaceutical preparation as bio-adhesive agents and thickeners (Mahajan et al. 2013).

1.5.2. Triethanolamine

Triethanolamine it is oily high viscous liquid with slight ammonia odor which is denser than water density. It used as alkalizing agent that neutralize the pH of material in the formulation so it is not considered as active ingredient and no

authorized indication. It should not exposure to light because it becomes brown (PubChem 2023c) (Iceri et al. 2022).

1.5.3. Co-solvents

1.5.3.1. Polyethylene Glycol

PEG has molecular weight 380 - 420, the USP32-NF express it as polymer of ethylene oxide and water, the grades of PEG (200-600) are liquid. PEG400 is clear viscous liquid, colorless or slightly yellow-colored also has slight odor and bitter material moreover a bit burning taste. The advantageous characteristics of PEG becomes introduce in different fields of pharmaceutical industries e.g., chemical stability, low immunogenicity, high tolerability, hydrophilicity, improving solubility and enhance permeability especially poorly soluble drugs (Derivatives 2022) (Shah et al. 2020).

1.5.3.2. Propylene Glycol

PG is a synthetic compound, colorless with sweet taste, practically odorless and viscous hygroscopic liquid. It is water miscible co-solvent also soluble in acetone and chloroform also acts as organic solvent in many pharmaceutical preparations (Shah et al. 2020) (PubChem 2023a)

1.5.4. Solubilizers / Penetration Enhancers

1.5.4.1. Polysorbate 80

Polysorbate 80 is a synthetic compound consist from fatty acid esters of polyoxyethylene sorbitan, it trades names Tween80. It has molecular weight of

1309 Da and the density is 1.064 g/ml also the appearance is amber colored liquid, moreover has hydrophilic -lipophilic properties (HLB) value (15) and the concentrations micelles formation over the critical micelles concentration of 0.01% (weight/volume) which it is used as solubilizer ,emulsifier and surfactant in many pharmaceutical formulations ,foods and cosmetics (Schwartzberg and Navari 2018) (Hassan 2015).

1.5.4.2 . Cremophor RH40 (Kolliphor RH40)

Cremophor RH40 (Polyoxyl 40 hydrogenated castor oil) has molecular weight 853.91 g/mole, it is non anionic emulsifying agent, solubilizer and penetration enhancer in order to solubilize the hydrophobic active ingredient in aqueous and the alcohol solvents which has hydrophilic -lipophilic balance (HLB) value (14-16) and the critical micelle concentration (CMC) 9mg/ml.

This substance forms “ex tempore” small (10-30nm) to coat the particles physically in order to solubilize them (Katona et al. 2022).

1.5.4.3 . PPG 15 Stearyl Ethers (Arlamol)

Polypropylene Glycol it is also known as Polyoxypropylene 15 Stearyl Ether. It is characteristic are clear, pale yellow, oily liquid. It is used in the formulations as surfactant, emulsifier and solubilizer agent used to enhance the solubility and permeation. It safe for use and nonirritant substance (Lanigan 2001) (Bergfeld et al. 2022).

Chapter Ⅱ: Problem Statement and Objectives

2.Problem and Objectives

2.1. Problem Statement

Tinidazole is used for the treatment of Amoebiasis, Giardiasis, and Trichomoniasis. In addition to its action on protozoans, it has bactericidal action against anaerobic bacteria. Tinidazole offers selective bactericidal activity against anaerobic bacteria that makes it of interest against periodontopathogen infections and other disorders.

Tinidazole is practically insoluble in water. A BCS Class II drug has low solubility and high permeability, and is marketed as prescription, oral tablet dosage form in 250mg and 500mg strengths. Most Tinidazole preparations for oral dosage forms lead to low concentration of Tinidazole in periodontal pocket and serious adverse reactions in gastrointestinal tract. Therefore, we will develop mucoadhesive gel with suitable and compatible excipients.

The introduction of mucoadhesive gel as buccal drug delivery by avoidance of first-pass metabolism and intermediate permeation properties makes it attractive for drugs which are sensitive to pH and enzymatic degradation in stomach so buccal delivery system is a promising new dosage form to increase the residence time in epithelium tissue, thus increasing the drug absorption which is an important factor in bioavailability. It achieves the desired pharmacological effects for low solubility drugs like Tinidazole.

2.2. Objectives

- Solubilization of Tinidazole in water by using the concept of mixed solvency for formulations purposes.
- Adoption of analytical test method like HPLC/UV for the accurate determination of Tinidazole in solution and semi-solid dosage form.
- Formulation of Tinidazole mucoadhesive gel as buccal drug delivery system.
- Evaluation of the mucoadhesive Tinidazole gel with respect to organoleptic properties, content, viscosity and pH.
- Studying the permeation behavior and release of the Tinidazole gel through synthetic membrane, dialysis membrane, Chicken eggshell membrane, Permeapad® membrane by utilizing Franz diffusion cell.
- Using chemical penetration enhancers to optimize the permeation of buccal mucoadhesive gel
- Studying the stability of the chosen preparation formulae at a long term and accelerated storage conditions.
- Analyzing data to control the quantity of drug that penetrates the membranes during prior experiments.
- Report the results of my analysis in my honor thesis.

Chapter Ⅲ: Research Methodology

3. Research Methodology

3.1. Equipment and Materials

3.1.1. Equipment and Tools in the Experiment

The equipment used in the current research are shown in table (4)

Table 4: Equipment and Tools Used in This Study

No.	Name item of Equipment /tool	Source/Model	Purpose
1.	HPLC	Agilent Technologies (1200 Series)	Analysis
2.	UV spectrophotometer	PerkinElmer, Lambda 25	Analysis test
3.	Franz diffusion cell	Orchid Science, Model no. FDC-06	Diffusion and permeation study
4.	Sonicator bath	Elma, S 300H, Elma Sonic	Solubilization and degassing
5.	pH Meter	HANNA instruments (pH/ORP mete)	Analysis, formulation &pH adjustment
6.	Rotary Viscometer	From Jerusalem Pharmaceuticals company	Analysis of viscosity
7.	Hot Plate stirrer	Fried Electric Model MH-4	Analysis & formulation
8.	Caliper	From Samih Darwazah Institute	Analysis, effective surface area determination &thickness measurement
9.	Volumetric flasks &beakers	Class A	Analysis
10.	Analytical balance	METTLER TOLEDO balance (5 digits), OHAUS®	Weighting &analysis
11.	Magnetic stirring bar	From Jerusalem pharmaceuticals company	Analysis &mixing

No.	Name item of Equipment /tool	Source/Model	Purpose
12.	Column: BDS Hypersil (C8,250*4.6mm, 5μm) Thermo scientific Part #: 28105-154630)	Thermo scientific Part #: 28105-154630)	Analysis
13.	HPLC vials with caps (500 vials and caps)	From Jerusalem pharmaceuticals company	Analysis
14.	1 ml syringe disposable (100 syringe)		Analysis
15.	Humidity champers at 40 ºC	Firlabo	Stability &compatibility study
16.	Humidity champers at 30 ºC	Biolab 30 /walking chamber	
17.	Refrigerator	beko®	Analysis, stability and storage
18.	Computer	HP Elite Desk 705 G2MT	For data collection and data analysis
19.	Volumetric & graduated pipettes (different sizes)	Class A	Formulation
20.	Micropipette	KIRGEN®	Formulation &analysis
21.	Plastic droppers& Plastic dishes	From Samih Darwazah Institute	Formulation
22.	Disposable Syringes		Formulation
23.	Flexible needles and syringes		Diffusion studies
24.	Para film	Bemis	Formulation and permeation test

No.	Name item of Equipment /tool	Source/Model	Purpose
25.	Thermometer	From Samih Darwazah Institute	Temperature measurement
26.	Glass slides	From Samih Darwazah Institute	Residence time test
27.	Tweezer	From Samih Darwazah Institute	Diffusion studies
28.	Centrifuge	Hermle/Amie	Centrifugation
29.	Polyamide membrane 0.45 µm	SUPELCO®	Release studies
30.	Dialysis membrane	Sigma-Aldrich	Release studies
31.	Chicken eggshell membrane	Chicken egg	Permeation studies
32.	Permeapad® membrane	InnoME GmbH/ Germany	Permeation studies

3.1.2. Formulation Materials and Reagents

All formulation materials used in this research were of pharmaceutical grade and described in table no. (5)

Table 5: Ingredients and their functions used in gel formulation

No.	Name of Component	Grade	Source	Function
1.	Tinidazole	BP/USP	Aarti drugs limited/India	Active Pharmaceutical Ing.
2.	Carbomer (Carbopol 934P)	BP/USP	Lubrizol	Gelling agent / mucoadhesive

No.	Name of Component	Grade	Source	Function
3.	PVP-K25	BP/USP	Zhongbao Chemicals Co. Ltd	Mucoadhesive
4.	Methocel K100M	BP/USP	Colorcon Limitd/DuPont de Nemours, Inc	Polymer
5.	Methocel E5	BP/USP	Colorcon Limitd/DuPont de Nemours, Inc	Polymer
6.	Xanthan gum	BP/USP	Zhongbao Chemicals Co. Ltd	Gelling agent
7.	Triethanolamine	BP/USP	Idesa Petroquimico	Alkalizing agent
8.	PEG 400	BP/USP	Dow Europe Co.	Co-solvent
9.	Propylene glycol	BP/USP	Dow Chemical's Co.	Co-solvent
10.	Polysorbate 80	BP/USP	Eicenmann &Veronelli	Solubilizer / Penetration enhancer
10.	Cremophor RH40	BP/USP	Sigma-Aldrich	Solubilizer / Penetration enhancer

No.	Name of Component	Grade	Source	Function
11.	PPG 15 Stearyl Ethers	BP/USP	Croda Europe Limited	Solubilizer / Penetration enhancer
12.	Purified Water	BP/USP	Jerusalem pharmaceuticals company	Vehicle

3.1.3. Analytical Materials

All reagents used were of analytical grade, table no. (6)

Table 6: Materials and reagents used in the current research

No.	Analytical material	Grade	Source	CAS No.
1.	Water Bidistilled	HPLC grade	Jerusalem Pharmaceuticals company	7732-18-5
2.	Tinidazole working standard	Analytical standard	Aarti drugs limited/India	19387-91-8
3.	Potassium dihydrogen monobasic phosphate	Analytical Grade	Merck KGaA	7778-770
4.	Sodium hydroxide	Analytical Grade	Merck KGaA	1310-73
5.	Hydrochloric Acid 37%	Analytical Grade	Merck KGaA	7647-001-0
6.	Methanol	Analytical Grade	J.T. Baker	67-56-1
7.	Sodium acetate	Analytical Grade	Merck KGaA	127-09-3

3.2. Solubilization of Tinidazole in Water by Using the Concept of Mixed Solvency

The concept of mixed of solvency based on enhancement the water solubility of poorly water dugs as Tinidazole with amount 1g, so employing this concept to add safe and efficient co-solvent to give enhanced solubility. The mixed of solvency may be used to set the concentrated mixture from each co-solvents, for example from Polyethylene glycol 400 as co-solvent and propylene glycol as wetting agent (Solanki, Soni, and Maheshwari 2013).

The technique is done by putting the sample vials of PEG 400, PG and also vials contains mixture of them as ratio (50:50) in refrigerator for one week to investigate the crystals formation.

3.3. Formulation of Tinidazole Mucoadhesive Gel as Buccal Drug Delivery System

3.3.1. Compatibility Studies (Drug-excipients interaction studies)

The study will focus on the compatibility of Tinidazole with excipients. Binary mixtures with ratio (1:1) will be prepared and the samples will be kept at ambient temperature at 25 °C / 60% RH and at 40 °C / 75% RH in closed samples.

Primary Packaging of samples**:** Glass Vial Type (I) with Rubber Closure and Aluminum Cap.

The procedure of Analysis of the Compatibility Study

The analysis was done by HPLC test method and the standard solution was prepared in methanol in 0.2mg/ml Tinidazole, while the test preparation was done by

dissolving the equivalent of 50mg of Tinidazole in 10 ml of methanol, then making the dilution (1ml to 25ml).

The samples were tested for assay, appearance, and degradation products at initial and after one-month storage as indicated in table (7).

Table 7: Compatibility study of TNZ at zero-time and after one-month at 25 ˚C and 40 ˚C

Components		**Initial at RT (Zero time)**	**One-month**	
			25 ˚C + 60%RH	**40 ˚C + 75%RH**
Tinidazole		Y	Y	Y
TNZ	Carbopol 934P	X	Y	Y
TNZ	TEA	X	Y	Y
TNZ	PEG 400	X	Y	Y
TNZ	PG	X	Y	Y
TNZ	Water	X	Y	Y
TNZ	PVP (Grade K25)	X	Y	Y
TNZ	Xanthan gum	X	Y	Y
TNZ	Methocel K100M	X	Y	Y
TNZ	Methocel E5	X	Y	Y
TNZ	Tween 80	X	Y	Y
TNZ	Cremophor RH40	X	Y	Y
TNZ	PPG 15 SE	X	Y	Y

Y: To be tested, X: not tested

3.3.2. Formulation Trials

3.3.2.1. General Formula of Mucoadhesive Gel

The components and their concentrations are described in table no. (8)

Table 8: Components of General Tinidazole Mucoadhesive Gel

Component	**Concentration w/w**
Tinidazole	1%
Gelling / mucoadhesive agent	0.2 - 2%
Alkalizing agent	0.5 -1%
Co-solvent/ wetting agent	5-60%
Solubilizer / Penetration enhancer	0.3-5%
Purified Water	Up to 100%

3.3.2.2. Method of Preparation

a. Put wetting agent in an appropriate beaker.

b. Distribute the gelling agent in wetting agent.

c. Heat up 90% of water to 85 ºC.

d. Insert the content of step [c] to the beaker in step [a] and blend until the dissolution is completed [Solution A].

e. With another acceptable beaker, dissolve Tinidazole in Co-solvent then add penetration enhancer agent [Solution B].

f. Add [Solution B] to [Solution A]. Mix till a plain gel is gained, then cool the mixture at room temperature.

The vehicle pH value is an agent to be regarded in the estimation of drug penetration from gels via membranes or buccal tissues (Kushla GP, Zatz JL. 1991). Therefore, gel formulations were calibrated to pH 6.2–7.6.

Table 9: Composition of Mucoadhesive Gel Formulations with Various Polymers

Component	**Formulations (% w/w)**				
	F1	**F2**	**F3**	**F4**	**F5**
Tinidazole	1	1	1	1	1
Carbopol 934P	0.5	0.5	0.5	0.5	0.5
TEA	0.5	0.5	0.5	0.5	0.5
PEG 400	5	5	5	5	5
Propylene glycol	5	5	5	5	5
PVP K25	2	2	2	2	2
Xanthan gum	-	0.2	0.5	-	1
Methocel E5	-	0.5	1	1	2
Methocel K100M	-	1	1.5	2	2
Purified water	86	84.3	83	83	81
Total weight	100	100	100	100	100

3.3.2.3. Formulation of Mucoadhesive Gel

Depending on the residence time of the mucoadhesive gel two formulations are selected for further studies:

3.3.2.3.A. Formulation of mucoadhesive gel without penetration enhancers

The components of F4 mucoadhesive gel without PEs are described in table no. (10)

Table 10: F4 Mucoadhesive Gel without PEs

Component	**Percent (w/w)**	**Qty (grams)**
Tinidazole	1%	1
Carbopol 934P	0.5%	0.5
TEA	0.5%	0.5
PEG 400	5%	5

Component	Percent (w/w)	Qty (grams)
Propylene glycol	5%	5
PVP K25	2%	2
Methocel E5	1%	1
Methocel K100M	2%	2
Purified water	83%	83
Total weight	100%	100

The components of F5 mucoadhesive gel without PEs are described in table no. (11)

Table 11: F5 Mucoadhesive Gel without PEs

Component	Percent (w/w)	Qty (grams)
Tinidazole	1%	1
Carbopol 934P	0.5%	0.5
TEA	0.5%	0.5
PEG 400	5%	5
Propylene glycol	5%	5
PVP K25	2%	2
Xanthan gum	1%	1
Methocel E5	2%	2
Methocel K100M	2%	2
Purified water	81%	81
Total weight	100%	100

In mucoadhesive Gel formulas F4 and F5 the concentrations of Tinidazole and other excipients are the same but the difference in the polymer concentrations and F5 formula contains 1% xanthan gum while F4 without it.

3.3.2.1.B. Formulation of optimized mucoadhesive gels with different penetration enhancers

The component of F4 mucoadhesive gel with different PEs are described in table no. (12)

Table 12: F4 Mucoadhesive Gel with Different PEs

Component %(w/w)	**F4 with Tween 80 (F4A)**	**F4 with Cremophor RH40 (F4B)**	**F4 with Arlamol (F4C)**
Tinidazole	1	1	1
Carbopol 934P	0.5	0.5	0.5
TEA	0.5	0.5	0.5
PEG 400	5	5	5
Propylene glycol	5	5	5
PVP K25	2	2	2
Methocel E5	1	1	1
Methocel K100M	2	2	2
Tween 80	0.3	-	-
Cremophor RH40	-	0.3	-
PPG 15 SE (Arlamol)	-	-	0.3
Purified water	82.7	82.7	82.7
Total weight	100	100	100

The component of F5 mucoadhesive gel with different PEs are described in table no. (13)

Table 13: F5 Mucoadhesive Gel with Different PEs

Component% (w/w)	**F5 with Tween 80 (F5A)**	**F5 with Cremophor RH40 (F5B)**	**F5 with Arlamol (F5C)**
Tinidazole	1	1	1
Carbopol 934P	0.5	0.5	0.5
TEA	0.5	0.5	0.5
PEG 400	5	5	5
Propylene glycol	5	5	5
PVP K25	2	2	2
Xanthan gum	1	1	1
Methocel E5	2	2	2
Methocel K100M	2	2	2
Tween 80	0.3	-	-
Cremophor RH40	-	0.3	-
PPG 15 SE (Arlamol)	-	-	0.3
Purified water	80.7	80.7	80.7
Total weight	100	100	100

3.4. Evaluation of the Mucoadhesive Tinidazole Gel

The buccal mucoadhesive gel is evaluated through the following characters:

3.4.1. Visual Description

Put about 2 g of gel on to a white dry and clean paper, then examine it visually (Sulayman 2011) (Fong Yen et al. 2015).

3.4.2. Identification Test (HPLC)

In the assay method, the retention time of the major peak in the chromatogram of the test preparation corresponds to that in the chromatogram of the standard preparation.

3.4.3. Viscosity Testing

Use rotary viscometer, equipped with T-spindle will be used to measure the viscosity at 20-25 ºC (Tian, Shen, and Jv 2016) (Sulayman 2011) (Wróblewska et al. 2020).

3.4.4. pH measurement

Mix up 10 g of gel with 90 ml of distilled water. Apply a calibrated pH meter with combined glass electrode. Insert the electrode into the solution, and record the readings (Sulayman 2011) (Wróblewska et al. 2020) (Maru et al. 2012).

3.4.5. Assay of Tinidazole

The concentration of Tinidazole gel is determined by reverse phase HPLC at wavelength (310 nm) and by using methanol HPLC Grade, column: BDS Hypersil C8, (250 mm X 4.6 mm ,5 um), (Thermo scientific Part #: 28105-154630). The column is kept at 30 ºC, the flow rate is 1.5 ml/min and the injection volume is 10µL. Record the responses (Tian, Shen, and Jv 2016) (Sulayman 2011) (Wróblewska et al. 2020). Calculate the assay (%) as in equation below:

$$\text{Assay (\%)} = \frac{\text{Area Test} \times \text{Conc. STD}}{\text{Area STD} \times \text{Conc. Test}} \times \text{Potency of STD}$$

3.5. Residence Time, *in-vitro* release and permeation of mucoadhesive Tinidazole gel through membranes

3.5.1. In Vitro Residence Time Study

3.5.1.1. Preparation of Rabbit Buccal Mucosa

In this study the buccal mucosa was used as a barrier membrane. Buccal tissue was applied from regional slaughter house from a freshly sacrificed rabbit and utilized immediately after sacrifice, then transferred to veterinary laboratory and the tissues were cut off and trimmed precisely from the sides then saved in PBS pH 7.4 upon collection to wash them. Epithelium was separated carefully from connective tissue, muscles and fat by surgical blade and scalpel so the buccal mucosa isolated from the underlying tissues and that described as shown in figure no. (13). Finally, the buccal membrane was used for the experiments (Penjuri, Damineni, and Ravouru 2015) (Mona Semalty and Kumar 2008) (Satishbabu and Srinivasan 2008).

Figure 13: Preparation Steps of Rabbit Buccal Mucosa.

3.5.1.2. In Vitro Residence Time Determination

The residence time in vitro was determined by using disintegration apparatus which contained 800 ml of phosphate buffer medium pH 7.4 and maintained at 37 ± 1°C which mimics the conditions in the oral cavity. The rabbit buccal mucosa, each of 2 cm width, was glued to glass slide by adhesive material as shown in figure no. (14). One gram of each formulation was spread over the buccal mucosa, colored with food color Ponceau Red E material to visualize the gel erosion by time, and then hydrated with phosphate buffer. The slides were held vertically in the disintegration apparatus to allow its movement up and down while the slides with gel were completely immersed in the PB, as described in figure no. (15). The time which needed for the erosion of the mucoadhesive gel formulations from the mucosal surface was recorded and that was considered the end point of the mucoadhesion (Penjuri, Damineni and Ravouru 2015) (Mona Semalty and Kumar 2008).

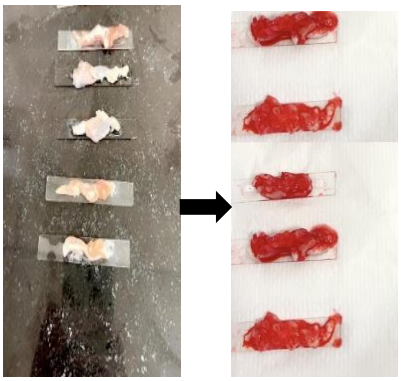


Figure 14: Mucoadhesive Gel Formulations on Buccal Mucosa Slides.

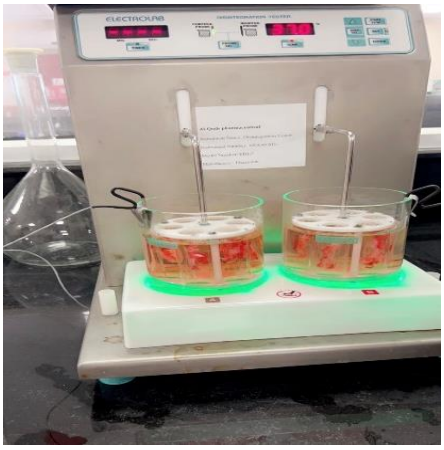


Figure 15: Residence time Determination by Using Disintegration Apparatus at 37 ± 1°C.

3.5.2. In Vitro Release Study

In vitro release study to determine the cumulative amount of drug release per unit of time by using Franz diffusion cell (FDC) at 600 rpm and phosphate buffer (6.8-7.4) with Tween 80 while keeping the temperature at 37°C, also use different types of membranes to comparison the results between the experiments. The medium should provide sink condition of Tinidazole. Then we obtained various samples at different periods (15,30 minutes and 1,2,3,4,5,6 hours). Then we replaced them with fresh volume from phosphate buffer. All the samples were diluted and assayed at (316nm) by UV methods. Based on solubility with keeping the sink condition and Tinidazole concentration that should never be more than 10% of its saturated solubility (Tian, Shen, and Jv 2016) (Wróblewska et al. 2020).

3.5.3. In Vitro Permeation Study

This study is usually conducted by using FDC and artificial membranes that mimic the periodontal membrane in vitro buccal permeation at determined temperature 37 ± 0.2°C. We use phosphate buffer (6.2-7.4) and use magnetic bar for stirring at 600 rpm. At a preset periods of time, one ml sample is withdrawn and tested for gel content at appropriate wavelength which was equal to (316 nm) by a UV spectrophotometer or HPLC method (Budhrani and Shadija, n.d.) (Fini, Bergamante, and Ceschel 2011).The % accumulative total per unit area (mg/cm^2) is drawn versus time (h), the slope of the linear position of the curve is the flux ($mg/cm^2/h$).

3.5.4. Franz Diffusion Cell Procedure

3.5.4.1. Receptor compartment Volume and Effective Diffusion Area Determinations

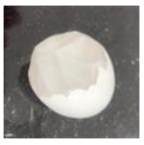
Receptor compartment which is called supplier donor, includes Tinidazole with receptor medium (Mustapha et al. 2011). We should be certain that the compartments and the cells are clean and dry, and we should ensure that the volume becomes full by putting the magnetic stirrer, and we can record the trials three times, and then take the mean of these trials (Ng et al. 2010).The application surface which is termed "effective diffusion area" (EDA) is 3.14 cm^2 , and the occlusion is provided by parafilm (Mustapha et al. 2011).

3.5.4.2. Membranes

3.5.4.2.1. Types of the Membranes

The synthetic, semi-permeable and biological membranes and their characteristics are indicated at table no. (14)

Table14: Types of the membrane and their characteristics

Types of the membrane	Polymer type	Thickness	Pore size	Diameter	Av. Flat Width	Manufacturer	Form of the membrane
Nylon 66	Synthetic (Polyamide)	100 µm	0.45 µm	47 mm	-	SUPELCO, Bellefonte	
Dialysis	Tubing cellulose (Semi permeable)	100 µm before soaked 160 µm after soaked	-	49 mm	76 mm (3inch)	Sigma-Aldrich	
Chicken Eggshell membrane	Biological membrane	80 µm	1-10 mm	-	-	Local supermarket	
Permeapad®	Biomimetic barrier	54µm	0.45 µm	25,0 + 0,2 mm	-	InnoME GmbH/ Germany	

3.5.4.2.2. Preparation of Chicken Eggshell Membrane

The whole chicken egg was added to sufficient quantity of hydrochloric acid solution about 5M. Wait until the bubbling stops and the foam disappears. The leftover substance is eggshell. Eggshell membrane was prepared by making an aperture at one end of egg and then yolk was completely removed, the remaining membrane was washed with distilled water and soaked in phosphate buffer (pH 7.4) for two hours before use as shown in figure no. (16).

Figure16: Chicken Eggshell Membrane.

3.5.4.2.3. Preparation of Permeapad® Membrane

Briefly, phospholipids (soy bean phosphatidylcholine S-100) a thin layer was put in a support sheet. The terminal barrier was formed by the support layer and a dry layer of lipid. Mechanically, the final barrier looks resistant and flexible and may be cut to size by scissors or by a punishing device (Di Cagno, Bibi, and Bauer-Brandl 2015).

Based on general model of phospholipid hydration, the lipids arranged in order to swell in contact with water then after minutes generates spheroids which appears as tightly packed layers and composed from stacks of bilayers, also with interacting with water which mimic the cell membrane. Moreover, the phospholipids vesicles closed to each other and thereby mimic tissue morphology. The lipid layers protect from the erosion also from escape the lipids to aqueous environment. Permeapad® membrane is ready to use , it is available as disk Permeapad® and in the form of inserts for 6-well plates also high-through put screening can be performed using 96-well plate Permeapad® Plate (Berben et al. 2018).

In general Permeapad® membrane it is considered fully artificial synthetic phospholipid-based biomimetic membrane with a structured layers as the form at figure no. (17) (Berben et al. 2018).

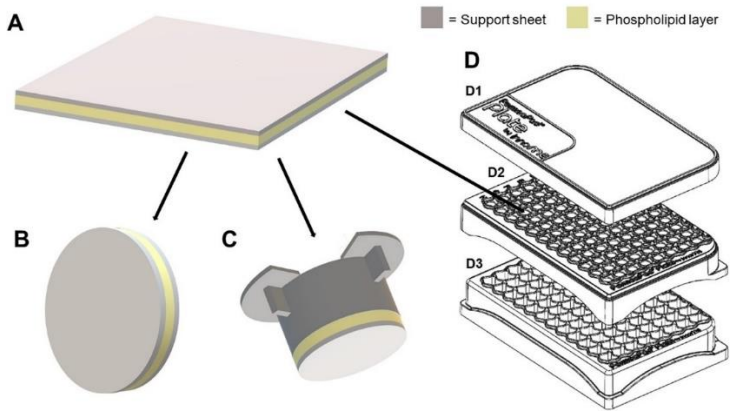


Figure17: Schematic Representation of the Available Formats of the Permeapad® Barrier. A. Bulk sheet of Permeapad®, B. Disk of Permeapad® compatible with side-by-side diffusion cells and Franz diffusion cells, C. Insert with Permeapad® for 6-well plates (surface area of 3.8 cm^2), D. Permeapad® plate, a 96-well plate with a surface area of 0.13 cm^2 for high throughput permeation screening with: D1 lid, D2 middle plate with Permeapad® as barrier and D3 bottom well-plate (Berben et al. 2018).

The Permeapad® that was used in our experiments had batch no. (2023-0002) and expiry date (02/2024) also should store at (20-25 ºC) and protect from light and UV radiation. Figure no. (18)

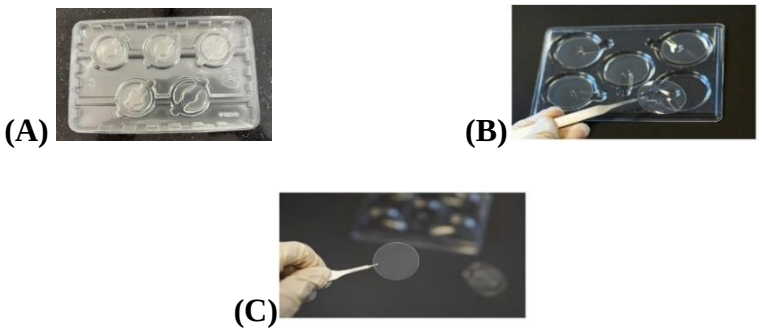


Figure 18: Permeapad® Membrane A.

B,C.(“Https://Permeapad.Com/En/Permeapad-Barrier/” 2020)

3.5.4.2.4. Membrane Treatment:

Membrane of diffusion is synthetic membrane or a mucus membrane. We must be certain that the diameter of the effective diffusion area is less than the diameter of the membrane. The membrane is soaked in the receptor fluid compartment for at least half an hour. When we place the diffusion membrane this stabilizes and maintains the cells that will occur into a water bath at 37 ºC. The temperature and homogeneity of the content in the lower compartment is maintained by bar magnet movement. The pH of the samples should be between 6.5-7 (Mustapha et al. 2011).The content with uniform mixtures with magnetic stirrer is filtered and analyzed according to the analyst’s directions (Pawar et al. 2013).

3.5.4.3. Solubility of Tinidazole in Receptor Fluid:

The lower compartment receives the medium buffer according to sink condition, and it should be as close as possible to physiological medium. So, this liquid

medium is utilized for releasing the Tinidazole in the phosphate buffer at pH 7.4. We determined the amount of the drug release which the volume is received which should be around 20 ml (Mustapha et al. 2011).

3.6. Solubility Profile Study

3.6.1. Solubility Determination

The determination of solubility is done by utilizing the shake-flask method. Shortly, an extra amount of the TNZ can be added to each one of conical flask which contains 20 ml of solvent whose number approx. 15 flasks every three of them contains the same medium under test. Mechanically, the complex is shaken for 24 h at 37 ºC with 40 rpm speed permit to stand for 24 h to attain equilibrium. After that, all mixtures were centrifuged at 3000 rpm until 15 minutes then withdraw 10 ml sample from each one then made filtration through nylon membrane filter (0.45 µm), followed by dilution with appropriate solvent as phosphate buffer at pH 7.4 which were analyzed by UV test method and measured the absorbance at 316 nm then took the average of absorbance for each medium (Pyka-Pająk, Parys, and Dołowy 2019).

3.6.2. Solubility Profile Preparation

Solubility determination of Tinidazole solubility by preparing different medium pHs buffer which were (purified water, Hydrochloric acid 0.1N, Acetate buffer pH 4.5, Phosphate buffer pH 6.8, Phosphate buffer pH 7.4) then made

saturation solubility by adding respective amount of Tinidazole (approximately about 1.0 g)

1) 0.1N HCl:

Dilute 8.5 ml of hydrochloric acid 37% w/v with water to 1000 ml

2) Acetate buffer pH 4.5:

Dissolve 2.99 g of Sodium Acetate in 500 ml of water then add 14 ml of 2M Acetic acid and dilute to 1000 ml of water.

3) Phosphate buffer pH 6.8:

Weighed 6.8g Potassium Dihydrogen monobasic Phosphate and 0.94g of Sodium Hydroxide then dissolve in 1000 ml water.

4) Phosphate buffer pH 7.4:

Weighed 6.8g Potassium Dihydrogen monobasic Phosphate and 1.5g of Sodium Hydroxide then dissolve in 1000 ml water.

3.6.3. Standard Preparation for Solubility Study

50 mg of Tinidazole was weighed and dissolved in about 2ml methanol in 100-ml volumetric flask then it was diluted with phosphate buffer pH 7.4 to concentration of 0.01 mg/ml. The absorbance was measured by UV at wavelength 316nm.

3.6.4. Preparation of Receptor Solution (Phosphate Buffer pH 7.4 with Tween 80 at 37 ºC)

The receptor solution shall fulfill the sink conditions. For this purpose, we prepared phosphate buffer pH 7.4 with different concentrations of Tween 80, and

measured the solubility of Tinidazole at 37 ºC. The concentrations of Tween 80 are indicated in the table no. (15)

Table 15: The Samples Name and Their Conc. of Tween 80

No.	**Sample name**
1.	Phosphate buffer pH 7.4 without Tween 80
2.	Phosphate buffer pH 7.4+ 0.05% Tween 80
3.	Phosphate buffer pH 7.4+ 0.1% Tween 80
4.	Phosphate buffer pH 7.4+ 0.15% Tween 80
5.	Phosphate buffer pH 7.4+ 0.3% Tween 80

3.6.5. Procedure of Sampling

Franz diffusion cell composed of 6-cell units. In general, the design of the cell includes the donor chamber, the membrane, the sampling port and the receptor chamber also used the disc and alignment ring over the membrane also used the clamps to held the membrane with other parts. The temperature should be 37 ºC within the cells and this maintained by a heating jacket and the temperature can be defined by using a calibrated infrared thermometer and the rate of stirring (regularly 600 rpm) and used magnetic bar for continuous stirring. The membrane purposed to keep the Tinidazole gel and the receptor medium separated and featured. Then filled the donor compartment with accurate amount of gel (2 ± 0.1g) which put on the membrane inside the cavity chamber which can be occluded with parafilm and before inserting the donor compartment make sure that all the system had reached the required experimental temperature and then checked again the calibration mark. During the procedure is necessary to certified that there are no air bubbles in the receptor medium or below the membrane that may escape from the sampling port

so we overcome from this issue through several ways by warming the medium at suitable temperature and make degassing also by tilting the tool in all the orientations. The volume at receptor chamber can be adapted with calibrated level on the arm of sampling port.

Usually, a set of 6 cells are prepared at one time (a single run) and the sampling occurred at over period of time (0.25,0.5,1,2,3,4,5,6 hrs.) then collect the aliquots (every one 1 ml) by a syringe subsequently diluted with phosphate buffer then analyze the samples through UV visible spectrometer furthermore the volume withdrawn through the sampling port necessary for replenishment with buffer medium.

To realize the sink conditions the receptor medium should have a high capacity to dissolve Tinidazole gel and its concentration at this medium at the last of the test ideally must be as low as possible. For each cell, the cumulative amount of drug released per unit area (mg / cm^2) at each sampling time is determined plotted versus time (h) (United States Pharmacopeial 2014).

3.7. Calibration Curve of Tinidazole

3.7.1. Phosphate Buffer pH 7.4 Preparation

Weighed 6.8g Potassium Dihydrogen monobasic Phosphate and 1.5g of Sodium Hydroxide and dissolve in 1000 ml purified water with 0.3g of Tween 80 and calibrated pH at 7.4 by pH meter.

3.7.2. Standard Preparation for Calibration Curve of Tinidazole

Weighed 50 mg of Tinidazole and dissolved in 2 ml of methanol then diluted in phosphate buffer pH 7.4 and 0.3% Tween 80 and preparation different concentrations of Tinidazole as (0.005,0.008,0.01,0.015,0.02,0.03,0.04 mg/ml) which were analyzed by UV spectrometer and determine the spectrum at wavelength (λ_{max} = 316 nm) then measured the absorbance of the concentration to make the calibration curve of Tinidazole.

3.8. In Vitro Drugs Release Kinetics Profile

The various mathematical models could apply for expression the mode of drug release kinetics and procedures of optimized mucoadhesive buccal gel formulations containing Tinidazole, the in vitro was estimated to find the appropriate mathematical model to suited the experimental results.

The kinetics of release the drug formulations depends on existing the best fit the release data to zero order , first order, Higuchi, Korsmeyer Peppas , Makoid-Banakar and Weibull release kinetic models (Penjuri, Damineni, and Ravouru 2015) (Syed et al. 2022) (Melike Ongun, Emre Tuncel, Esra Kodan, Fatmanur Tugcu-Demiroz 2020) and the determination of rate constants by using Excel Add-In Program DD Solver®

(Hashmat et al. 2020). The value of experimental results were compared and the model describing the highest value of coefficient of regression (R^2) which was considered the best fitted model of drug release kinetics (Syed et al. 2022) (Hashmat et al. 2020).

The equations of models are as follows:

Zero-order:

$$Qt = Qo + kot$$

where Q_o and Q_t represent the initial amount of drug in dosage form and amount of drug at time t, respectively. k_o is a zero-order rate constant.

First -order:

$$\log Q_t = \log Q_o + k_1 t/2.303$$

Where k_1 is the first order rate constant.

Higuchi model:

$$Qt = kHt1/2$$

Where k_H is the Higuchi rate constant.

Korsmeyer-Peppas model:

$$Mt/M\infty = Ktn$$

Where, M_t/ M_∞ is the fraction of the drug released at time t, K is rate constant and n is the release exponent indicating the drug release mechanism.

Makoid-Banakar model:

$$Mt/M\infty = k\ MB\ tn\ e\text{^}((-ct))$$

Where, K_{MB}, n, and care empirical parameter (K_{MB}, n, c > 0) and M_t/M_∞ is the accumulation fraction of the drug in solution at time t.

Weibull model:

$$m = 1 - exp[-(t - Tl)\ \beta\alpha]$$

Where ‘m’ is drug accumulated fraction in solution at any time t. The scale parameter is, α, defining time scale process. Lag time is presented by T_l i.e., the time required before the onset of drug release, in most cases, it will be zero. β is considered as a shape parameter and expresses curve.

3.9. Diffusion Parameters

Calculations

At each sampling time the concentration of solution in the receptor compartment is determined by ultraviolet spectrophotometer, and the cumulative amount of drug (Q) in the receiver compartment is determined, then the cumulative amount per unit area (m) is calculated by dividing the cumulative amount over the surface area of the membrane (A)

$M_t = Q_t/A$

We plot a curve resembling the time course of drug permeation and we calculate the penetration parameters and the enhancement ratio of penetration.

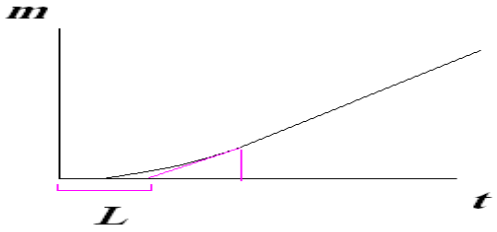


Figure 19: The Time Course of Drug Permeation.

At long times the plot approaches a straight line and steady state is obtained and expressed as follows:

$J = dm/dt = -DC_oK/h$ .....(1)

J=Flux: slope of the steady state Plot in fig (6).

D= Diffusion coefficient

K= membrane vehicle partition coefficient

h= membrane thickness

C_o=constant: concentration in donor compartment

The steady state may be expressed mathematically as

$Y = ax + b$……... (2)

Where Y is equal to m_t and x is the time in hours.

If the steady state plot is extrapolated to the time axis, the intercept is obtained which equals the lag time (T_L).

According to Fick's second law of diffusion

$T_L = h^2/6D$…… (3)

We can calculate the diffusion coefficient (D) in cm^2/hr.

Lag time (T_L) is calculated from the plot, h is known we calculate D,

We can calculate K by substitution in equation (1)

We calculate the permeability coefficient from equation (4)

P=J/C and that …… (4)

Substituting equation (1) instead of (J) we can calculate (P)

P= KD/h……... (5)

Then we calculate the enhancing ration from equation (6)

E.R (enhancement ratio): = (P" penetration enhancer")/ (P" without enhancer") …… (6)

Table no. (16) Presents a summary of the diffusion parameters and their calculation.

Table 16: Summary of Diffusion Parameters and Their Calculation

Slope	Lag Time (T_L) [min]	Diffusion Coefficient [cm^2 / min]	Permeability Coefficient [cm/sec]	Partition Coefficient	Enhancement Ratio
Estimated from the graph	Intercept with x axis	$\frac{h^2}{6TL}$	Slope/C_d	$\frac{P.h}{D}$	Permeability with enhancer/ permeability without enhancer

3.10. Stability Studies

For the stability studies, the mucoadhesive gel was filled in aluminum tubes, sealed and stored for different intervals in stability chambers for 4 months at intermediate stability (30°C ± 2°C/65% RH ± 5% RH) and at accelerated stability (40 ºC ± 2 ºC/ 75% RH ± 5% RH). These samples are tested for assay , pH , viscosity and degradation (Sulayman 2011) (Charyulu et al. 2013) (Goudanavar et al. 2021) In the stability study fill each formulation into individual vials (type A) protected from light for each period of time for each storage conditions. For analysis using HPLC by using column (C8,250*4.6mm,5µm), and the mobile phase (CH3OH: H2O) (20:80) at flow rate 1.5ml/min. The standards were prepared by put 20 mg of Tinidazole in 100 ml CH3OH which were taken 6 times at zero time and before the analysis the formulations after incubation for 4 months while the test prepared by

weighing 2 g of sample of each formula (as mucoadhesive oral gel) which was equal 20 mg Tinidazole in 100 ml CH3OH.

To calculate the assay (%) degradation (%) and for formulations by equations below

$$\text{Assay (\%)} = \frac{\text{Area Test} \times \text{Conc. STD}}{\text{Area STD} \times \text{Conc. Test}} \times \text{Potency of STD}$$

$$\text{Degradation (\%)} = \frac{\text{Impurity area}}{\text{Total area}} \times 100\%$$

Chapter Ⅳ: Results and Discussion

4. Results and Discussion

4.1. Solubilization of Tinidazole in Water by Using the Concept of Mixed Solvency Results

The results after placing the sample vials for one week in the refrigerator, we observed that the sample vials contained PEG 400 and PG alone had crystals formation while the mixture from both of them had no crystals formation, therefore the binary of mixture as ratio (50:50) from PEG 400 and PG was used for the formulation trials.

4.2. Compatibility Study Results

The compatibility profile showed no significant changes and there was no interaction between the drug and the excipients in their physical mixtures as shown in table no. (17)

Table 17: Assay, Appearance & Degradation of Compatibility Study Results

Components		**Assay (%)**			**Appearance**		**Degradation (%)**	
		Zero time	**One-month**		**One-month**		**One-month**	
			25 ºC+ 60%RH	**40 ºC+ 75%RH**	**25 ºC+ 60%RH**	**40 ºC+ 75%RH**	**25 ºC+ 60%RH**	**40 ºC+ 75%RH**
Tinidazole		100	100.06	97.67	Off-white	Off-white	-	-
TNZ	Carbopol 934P	100	99.3	97.9	Pale yellow	Pale yellow	-	-
TNZ	TEA	100	94.66	89.48	Pale yellow	Pale yellow	0.69	4.29
TNZ	PEG 400	100	98.51	95.18	White	Off-white	-	-
TNZ	PG	100	99.15	97.35	No significant changes were observed	No significant changes were observed	-	-
TNZ	Water	100	97.02	101.86	Light yellow	Light yellow	0.01	0.07

TNZ	PVP (Grade K25)	100	102.37	104.76	No significant changes were observed	No significant changes were observed	-	-
TNZ	Xanthan gum	100	101.08	98.84	Pale yellow	Pale yellow	-	-
TNZ	Methocel K100M	100	107.33	102.44	No significant changes were observed	No significant changes were observed	-	-
TNZ	Methocel E5	100	102.20	101.74	No significant changes were observed	No significant changes were observed	-	-
TNZ	Tween 80	100	95.55	97.45	No significant changes were observed	No significant changes were observed	-	0.06
TNZ	Cremophor RH40	100	101.06	99.90	No significant changes were observed	Pale yellow	0.04	0.17
TNZ	PPG 15 Stearyl Ethers	100	103.05	101.75	No significant changes were observed	No significant changes were observed	-	-

4.3. Physiochemical Properties of Mucoadhesive Gel Formulations

The mucoadhesive gel were evaluated for pH, viscosity, texture, color, smell, transparency and taste. For all formulation trials: the pH results were in the range of 6.4-7.4, the viscosity results were more than $2*10^6$ cp, the texture of trials were smooth, the color of trials were white to pale yellow, formulations were transparent and had no smell with bitter taste. The details were shown in table no. (18)

Table 18: Results of Physiochemical Properties of Mucoadhesive Gel Formulations

Tests	**Formulation**						
	F1	**F2**	**F3**	**F4**	**F5**	**SD**	**RSD (%)**
pH	6.8	6.4	6.5	7.4	6.8	0.39	5.75
Viscosity (centipoise)	$>2*10^6$ cp	$>2*10^6$ cp	$>2*10^6$ cp	$>2*10^6$ cp	$>2*10^6$ cp	0	0
Texture	Smooth	Smooth	Smooth	Smooth	Smooth		
Color	White	Pale yellow	Pale yellow	Off white	Pale yellow		
Smell	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic		
Transparency	Transparent	Transparent	Transparent	Transparent	Transparent		
Taste	Bitter	Bitter	Bitter	Bitter	Bitter		

4.4. In vitro Residence Time Determination Results

Residence time determination according to disintegration apparatus tester at 37 ± 1˚C for the five formulations in phosphate buffer medium pH 7.4 to measure and compare the residence time for all formulations which they remain per periods of time until they completely dissolved and the time recorded for each formula.

Moreover, rabbit buccal mucosa was selected because it considered more suitable than others, therefore it includes non-keratinized buccal mucosa. Also, the buccal mucosa thickness is (600 μm) with comparable to that of humans buccal (500–800 μm) (Kraan et al. 2014). The optimum concentration of polymer corresponding to the best mucoadhesion was determined. Many factors affect mucoadhesion and the residence time such as initial force of application therefore, higher forces lead to enhanced interpenetration and high bioadhesive strength. Additionally, the

greater the initial contact time between substrate and bioadhesive, the greater the swelling and interpenetration of polymer chains (Shaikh et al. 2011).

The presence of PVP K25 and xanthan gum in the previous formulations has paramount effect on the residence time

The mucoadhesion time was measured for all formulations (F1, F2, F3, F4, and F5) and we noticed that all of them reached magnitude of residence time shown in table no. (19), all formulations contain Carbopol 934P which has good mucoadhesive properties and hydrophilicity due to interpenetration of polymeric chains in the mucosal membrane so it showed lower rate of release (Sulayman 2011). The significant variations between all formulations come from the addition of HPMC grades (Methocel E5 and Methocel K100M) with different concentrations and that agree with literature. Sharma et al prepared thermosensitive in situ gel of Tinidazole and found that the (20% poloxamer 407 and HPMC) had the highest mucoadhesion as compared to the formulations containing Carbopol (S. Sharma and Kaushal 2014). Cho et al mentioned the effects of HPMC polymer. A bioadhesive transdermal Bupivacaine gels were developed for enhanced local anesthetic action and the increase in the concentration of HPMC grade (K100M) increased the viscosity and the bioadhesion (Cho, Kim, and Shin 2012). The results of residence time shown in table no. (19), prevail that F4 and F5 formulation exhibited the highest residence time (4, 4.30 hours) respectively possibly due to the presence of xanthan gum. These results are in agreement with scientific studies which showed that xanthan

gum had the longest residence time when it was compared with other polymers, according to the following rank order CP < HEC, HA, ALG, PCP < CMC < XTGM (Bernkop-Schnürch 2019).

The results of residence time measurement are shown in table no. (19)

Table 19: In vitro Residence Time Determination Results

Formulations	**Time**
F1	40 min
F2	2hrs, 5min
F3	2hrs, 10min
F4	4hrs
F5	4hrs, 30min

4.5. Solubility Profile Results

4.5.1. Solubility Determination Results of Tinidazole in Different Solutions At 37 ^{0}C

According to solubility determination results of Tinidazole in different solutions at 37 ^{0}C as in table no. (20), the solubility of Tinidazole in HCl 0.1N was the highest one (0.288g/100ml). Phosphate buffer pH 7.4 was used for formulation because it mimics the characteristic of oral environment especially in the physiological environment, saliva has a normal pH range of (6.2–7.6), so the best medium for Tinidazole is phosphate buffer pH 7.4. The solubility was found to best (0.104g/100ml). The solubility results are shown in table no. (20)

Table 20: Solubility Results of Tinidazole in Different Solutions at 37 ^{0}C

Medium	**Conc.(mg/ml) (conc. in initial conc C_0)**	**Solubility (g/100ml)**
Water	0.658	0.066 ± 0.6
HCl 0.1 N	2.880	0.288 ± 0.1
Acetate buffer pH 4.5	0.965	0.097 ± 0.3
Phosphate buffer pH 6.8	0.968	0.097 ± 0.7
Phosphate buffer pH 7.4	1.044	0.104 ± 0.7

4.5.2. Solubility of Tinidazole According to Sink Conditions

The lower compartment receives the medium buffer according to sink condition which should have a high capacity to dissolve Tinidazole gel and it should be as close as possible to physiological medium based on the results of solubility of Tinidazole (g/100 ml) at different conc. of Tween 80 at 37ºC, solubility of Tinidazole in phosphate buffer pH 7.4+0.3% Tween 80 has the highest value which equal (3.184 g/100 ml), so that the sink conditions is maintained when using penetration enhancers such as Tween 80. The solubility of Tinidazole (g/100 ml) is shown in table no. (21)

Table 21: Solubility of Tinidazole (g/100 ml) at different conc. of Tween 80 at 37 ^{0}C

No.	**Sample name**	**Saturation concentration (C_0) (mg/ml)**	**Solubility of Tinidazole (g/100 ml)**
1.	Phosphate buffer pH 7.4 without Tween 80	1.044	0.104 ± 0.01
2.	Phosphate buffer pH 7.4+ 0.05% Tween 80	9.204	0.920 ± 0.6
3.	Phosphate buffer pH 7.4+0.1% Tween 80	8.934	0.893 ± 0.5
4.	Phosphate buffer pH 7.4+0.15% Tween 80	16.282	1.628 ± 1.0
5.	Phosphate buffer pH 7.4+0.3% Tween 80	31.844	3.184 ± 0.1

4.5.3. Selection of Analytical Wavelength

The spectrum of Tinidazole was recorded and λ_{max} for Tinidazole was 316 nm as shown in figure no. (20)

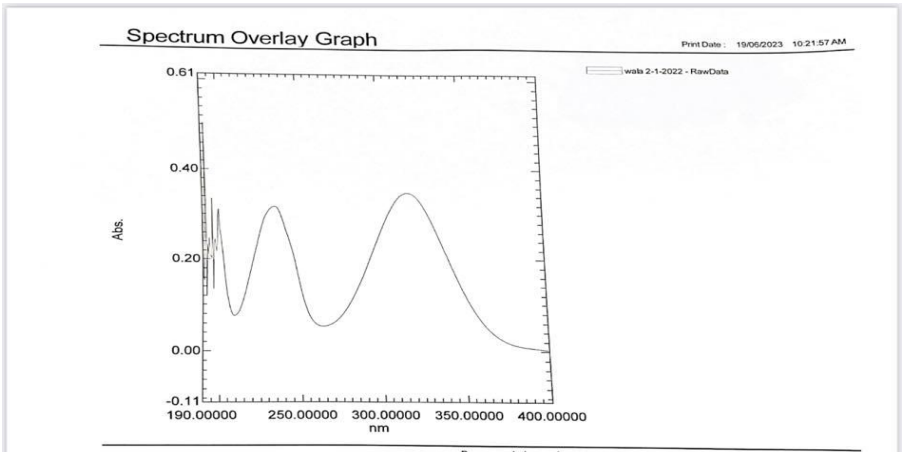


Figure 20: Overlay Spectra of TNZ

4.5.4. Linearity Results of Tinidazole Absorbance

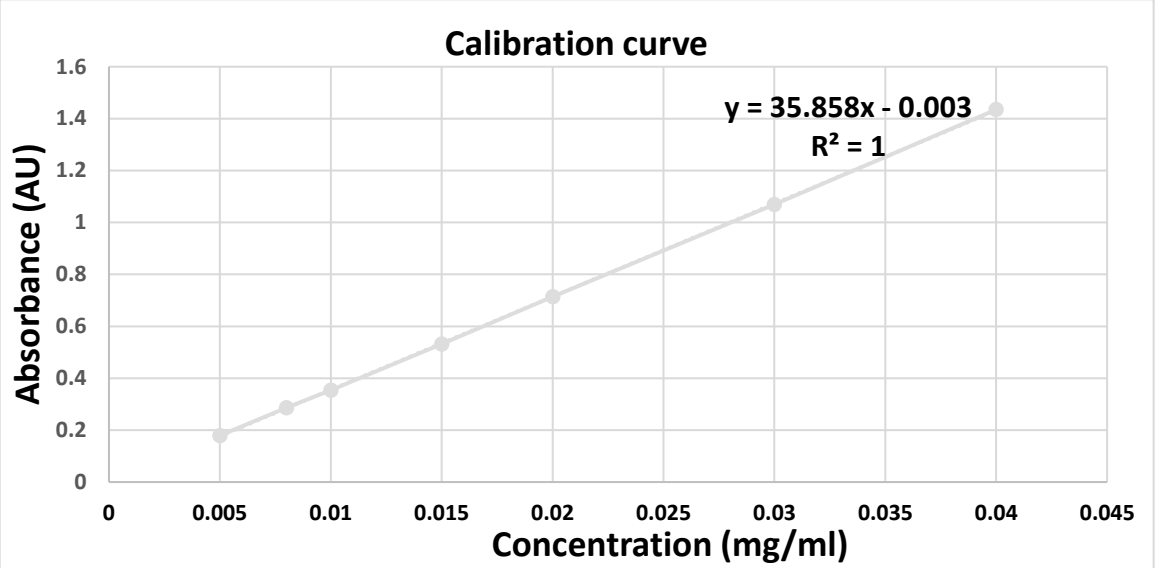


Figure 21: Calibration Curve of Tinidazole at UV 316 nm.

4.6. Results of Diffusion Study

4.6.1. In Vitro Release Study of F4 formulations using synthetic membrane (polyamide membrane)

The release of F4 mucoadhesive gel without penetration enhancer through polyamide membrane shows that the cumulative amount released per unit area over periods of time around 6 hours which was equal to (2.188 mg/cm²) and the (%) drug released versus time was equal (34.35%). Table no. (22) displays the detailed results.

Table 22: Data obtained via diffusion of F4 mucoadhesive gel (F4 without PEs) through polyamide membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.201	0.205	0.210	0.205	0.004	2.196	0.006	10	0.058	1.162	0.058	1.162	0.370
0.5	0.394	0.395	0.416	0.402	0.012	3.093	0.011	10	0.113	2.257	0.113	2.315	0.737
1.0	0.517	0.520	0.563	0.533	0.026	4.825	0.014	10	0.149	2.991	0.149	3.104	0.989
2.0	0.655	0.700	0.685	0.680	0.023	3.369	0.019	10	0.190	3.809	0.190	3.959	1.261
3.0	0.824	0.857	0.882	0.854	0.029	3.405	0.024	10	0.239	4.782	0.239	4.972	1.583
4.0	0.935	0.973	0.980	0.963	0.024	2.515	0.027	10	0.269	5.386	0.269	5.625	1.791
5.0	0.520	0.535	0.548	0.534	0.014	2.622	0.015	20	0.299	5.994	0.299	6.263	1.995
6.0	0.575	0.584	0.599	0.586	0.012	2.069	0.016	20	0.328	6.570	0.328	6.870	2.188

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F4 mucoadhesive gel with Tween 80 (F4A) as penetration enhancer through polyamide membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.819 mg/cm²) and the (%) drug released versus time was equal (28.56%). Table no. (23) displays the detailed results.

Table 23: Data obtained via diffusion of F4 mucoadhesive gel with Tween 80 (F4A) as penetration enhancer through polyamide membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.145	0.144	0.163	0.150	0.011	7.097	0.004	10	0.043	0.857	0.043	0.857	0.273
0.5	0.297	0.293	0.301	0.297	0.004	1.347	0.008	10	0.084	1.673	0.084	1.716	0.547
1.0	0.333	0.331	0.340	0.335	0.005	1.412	0.009	10	0.094	1.883	0.094	1.967	0.626
2.0	0.436	0.429	0.446	0.437	0.009	1.955	0.012	10	0.123	2.454	0.123	2.548	0.811
3.0	0.561	0.525	0.585	0.557	0.030	5.422	0.016	10	0.156	3.123	0.156	3.246	1.034
4.0	0.665	0.655	0.704	0.675	0.026	3.837	0.019	10	0.189	3.780	0.189	3.935	1.253
5.0	0.850	0.741	0.887	0.826	0.076	9.189	0.023	10	0.231	4.624	0.231	4.813	1.533
6.0	0.991	0.950	0.998	0.979	0.026	2.647	0.027	10	0.274	5.481	0.274	5.712	1.819

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F4 mucoadhesive gel with Cremophor RH40 (F4B) as penetration enhancer through polyamide membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.942 mg/cm²) and the (%) drug released versus time was equal (30.48%). Table no. (24) displays detailed results.

Table 24: Data obtained via diffusion of F4 mucoadhesive gel with Cremophor RH40 (F4B) as penetration enhancer through polyamide membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.150	0.151	0.151	0.151	0.001	0.383	0.151	10	0.043	0.857	0.043	0.857	0.273
0.5	0.327	0.301	0.326	0.318	0.015	4.632	0.318	10	0.090	1.790	0.090	1.833	0.584
1.0	0.477	0.379	0.465	0.440	0.053	12.139	0.440	10	0.124	2.472	0.124	2.562	0.816
2.0	0.666	0.666	0.639	0.657	0.016	2.373	0.657	10	0.184	3.681	0.184	3.805	1.212
3.0	0.791	0.700	0.826	0.772	0.065	8.421	0.772	10	0.216	4.324	0.216	4.509	1.436
4.0	0.951	0.816	0.991	0.919	0.0917	9.974	0.026	10	0.257	5.144	0.257	5.361	1.707
5.0	0.991	0.950	0.992	0.978	0.0240	2.451	0.027	10	0.273	5.470	0.273	5.727	1.824
6.0	0.518	0.510	0.529	0.519	0.009	1.838	0.015	20	0.291	5.823	0.291	6.096	1.942

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F4 mucoadhesive gel with Arlamol (F4C) as penetration enhancer through polyamide membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (2.631 mg/cm²) and the (%) drug released versus time was equal (41.30%). Table no. (25) displays detailed results.

Table 25: Data obtained via diffusion of F4 mucoadhesive gel with Arlamol (F4C) as penetration enhancer through polyamide membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.161	0.156	0.163	0.160	0.004	2.253	0.005	10	0.045	0.909	0.045	0.909	0.290
0.5	0.330	0.304	0.354	0.329	0.025	7.593	0.009	10	0.093	1.854	0.093	1.899	0.605
1.0	0.444	0.424	0.466	0.445	0.021	4.724	0.012	10	0.125	2.497	0.125	2.589	0.825
2.0	0.655	0.650	0.667	0.657	0.009	1.329	0.018	10	0.184	3.683	0.184	3.808	1.213
3.0	0.801	0.793	0.820	0.805	0.014	1.723	0.023	10	0.225	4.505	0.225	4.689	1.493
4.0	0.511	0.504	0.530	0.515	0.013	2.612	0.014	20	0.289	5.779	0.289	6.003	1.912
5.0	0.604	0.595	0.625	0.608	0.015	2.532	0.017	20	0.341	6.816	0.341	7.105	2.263
6.0	0.714	0.702	0.705	0.707	0.006	0.883	0.020	20	0.396	7.920	0.396	8.261	2.631

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

All formulations of the mucoadhesive gel F4 contained the same formulations but the difference in the penetration enhancer types which were (F4 without PEs, F4 with Tween 80 (F4A), F4 with Cremophor RH40 (F4B), F4 with Arlamol(F4C)). The cumulative amount released per unit area after 6 hours for F4 with Arlamol by using synthetic membrane (polyamide) has the highest value which

was equal (2.631 mg/cm²) and the (%) drug released versus time that was equal (41.30%). Figure no. (22) was shown the (%) drug released versus time(hr.). The descending order of (Q) per unit area for F4 by using synthetic membrane: F4 with Arlamol (F4C) >F4 without PEs >F4 with Cremophor RH40 (F4B) >F4 with Tween 80 (F4A).

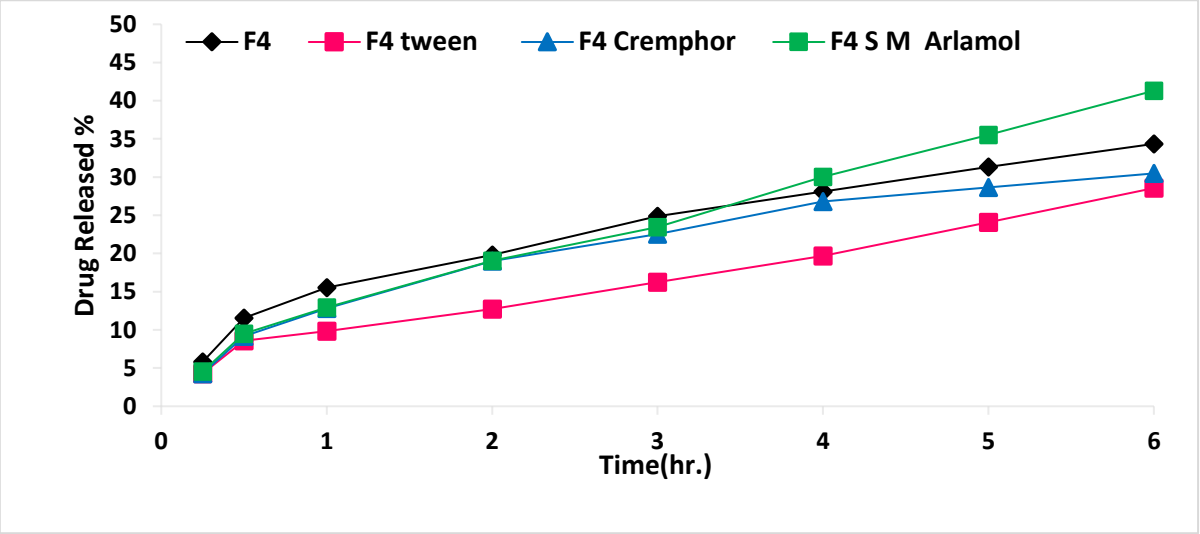


Figure 22: In vitro release the (%) drug released versus time of mucoadhesive gel (F4 without PEs, F4 with Tween 80 (F4A), F4 with Cremophor RH40 (F4B), F4 with Arlamol (F4C)) per unit area of polyamide membrane (mg/cm²).

4.6.2. In Vitro Release Study of F5 formulations using synthetic membrane (polyamide membrane)

The release of F5 mucoadhesive gel without penetration enhancers through polyamide membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (2.106 mg/cm²) and the (%) drug released versus time was equal (33.1%). Table no. (26) displays the detailed results.

Table 26: Data obtained via diffusion of F5 mucoadhesive gel (F5 without PEs) through polyamide membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.190	0.199	0.196	0.195	0.005	2.350	0.006	10	0.055	1.104	0.055	1.104	0.352
0.5	0.367	0.410	0.396	0.391	0.0219	5.609	0.011	10	0.110	2.198	0.110	2.253	0.717
1.0	0.530	0.553	0.543	0.542	0.012	2.128	0.015	10	0.152	3.040	0.152	3.150	1.003
2.0	0.650	0.690	0.681	0.674	0.021	3.115	0.019	10	0.189	3.774	0.189	3.926	1.250
3.0	0.851	0.875	0.870	0.865	0.013	1.463	0.024	10	0.242	4.843	0.242	5.032	1.602
4.0	0.942	0.981	0.951	0.958	0.020	2.132	0.027	10	0.268	5.360	0.268	5.602	1.784
5.0	0.500	0.530	0.515	0.515	0.015	2.913	0.014	20	0.289	5.778	0.289	6.046	1.926
6.0	0.558	0.569	0.565	0.564	0.006	0.987	0.016	20	0.316	6.325	0.316	6.614	2.106

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F5 mucoadhesive gel with Tween 80 (F5A) as penetration enhancer through polyamide membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.749 mg/cm²) and the (%) drug released versus time was equal (27.45%). Table no. (27) displays the detailed results.

Table 27: Data obtained via diffusion of F5 mucoadhesive gel with Tween 80 (F5A) as penetration enhancer through polyamide membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.122	0.124	0.154	0.133	0.018	13.444	0.004	10	0.038	0.760	0.038	0.760	0.242
0.5	0.235	0.308	0.315	0.286	0.044	15.491	0.008	10	0.081	1.612	0.081	1.650	0.525
1.0	0.341	0.387	0.409	0.379	0.035	9.155	0.011	10	0.107	2.130	0.107	2.211	0.704
2.0	0.462	0.519	0.531	0.504	0.037	7.314	0.014	10	0.141	2.828	0.141	2.934	0.935
3.0	0.665	0.691	0.723	0.693	0.029	4.192	0.019	10	0.194	3.882	0.194	4.023	1.281
4.0	0.762	0.778	0.780	0.773	0.010	1.276	0.022	10	0.217	4.330	0.217	4.5241	1.441
5.0	0.833	0.851	0.856	0.847	0.012	1.429	0.024	10	0.237	4.739	0.237	4.956	1.578
6.0	0.893	0.944	0.980	0.939	0.044	4.655	0.026	10	0.263	5.254	0.263	5.491	1.749

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F5 mucoadhesive gel with Cremophor RH40 (F5B) as penetration enhancer through polyamide membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.871 mg/cm^2) and the (%) drug released versus time was equal (29.4%). Table no. (28) displays the detailed results.

Table 28: Data obtained via diffusion of F5 mucoadhesive gel with Cremophor RH40 (F5B) as penetration enhancer through polyamide membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm^2)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.168	0.162	0.165	0.165	0.003	1.818	0.005	10	0.047	0.937	0.047	0.937	0.298
0.5	0.340	0.344	0.350	0.345	0.005	1.460	0.010	10	0.097	1.939	0.097	1.986	0.632
1.0	0.517	0.525	0.490	0.511	0.018	3.591	0.014	10	0.143	2.865	0.143	2.962	0.943
2.0	0.644	0.685	0.663	0.664	0.021	3.090	0.019	10	0.186	3.720	0.186	3.864	1.230
3.0	0.846	0.734	0.780	0.787	0.056	7.156	0.022	10	0.220	4.404	0.220	4.590	1.462
4.0	0.905	0.848	0.874	0.876	0.029	3.259	0.025	10	0.245	4.901	0.245	5.121	1.631
5.0	0.924	0.952	0.967	0.948	0.022	2.303	0.027	10	0.265	5.302	0.265	5.547	1.767
6.0	0.490	0.500	0.510	0.500	0.010	2.000	0.014	20	0.281	5.611	0.281	5.876	1.871

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F5 mucoadhesive gel with Arlamol (F5C) as penetration enhancer through polyamide membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (2.387 mg/cm²) and the (%) drug released versus time was equal (37.5%). Table no. (29) displays the detailed results.

Table 29: Data obtained via diffusion of F5 mucoadhesive gel with Arlamol (F5C) as penetration enhancer through polyamide membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.167	0.164	0.170	0.167	0.003	1.796	0.005	10	0.047	0.948	0.047	0.948	0.302
0.5	0.349	0.331	0.350	0.343	0.011	3.114	0.010	10	0.097	1.931	0.097	1.980	0.630
1.0	0.449	0.448	0.456	0.451	0.004	0.966	0.013	10	0.127	2.532	0.127	2.629	0.837
2.0	0.708	0.670	0.716	0.698	0.025	3.521	0.020	10	0.195	3.910	0.195	4.036	1.286
3.0	0.821	0.812	0.828	0.820	0.008	0.978	0.023	10	0.230	4.592	0.230	4.788	1.525
4.0	0.455	0.450	0.460	0.455	0.005	1.099	0.013	20	0.255	5.109	0.255	5.339	1.700
5.0	0.540	0.535	0.555	0.543	0.010	1.916	0.015	20	0.305	6.094	0.305	6.350	2.022
6.0	0.650	0.645	0.630	0.642	0.010	1.622	0.018	20	0.360	7.191	0.360	7.496	2.387

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

All formulations of the mucoadhesive gel F5 contained the same formulations but the difference in the penetration enhancer types which were (F5 without PEs, F5 with Tween 80 (F4A), F5 with Cremophor RH40 (F4B), F5 with Arlamol F5C)). The cumulative amount released per unit area after 6 hours for F5 with Arlamol by using polyamide membrane has the highest value that was equal (2.387 mg/cm²) and the (%) drug released versus time was equal (37.48 %). Figure no. (23) was shown the (%) drug released versus time(hrs.). The descending order of (Q) per unit area for F5 by using synthetic membrane:

F5 with Arlamol (F5C) > F5 without PEs > F5 with Cremophor RH40 (F5B) >F5 with Tween 80 (F5A)

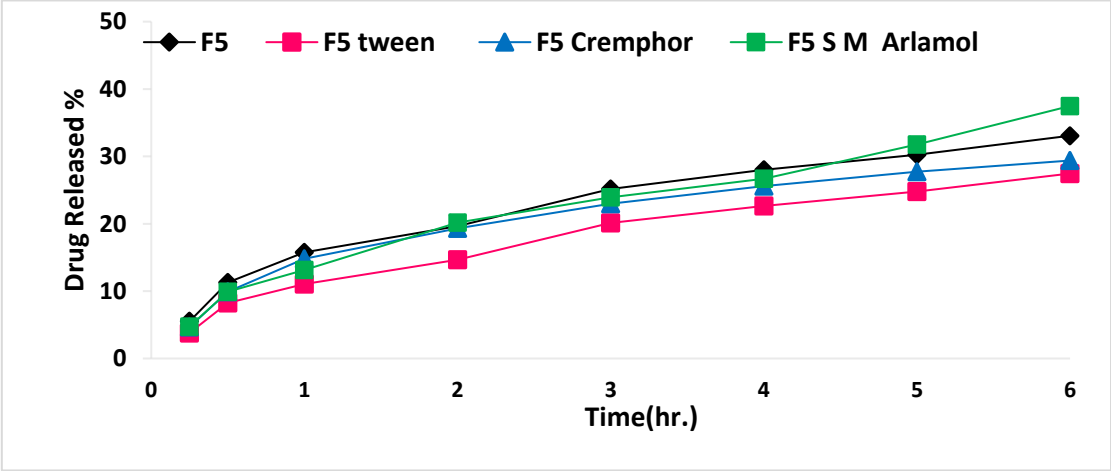


Figure 23: In vitro release the (%) drug released versus time of mucoadhesive gel (F5 without PEs, F5 with Tween 80 (F4A), F5 with Cremophor RH40 (F4B), F5 with Arlamol F5C)). per unit area of polyamide membrane (mg/cm²).

4.6.3. In Vitro Release Study of F4 formulations using dialysis membrane

The release of F4 mucoadhesive gel without penetration enhancer through dialysis membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.588 mg/cm²) and the (%) drug released versus time was equal (24.9 %). Table no. (30) displays the detailed results.

Table 30: Data obtained via diffusion of F4 mucoadhesive gel (F4 without PEs) through dialysis membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.184	0.205	0.164	0.184	0.021	11.122	0.005	10	0.052	1.045	0.052	1.045	0.333
0.5	0.264	0.273	0.255	0.264	0.009	3.409	0.007	10	0.074	1.489	0.074	1.541	0.491
1.0	0.383	0.388	0.378	0.383	0.005	1.305	0.011	10	0.108	2.152	0.108	2.227	0.709
2.0	0.539	0.589	0.49	0.539	0.050	9.178	0.015	10	0.151	3.025	0.151	3.133	0.998
3.0	0.671	0.675	0.667	0.671	0.004	0.596	0.019	10	0.188	3.759	0.188	3.911	1.245
4.0	0.799	0.834	0.765	0.799	0.035	4.316	0.022	10	0.224	4.475	0.224	4.663	1.485
5.0	0.820	0.850	0.790	0.820	0.030	3.659	0.023	10	0.230	4.590	0.230	4.814	1.533
6.0	0.855	0.890	0.805	0.850	0.043	5.026	0.024	10	0.238	4.758	0.238	4.987	1.588

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F4 mucoadhesive gel with Tween 80 (F4A) as penetration enhancer through dialysis membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.463 mg/cm^2) and the (%) drug released versus time was equal (22.9 %). Table no. (31) displays the detailed results.

Table 31: Data obtained via diffusion of F4 mucoadhesive gel with Tween 80 (F4A) as penetration enhancer through dialysis membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm^2)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.081	0.049	0.050	0.060	0.0182	30.322	0.002	10	0.018	0.351	0.018	0.351	0.112
0.5	0.096	0.081	0.096	0.091	0.009	9.517	0.003	10	0.026	0.524	0.026	0.542	0.173
1.0	0.189	0.134	0.131	0.151	0.033	21.578	0.004	10	0.043	0.861	0.043	0.887	0.282
2.0	0.280	0.272	0.270	0.274	0.005	1.931	0.008	10	0.077	1.545	0.077	1.588	0.506
3.0	0.399	0.378	0.375	0.384	0.013	3.405	0.011	10	0.108	2.159	0.108	2.236	0.712
4.0	0.515	0.504	0.504	0.507	0.006	1.251	0.014	10	0.142	2.848	0.142	2.956	0.941
5.0	0.685	0.680	0.656	0.673	0.016	2.301	0.019	10	0.189	3.774	0.189	3.917	1.247
6.0	0.801	0.789	0.770	0.786	0.016	1.987	0.022	10	0.220	4.404	0.220	4.593	1.463

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F4 mucoadhesive gel with Cremophor RH40 (F4B) as penetration enhancer through dialysis membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.028 mg/cm²) and the (%) drug released versus time was equal (16.1%). Table no. (32) displays the detailed results.

Table 32: Data obtained via diffusion of F4 mucoadhesive gel with Cremophor RH40 (F4B) as penetration enhancer through dialysis membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.079	0.081	0.116	0.092	0.021	22.618	0.003	10	0.026	0.530	0.026	0.530	0.169
0.5	0.103	0.086	0.119	0.103	0.017	16.074	0.003	10	0.029	0.590	0.029	0.616	0.196
1.0	0.194	0.097	0.133	0.141	0.049	34.694	0.004	10	0.040	0.805	0.040	0.834	0.266
2.0	0.220	0.183	0.176	0.193	0.024	12.250	0.005	10	0.055	1.093	0.055	1.133	0.361
3.0	0.370	0.238	0.218	0.275	0.083	29.997	0.008	10	0.078	1.552	0.078	1.607	0.512
4.0	0.448	0.355	0.384	0.396	0.048	12.027	0.011	10	0.111	2.224	0.111	2.301	0.733
5.0	0.556	0.451	0.482	0.496	0.054	10.869	0.014	10	0.139	2.785	0.139	2.896	0.922
6.0	0.587	0.513	0.553	0.551	0.037	6.722	0.015	10	0.154	3.090	0.154	3.229	1.028

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of the mucoadhesive gel F4 with Arlamol (F4C) as penetration enhancer through dialysis membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.592 mg/cm²) and the (%) drug released versus time was equal (25.03 %). Table no. (33) displays the detailed results.

Table 33: Data obtained via diffusion of mucoadhesive gel F4 with Arlamol (F4C) as penetration enhancer through dialysis membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.166	0.117	0.141	0.141	0.025	17.336	0.004	10	0.040	0.805	0.040	0.805	0.256
0.5	0.292	0.229	0.260	0.260	0.036	12.100	0.007	10	0.073	1.469	0.073	1.509	0.481
1.0	0.349	0.311	0.330	0.330	0.019	5.758	0.009	10	0.093	1.857	0.093	1.931	0.615
2.0	0.400	0.359	0.379	0.379	0.021	5.405	0.011	10	0.107	2.132	0.107	2.225	0.709
3.0	0.455	0.434	0.442	0.444	0.011	2.389	0.012	10	0.125	2.491	0.125	2.598	0.827
4.0	0.574	0.557	0.565	0.565	0.009	1.504	0.016	10	0.158	3.170	0.158	3.294	1.049
5.0	0.712	0.699	0.702	0.704	0.007	0.966	0.020	10	0.197	3.945	0.197	4.104	1.307
6.0	0.870	0.855	0.849	0.858	0.011	1.261	0.024	10	0.240	4.802	0.240	4.910	1.592

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The formulations of mucoadhesive gel F4 which were (F4 without PEs, F4 with Tween 80 (F4A), F4 with Cremophor RH40 (F4B), F4 with Arlamol (F4C)) were released through dialysis membrane. The cumulative amount per unit area after 6 hours for F4 with Arlamol has the highest value which was equal (1.592 mg/cm²) and the (%) drug released versus time that was equal (25.03%). Figure no. (24) was shown the (%) drug released versus time(hr.).

The descending order of (Q) per unit area for F4 by using dialysis membrane: F4 with Arlamol (F4C) >F4 without PEs>F4 with Tween 80 (F4A) >F4 with Cremophor RH40 (F4B)

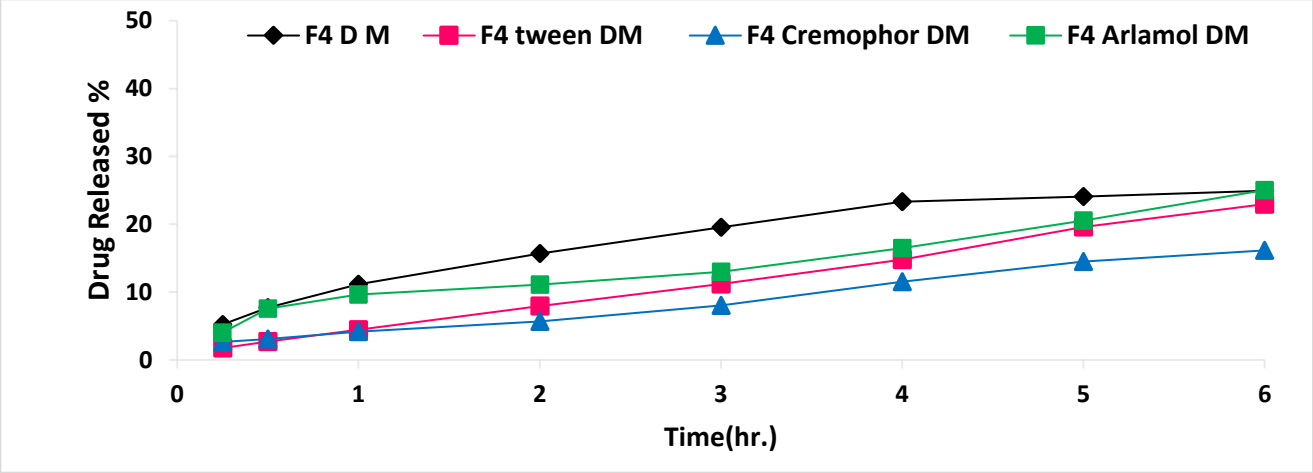


Figure 24: In vitro release the (%) drug released versus time of mucoadhesive gel (F4A), F4 with Cremophor RH40 (F4B), F4 with Arlamol (F4C)) per unit area of dialysis membrane (mg/cm²).

4.6.4. In Vitro Release Study of F5 formulations using dialysis membrane

The release of F5 mucoadhesive gel without penetration enhancer through dialysis membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.378 mg/cm²) and the (%) drug released versus time was equal (21.6%). Table no. (34) displays the detailed results.

Table 34: Data obtained via diffusion of mucoadhesive gel F5 (F5 without PEs) through dialysis membrane by Franz diffusion cell.

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.180	0.204	0.158	0.181	0.023	12.735	0.005	10	0.051	1.024	0.051	1.024	0.326
0.5	0.242	0.272	0.213	0.242	0.030	12.174	0.007	10	0.068	1.368	0.068	1.420	0.452
1.0	0.354	0.360	0.350	0.355	0.005	1.419	0.010	10	0.100	1.995	0.100	2.063	0.657
2.0	0.512	0.534	0.493	0.513	0.021	3.910	0.014	10	0.144	2.878	0.144	2.978	0.948
3.0	0.584	0.585	0.553	0.574	0.018	3.169	0.016	10	0.161	3.218	0.161	3.362	1.071
4.0	0.681	0.682	0.671	0.678	0.006	0.897	0.019	10	0.190	3.798	0.190	3.959	1.261
5.0	0.700	0.709	0.697	0.702	0.006	0.890	0.020	10	0.197	3.932	0.197	4.122	1.313
6.0	0.723	0.751	0.739	0.738	0.014	1.904	0.021	10	0.207	4.131	0.207	4.328	1.378

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F5 mucoadhesive gel with Tween 80 (F5A) as penetration enhancer through dialysis membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.462 mg/cm²) and the (%) drug released versus time was equal (22.9%). Table no. (35) displays the detailed results.

Table 35: Data obtained via diffusion of F5 mucoadhesive gel with Tween 80 (F5A) as penetration enhancer through dialysis membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.091	0.075	0.064	0.077	0.014	17.709	0.002	10	0.022	0.444	0.022	0.444	0.141
0.5	0.108	0.094	0.095	0.099	0.008	7.889	0.003	10	0.028	0.569	0.028	0.591	0.188
1.0	0.193	0.207	0.199	0.200	0.007	3.518	0.006	10	0.057	1.130	0.057	1.159	0.369
2.0	0.344	0.210	0.326	0.293	0.073	24.794	0.008	10	0.083	1.653	0.083	1.709	0.544
3.0	0.513	0.461	0.503	0.492	0.028	5.604	0.014	10	0.138	2.763	0.138	2.845	0.906
4.0	0.589	0.552	0.607	0.583	0.028	4.813	0.016	10	0.163	3.267	0.163	3.405	1.084
5.0	0.695	0.686	0.698	0.693	0.006	0.901	0.019	10	0.194	3.882	0.194	4.045	1.288
6.0	0.784	0.780	0.792	0.785	0.006	0.778	0.0220	10	0.220	4.397	0.220	4.591	1.462

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F5 mucoadhesive gel with Cremophor RH40 (F5B) as penetration enhancer through dialysis membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.005mg/cm²) and the (%) drug released versus time was equal (15.8%). Table no. (36) displays the detailed results.

Table 36: Data obtained via diffusion of F5 mucoadhesive gel with Cremophor RH40 (F5B) as penetration enhancer through dialysis membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.068	0.065	0.071	0.068	0.003	4.412	0.002	10	0.020	0.397	0.020	0.396	0.126
0.5	0.100	0.091	0.110	0.100	0.010	9.473	0.003	10	0.029	0.576	0.029	0.596	0.190
1.0	0.119	0.103	0.151	0.124	0.024	19.657	0.004	10	0.036	0.710	0.036	0.739	0.235
2.0	0.189	0.208	0.214	0.204	0.013	6.408	0.006	10	0.058	1.153	0.058	1.188	0.378
3.0	0.276	0.270	0.280	0.275	0.005	1.828	0.008	10	0.078	1.552	0.078	1.610	0.513
4.0	0.398	0.400	0.407	0.402	0.005	1.177	0.011	10	0.113	2.257	0.113	2.333	0.743
5.0	0.460	0.470	0.483	0.471	0.012	2.449	0.013	10	0.132	2.644	0.132	2.757	0.878
6.0	0.537	0.550	0.530	0.539	0.010	1.883	0.015	10	0.151	3.023	0.151	3.155	1.005

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The release of F5 mucoadhesive gel with Arlamol (F5C) as penetration enhancer through dialysis membrane, the cumulative amount released per unit area over periods of time around 6 hours which was equal (1.531 mg/cm²) and the (%) drug released versus time was equal (24.04%). Table no. (37) displays the detailed results.

Table 37: Data obtained via diffusion of F5 mucoadhesive gel with Arlamol (F5C) as penetration enhancer through dialysis membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.163	0.175	0.169	0.169	0.006	3.550	0.005	10	0.0480	0.959	0.048	0.959	0.306
0.5	0.208	0.233	0.220	0.220	0.012	5.675	0.006	10	0.062	1.246	0.062	1.294	0.412
1.0	0.309	0.334	0.332	0.325	0.014	4.275	0.009	10	0.091	1.829	0.091	1.892	0.602
2.0	0.437	0.458	0.459	0.451	0.012	2.753	0.013	10	0.127	2.534	0.127	2.626	0.836
3.0	0.517	0.533	0.539	0.530	0.011	2.147	0.015	10	0.149	2.971	0.149	3.098	0.987
4.0	0.559	0.647	0.650	0.619	0.052	8.356	0.017	10	0.173	3.467	0.173	3.616	1.152
5.0	0.687	0.704	0.710	0.700	0.012	1.704	0.020	10	0.196	3.923	0.196	4.096	1.304
6.0	0.820	0.824	0.828	0.824	0.004	0.485	0.023	10	0.231	4.613	0.231	4.809	1.531

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The mucoadhesive gel F5 with all formulations which were (F5 without PEs, F5 with Tween 80 (F5A), F5 with Cremophor RH40 (F5B), F5 with Arlamol (F5C)) through dialysis membrane had affected in presence of penetration enhancers. The highest value of cumulative amount released per unit area was F5 with Arlamol that was equal (1.531 mg/cm²) which was more than F5 without penetration enhancer. Figure no. (25) was shown the results.

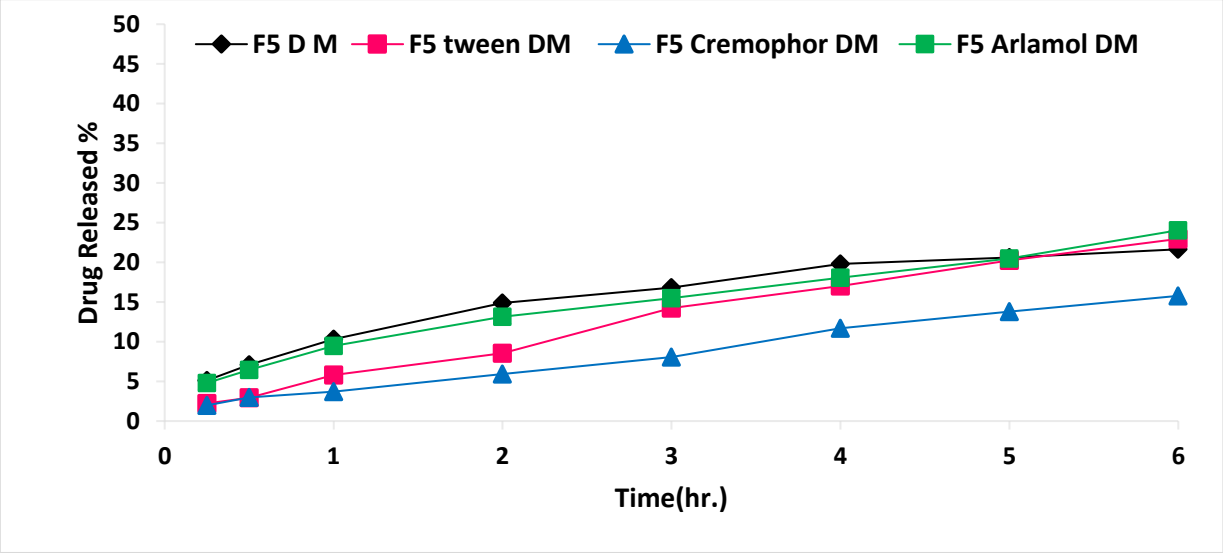


Figure 25: In vitro release the (%) drug released versus time of mucoadhesive gel *(*F5 without PEs, F5 with Tween 80 (F5A), F5 with Cremophor RH40 (F5B), F5 with Arlamol (F5C)) per unit area of dialysis membrane (mg/cm²)

4.6.5. Effects of PEs with different types on the release of mucoadhesive Tinidazole gel through synthetic (polyamide) and dialysis membrane

Tinidazole release from mucoadhesive gel (F4 without PEs, F4 with Tween 80 (F4A), F4 with Cremophor RH40 (F4B), F4 with Arlamol (F4C)) and the mucoadhesive gel F5 with all formulations which were (F5 without PEs, F5 with Tween 80 (F5A), F5 with Cremophor RH40 (F5B), F5 with Arlamol (F5C)) was examined through synthetic and dialysis membranes. The penetration enhancers concentrations were maintained at 0.3% for all formulations, it was found that formulation F4C has the highest amount of cumulative released per unit area during 6 hours using synthetic membrane. Hence, Arlamol (PPG 15 Stearyl Ether) is used as surfactant, emulsifier and solubilizer agent and generally it is used to enhance the solubility , release and permeation (Lanigan 2001) (Bergfeld et al. 2022). In this study the use of Polysorbate 80 (Tween 80) and Cremophor RH40 (Kolliphor RH40) as penetration enhancers resulted in decrease of the cumulative amount of drug released per unit area.

Tween 80 is used as solubilizer ,emulsifier and surfactant in many pharmaceutical formulations (Schwartzberg and Navari 2018) (Hassan 2015), when used it in the formulation it was found to behave as penetration inhibitor due to possible increase of solubility of Tinidazole in the formulation. Low concentrations of Polysorbate 80 were used to mitigate any possible toxicity and to maintain the physical properties of the formulation. Shahsavandi et al investigated the toxicity of various concentrations of Tween 80 against chicken embryos , high concentrations induced mortality of chicken (Shahsavandi et al. 2020). In our study we used low

concentrations of Tween 80 (0.3%) which is considered safe and enhanced the solubility of drug, with minimum effect on taste.

Cremophor RH40 is non anionic emulsifying agent, solubilizer and penetration enhancer in order to solubilize the hydrophobic active ingredient in the solvent. This substance forms “ex tempore” small (10-30nm) to coat the particles physically in order to solubilize them (Katona et al. 2022). In the formulations of Tinidazole gel Cremophor RH40 was acted as penetration inhibitor so the drug released was slower than the formulations without PEs.

4.7. In Vitro Drugs Release Kinetics Results

4.7.1. In Vitro Drugs Release Kinetics for formulations by using synthetic (polyamide)

We noticed that all results were belonged to **Makoid-Banakar** model or **Weibull** model which were considered goodness of fit according to highest value of coefficient regression **(R^2)** and the best value for the model release type. The results for in vitro drugs release that was followed **Makoid-Banakar** model (F4 with Arlamol) the release exponent **(n)** was considered the indicator for the of diffusion followed by these formulations. In this study **(R^2)** value (**0.9965**) whereas (**n)** value **(0.505)** which regarded non-Fickian diffusion and c values approached to zero. These results were observed in previous studies as Lornoxicam controlled release transdermal patch gel that **(R^2)** values which in the range of (0.998-0.999) and (n) value which were found between (0.5-1) also c values approached to zero.

While for the results for in vitro drugs release that were followed **Weibull** model, which were (F without PEs, F4 with Tween 80, F4 with Cremophor RH40, F5 without PEs, F5 with Tween 80, F5 Cremophor RH 40, F5 with Arlamol). In this study **(R^2)** values in the range of **(0.9919-0.9998)**, and **β** was found in the range of **(0.400-1.309)** so all results belonged to **β ≤ 0.75** was correlated with the Fickian diffusion that but only **β (1.309) >1** that represented the release mechanism is quite complex**.**

In previous studies showed that of **β ≤ 0.75** was correlated with the Fick diffusion while β values in the range of range of **(0.75 < β < 1)** were correlated with a combined mechanism (Fick diffusion and swelling controlled transport). For the shape factor **β > 1**, the release mechanism is quite complex: at first the rate of release increased non-linearly up to the inflection point and after that decreased asymptotically (Corsaro et al. 2021).

Table 38: Model fitting of Tinidazole mucoadhesive buccal gel by using synthetic (polyamide) membrane

Mathematical models	F4 without PEs	F4 with Tween 80	F4 with Cremophor	F4 with Arlamol	F5 without PEs	F5 with Tween 80	F5 with Cremophor	F5 with Arlamol
Zero-order								
R^2	0.6187	0.8284	0.6899	0.9122	0.5690	0.7430	0.5154	0.8134
k_0 (h^{-1})	6.704	5.035	6.105	7.368	6.561	5.311	5.978	6.810
First-order								
R^2	0.7334	0.8638	0.7859	0.9519	0.6934	0.8135	0.6393	0.8796
k_1 (h^{-1})	0.083	0.058	0.074	0.092	0.081	0.062	0.072	0.084
Higuchi								
R^2	0.9909	0.9556	0.9883	0.9579	0.9842	0.9895	0.9738	0.9803
k_H ($h^{-1/2}$)	14.165	10.451	12.859	15.178	13.905	11.139	12.710	14.193
Korsmeyer Peppas								
R^2	0.9923	0.9680	0.9884	0.9921	0.9879	0.9913	0.9808	0.9888
Kkp $^{(h-n)}$	14.605	9.283	12.725	12.197	14.595	10.715	13.560	12.941
n	0.476	0.590	0.508	0.664	0.463	0.530	0.450	0.570
Makoid-Banakar								
R^2	0.9925	0.9910	0.9961	**0.9965**	0.9908	0.9923	0.9920	0.9900
n	0.497	0.300	0.665	0.505	0.543	0.588	0.607	0.502
k_{MB}	14.745	8.369	13.524	11.726	15.151	10.947	14.621	12.622
c	0.009	-0.114	0.063	-0.059	0.034	0.023	0.067	-0.027
Weibull								
R^2	**0.9970**	**0.9919**	**0.9989**	0.9962	**0.9976**	**0.9967**	**0.9998**	**0.9934**
Td (h)	0.106	-2.075	0.176	-0.544	0.167	0.110	0.209	-0.044
α	5.815	46.683	6.357	12.203	5.540	8.082	5.784	7.456
β	0.490	1.309	0.484	0.984	0.444	0.532	0.400	0.661
Goodness of fit	**Weibull**	**Weibull**	**Weibull**	**Makoid-Banakar**	**Weibull**	**Weibull**	**Weibull**	**Weibull**

4.7.2. In Vitro Drugs Release Kinetics for formulations by using dialysis membrane

By using dialysis membrane all results were belonged to **Makoid-Banakar** model or **Weibull** model which were considered goodness of fit according to highest value of coefficient regression **(R^2)** and the best value for the model release type. The results for in vitro drugs release that were followed **Makoid-Banakar** model (F5 without PEs), the coefficient regression **(R^2)** value **(0.9975)** and the release exponent **(n)** which indicated the diffusion **(0.568)** while **(c)** values approached

zero. However, the remained formulations followed **Weibull** model, which were (F4 without PEs, F4 with Tween 80, F4 with Cremophor RH 40, F4 with Arlamol, F5 with Tween 80, F5 Cremophor RH 40, F5 with Arlamol). In this study **(R^2)** values in the range of **(0.9859-0.9993)**. **β** was found in the range of **(0.478 -2.455)**. The formulations were had **β (0.478,0.653)** belonged to $\beta \leq 0.75$ was correlated with the Fick diffusion while **β** equal **(0.902)** ($0.75 < \beta < 1$) were correlated with a combined mechanism (Fick diffusion and swelling controlled transport). Other formulations were followed $\beta > 1$, the release mechanism is quite complex: at first the rate of release increased non-linearly up to the inflection point and after that decreased asymptotically. as discussed above from previous literature (Corsaro et al. 2021).

Table 39: Model fitting of Tinidazole mucoadhesive buccal gel by using dialysis membrane

Mathematical models	F4 without PEs	F4 with Tween 80	F4 with Cremophor	F4 with Arlamol	F5 without PEs	F5 with Tween 80	F5 with Cremophor	F5 with Arlamol
Zero-order								
R^2	0.5958	0.9948	0.9515	0.7777	0.4528	0.9728	0.9669	0.7247
k_0 (h^{-1})	5.142	3.838	2.816	4.319	4.462	4.102	2.760	4.442
First-order								
R^2	0.6925	0.9938	0.9567	0.8074	0.5535	0.9851	0.9730	0.7808
k_1 (h^{-1})	0.060	0.042	0.030	0.049	0.051	0.046	0.030	0.051
Higuchi								
R^2	0.9854	0.8656	0.8956	0.9324	0.9756	0.9135	0.9063	0.9926
k_H ($h^{-1/2}$)	10.885	7.698	5.726	8.983	9.506	8.332	5.607	9.318
Korsmeyer Peppas								
R^2	0.9871	0.9956	0.9711	0.9390	0.9890	0.9946	0.9861	0.9932
Kkp $^{(h-n)}$	11.263	4.128	3.817	8.257	10.356	5.570	3.713	9.124
n	0.474	0.952	0.798	0.564	0.434	0.798	0.804	0.516
Makoid-Banakar								
R^2	0.9955	0.9986	0.9879	0.9815	**0.9975**	0.9948	0.9921	0.9970
n	0.625	0.701	0.385	0.205	0.568	0.846	0.540	0.411
k_{MB}	11.972	4.176	3.694	7.103	11.030	5.583	3.651	8.731
c	0.062	-0.077	-0.137	-0.146	0.057	0.016	-0.087	-0.043
Weibull								
R^2	**0.9956**	**0.9993**	**0.9965**	**0.9859**	0.9970	**0.9975**	**0.9975**	**0.9971**
Td (h)	0.104	-0.660	-1.909	-6.671	0.122	-0.102	-0.904	-0.269
α	7.761	44.469	122.572	1799.144	8.410	19.335	55.706	12.541
β	0.478	1.295	1.502	2.455	0.422	0.902	1.179	0.653
Goodness of fit	**Weibull**	**Weibull**	**Weibull**	**Weibull**	**Makoid-Banakar**	**Weibull**	**Weibull**	**Weibull**

The kinetics model was evaluated the Tinidazole mucoadhesive buccal gel release through synthetic and dialysis membranes so the highest value of regression coefficient R^2 expressed the best fitted model of release through synthetic (polyamide) membrane and through dialysis membrane. In prior studies showed that used gelling agents effected on the released of Lornoxicam as controlled release transdermal gel patch and when increase the concentrations of Carbopol in the range of (0.5-1%) that was observed that when increased the conc. of gelling agents, the drug released was decreased. In another study showed that. Also, another study

when used Tinidazole as mucoadhesive oral gel showed that formulas containing carbomer exhibited maximum swelling values with lower release rates and best mucoadhesion (Sulayman 2011). In this study F4 showed more drug released when comparison with F5 because the concentrations of gelling agent as HPMC grades more than in F5 also F5 contained Xanthan gum while F4 without it (Hashmat et al. 2020).

Moreover , the cumulative amount of mucoadhesive gel that released per unit area of polyamide membrane more than the cumulative amount mucoadhesive gel that released per unit area of dialysis membrane and that was due to the characteristics of polyamide membrane which considered synthetic membrane , the thickness 100 μm ,pore size 0.45 μm and the diameter 47 mm while the dialysis membrane which considered tubing cellulose , the thickness 160 μm, the diameter 49 mm and average flat width 76mm. Figures (22 and 23) were shown the mucoadhesive gel release by synthetic (polyamide) membrane while figures (24 and 25) were shown the mucoadhesive gel release by dialysis membrane.

4.8. Results of Permeation study

4.8.1. In Vitro Permeation Study of F4 formulations using Chicken Eggshell as Biological Membrane

The permeation of F4 mucoadhesive gel without penetration enhancer through Chicken eggshell as biological membrane, the cumulative amount permeated per unit area over periods of time around 6 hours which was equal (1.963mg/cm^2). Table no. (40) shows the detailed results.

Table 40: Data obtained via permeation of F4 mucoadhesive gel (F4 without penetration enhancer) through Chicken Eggshell membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm^2)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.218	0.22	0.248	0.229	0.017	7.335	0.006	10	0.065	1.292	0.065	1.292	0.412
0.5	0.311	0.313	0.314	0.313	0.002	0.489	0.009	10	0.088	1.761	0.088	1.825	0.581
1.0	0.393	0.395	0.396	0.394	0.002	0.387	0.011	10	0.111	2.218	0.111	2.306	0.734
2.0	0.481	0.483	0.484	0.483	0.002	0.316	0.014	10	0.135	2.709	0.135	2.820	0.898
3.0	0.637	0.645	0.646	0.643	0.005	0.768	0.018	10	0.180	3.601	0.180	3.737	1.190
4.0	0.809	0.812	0.821	0.814	0.006	0.767	0.023	10	0.228	4.559	0.228	4.737	1.509
5.0	0.439	0.457	0.469	0.455	0.015	3.319	0.013	20	0.255	5.109	0.255	5.337	1.610
6.0	0.524	0.526	0.530	0.527	0.003	0.580	0.015	20	0.295	5.908	0.295	6.164	1.963

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The permeation of F4 mucoadhesive gel (F4C) with Arlamol as penetration enhancer through Chicken eggshell as biological membrane, the cumulative amount permeated per unit area over periods of time around 6 hours which was equal (2.582 mg/cm²). Table no. (41) shows the detailed results.

Table 41: Data obtained via permeation of F4 mucoadhesive gel with Arlamol (F4C) as penetration enhancer through Chicken Eggshell membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.161	0.163	0.176	0.167	0.008	4.887	0.005	10	0.047	0.946	0.047	0.946	0.301
0.5	0.375	0.389	0.390	0.385	0.008	2.180	0.011	10	0.108	2.162	0.108	2.210	0.704
1.0	0.425	0.442	0.459	0.442	0.017	3.846	0.012	10	0.124	2.482	0.124	2.590	0.825
2.0	0.680	0.684	0.686	0.683	0.003	0.447	0.019	10	0.191	3.828	0.191	3.952	1.259
3.0	0.839	0.842	0.843	0.841	0.002	0.247	0.024	10	0.235	4.709	0.235	4.901	1.561
4.0	0.950	0.955	0.965	0.957	0.008	0.798	0.027	10	0.268	5.353	0.268	5.588	1.780
5.0	0.590	0.595	0.598	0.594	0.004	0.680	0.017	20	0.333	6.663	0.333	6.931	2.207
6.0	0.690	0.693	0.699	0.694	0.005	0.660	0.019	20	0.389	7.775	0.389	8.108	2.582

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

Tables no. (40 and 41) was expressed the results of the formulations of mucoadhesive gel F4 which were (F4 without PEs, F4 with Arlamol (F4C)) were permeated through Chicken eggshell membrane. The cumulative amount permeated per unit area after 6 hours for F4 with Arlamol (F4C) was more than F4 without penetration enhancer and the value was equal (2.582 mg/cm²). Figure no. (26) was shown the in vitro permeation the cumulative amount versus time of mucoadhesive.

The descending order of (Q) per unit area for F4 by using Chicken eggshell membrane: F4with Arlamol (F4C) >F4without PEs

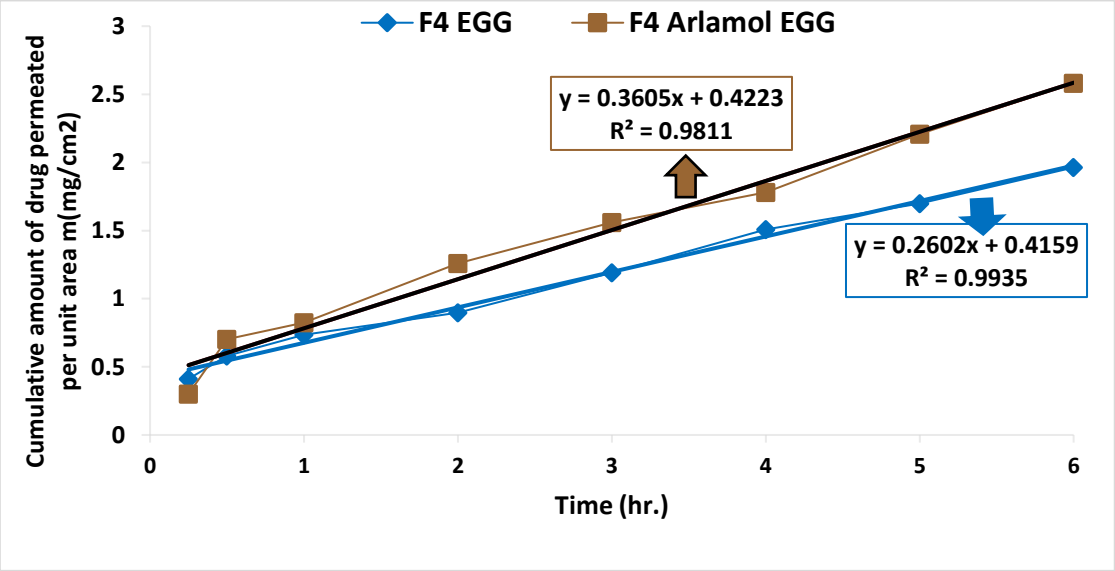


Figure 26: In vitro permeation the cumulative amount versus time of mucoadhesive gel (F4 without PE & F4 with Arlamol as PE) per unit area of Chicken Eggshell membrane (mg/cm²).

4.8.2. In Vitro Permeation Study of F5 formulations using Chicken Eggshell as Biological Membrane

The permeation of F5 mucoadhesive gel without penetration enhancer through Chicken eggshell as biological membrane, the cumulative amount permeated per unit area over periods of time around 6 hours which was equal (1.494 mg/cm²). Table no. (42) shows the detailed results.

Table 42: Data obtained via permeation of F5 mucoadhesive gel (F5 without penetration enhancer) through Chicken Eggshell membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.121	0.122	0.107	0.117	0.008	7.188	0.003	10	0.033	0.667	0.033	0.667	0.213
0.5	0.165	0.167	0.169	0.167	0.002	1.198	0.005	10	0.047	0.948	0.047	0.981	0.313
1.0	0.210	0.215	0.223	0.216	0.007	3.036	0.006	10	0.061	1.221	0.061	1.269	0.404
2.0	0.319	0.318	0.311	0.316	0.004	1.379	0.009	10	0.089	1.779	0.089	1.860	0.592
3.0	0.456	0.429	0.423	0.436	0.018	4.032	0.012	10	0.122	2.449	0.122	2.538	0.808
4.0	0.585	0.580	0.573	0.579	0.006	1.040	0.016	10	0.162	3.248	0.162	3.370	1.073
5.0	0.710	0.712	0.725	0.716	0.008	1.138	0.020	10	0.200	4.008	0.200	4.171	1.328
6.0	0.802	0.793	0.812	0.802	0.010	1.185	0.022	10	0.225	4.492	0.225	4.692	1.494

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The permeation of F5 mucoadhesive gel with Arlamol (F5C) as penetration enhancer through Chicken eggshell as biological membrane, the cumulative amount permeated per unit area over periods of time around 6 hours which was equal (1.851 mg/cm²). Table no. (43) shows the detailed results.

Table 43: Data obtained via permeation of F5 mucoadhesive gel with Arlamol (F5C) as penetration enhancer through Chicken Eggshell membrane by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.131	0.125	0.135	0.130	0.005	3.862	0.004	10	0.037	0.744	0.037	0.744	0.237
0.5	0.199	0.198	0.201	0.199	0.002	0.766	0.006	10	0.056	1.300	0.056	1.166	0.371
1.0	0.252	0.255	0.260	0.256	0.004	1.581	0.007	10	0.072	1.443	0.072	1.499	0.477
2.0	0.415	0.422	0.423	0.420	0.004	1.038	0.012	10	0.118	2.359	0.118	2.431	0.774
3.0	0.551	0.555	0.559	0.555	0.004	0.721	0.016	10	0.156	3.112	0.156	3.230	1.029
4.0	0.711	0.715	0.718	0.715	0.003	0.491	0.020	10	0.200	4.003	0.200	4.169	1.328
5.0	0.860	0.861	0.862	0.861	0.001	0.116	0.024	10	0.241	4.819	0.241	5.019	1.598
6.0	0.999	0.998	0.990	0.996	0.005	0.495	0.028	10	0.279	5.570	0.279	5.811	1.851

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

Tables no. (42 and 43) were shown the results of the formulations of mucoadhesive gel F5 which were (F5 without PEs, F5 with Arlamol) were permeated through Chicken eggshell membrane. The cumulative amount permeated per unit area after 6 hours for F5 with Arlamol had the highest value which was equal (1.851 mg/cm²). Figure no. (27) was shown the In vitro permeation the cumulative amount versus time of mucoadhesive.

The descending order of (Q) per unit area for F5 by using Chicken eggshell membrane: F5with Arlamol (F5C) >F5without PEs

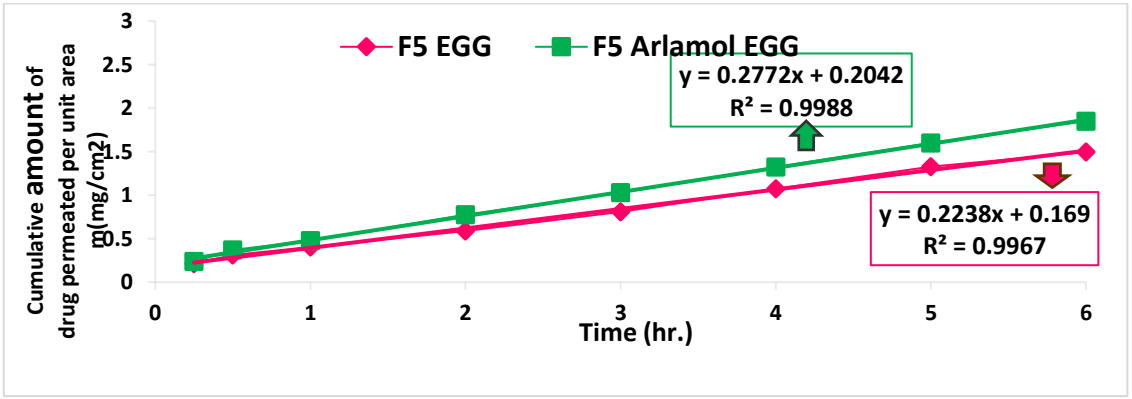


Figure 27: In vitro permeation the cumulative amount versus time of mucoadhesive gel (F5 without PE &F5 with Arlamol as PE) per unit area of Chicken Eggshell membrane (mg/cm²).

In conclusion the optimized formula from in vitro release of mucoadhesive gels by using the synthetic and the dialysis membrane which were taken and applied for in vitro permeation of mucoadhesive gels which were (F4 without PEs, F4 with Arlamol, F5 without PEs, F5 with Arlamol) and that was done by using Chicken eggshell as biological membrane we noticed that the F4 with penetration enhancers (Arlamol) provided the best results according to permeation parameters. Figure no. (28) was shown the results. The descending order for formulations by using Chicken eggshell membrane:

F4 with Arlamol (F4C) > F4 without PEs > F5 with Arlamol (F5C) > F5 without PEs

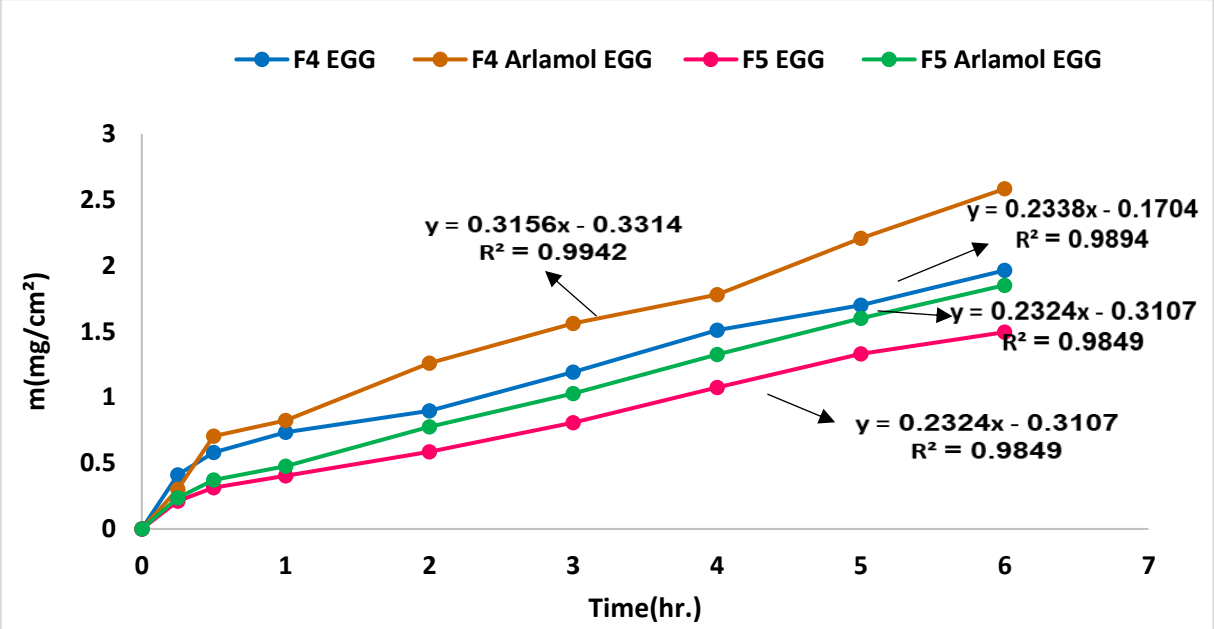


Figure 28: m (mg/cm²) per time(hr.) for all formulations through Chicken Eggshell membrane.

4.8.3. Results of Diffusion Parameters for Chicken Eggshell Membrane

The previous literature agreement the results when used Salbutamol as mucoadhesive buccal patch the permeation increased when used different

concentrations of penetration enhancer (5% w/w) through pig buccal mucosa so with the comparison between formulation (with and without PEs) observed that in the presence of PEs produced better permeation in these formulations (Prasanth et al. 2014). In our study we used Arlamol as penetration enhancer and the concentration was (0.3% w/w) by using Chicken eggshell membrane therefore, Tinidazole showed less permeation in the formulations without penetration enhancers than the formulations contained Arlamol as penetration when used Chicken eggshell membrane because Arlamol used in the formulations to enhance the permeability. The flux (Jss) for F4 with Arlamol and F5 with Arlamol were (0.3605 mg/cm²/h), (0.2772 mg/cm²/h) respectively, also the permeability coefficient (P) for F4 with Arlamol was (0.0058 cm/hr.) and for F5 with Arlamol was (0.0044 cm/hr.) as a result of this F4 with Arlamol higher than F5 with Arlamol. The table no. (44) shows the diffusion parameters calculations.

Table 44: Diffusion Parameters Calculations for formulations of Chicken Eggshell Membrane

Formulation trial	**Slope (flux) Jss (mg/cm^2/h)**	**Permeability Coefficient (P) (cm/hr.)**
F4 without PEs	0.2602	0.0042
F4 with Arlamol (F4C)	0.3605	0.0058
F5 without PEs	0.2383	0.0038
F5 with Arlamol (F5C)	0.2772	0.0044

4.8.4. In Vitro Permeation Study of F4 formulations using Permeapad® as Biomimetic Barrier

The permeation of mucoadhesive gel F4 without penetration enhancer through Permeapad® barrier as biomimetic membrane, the cumulative amount permeated per unit area over periods of time around 6 hours which was equal (1.573 mg/cm²). Table no. (45) shows the detailed results.

Table 45: Data obtained via permeation of F4 mucoadhesive gel (F4 without penetration enhancer) through Permeapad® barrier by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.084	0.078	0.091	0.084	0.007	7.715	0.002	10	0.024	0.487	0.024	0.487	0.155
0.5	0.142	0.133	0.143	0.139	0.006	3.953	0.004	10	0.040	0.794	0.040	0.818	0.261
1.0	0.196	0.215	0.240	0.217	0.022	10.170	0.006	10	0.061	1.227	0.061	1.267	0.403
2.0	0.297	0.318	0.317	0.311	0.019	3.813	0.009	10	0.087	1.749	0.087	1.811	0.577
3.0	0.422	0.438	0.437	0.432	0.009	2.073	0.012	10	0.121	2.428	0.121	2.516	0.801
4.0	0.545	0.555	0.559	0.553	0.007	1.304	0.016	10	0.155	3.101	0.155	3.222	1.026
5.0	0.700	0.722	0.731	0.718	0.016	2.222	0.020	10	0.201	4.020	0.201	4.175	1.329
6.0	0.845	0.843	0.852	0.847	0.005	0.558	0.0237	10	0.237	4.739	0.237	4.940	1.573

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The permeation of mucoadhesive gel F4 with Arlamol (F4C) as penetration enhancer through Permeapad® barrier as biomimetic membrane, the cumulative amount permeated per unit area over periods of time around 6 hours which was equal (2.614 mg/cm²). Table no. (46) shows the detailed results.

Table 46: Data obtained via permeation of F4 mucoadhesive gel with Arlamol (F4C) as penetration enhancer through Permeapad® barrier by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.139	0.118	0.119	0.125	0.012	9.452	0.004	10	0.036	0.716	0.036	0.716	0.228
0.5	0.160	0.166	0.154	0.160	0.006	3.750	0.005	10	0.045	0.909	0.045	0.945	0.301
1.0	0.250	0.249	0.260	0.253	0.006	2.404	0.007	10	0.071	1.428	0.071	1.473	0.469
2.0	0.430	0.459	0.449	0.446	0.015	3.302	0.013	10	0.125	2.504	0.125	2.576	0.820
3.0	0.620	0.630	0.645	0.631	0.012	1.992	0.018	10	0.177	3.540	0.177	3.665	1.167
4.0	0.860	0.865	0.869	0.865	0.005	0.522	0.024	10	0.242	4.839	0.242	5.016	1.598
5.0	0.540	0.545	0.559	0.548	0.010	1.797	0.015	20	0.307	6.146	0.307	6.388	2.035
6.0	0.701	0.705	0.710	0.705	0.005	0.639	0.020	20	0.395	7.902	0.395	8.209	2.614

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

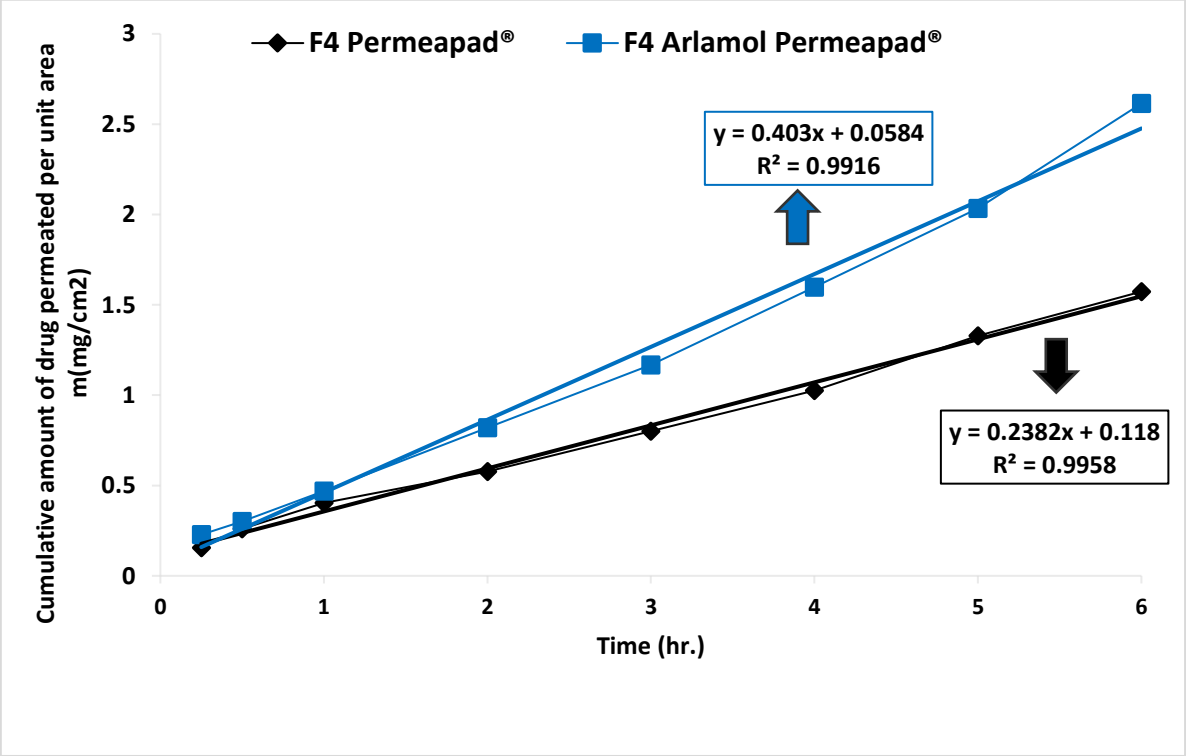


Figure 29**:** In vitro permeation the cumulative amount versus time of mucoadhesive gel (F4 without PE & F4 with Arlamol as PE) per unit area of Permeapad® membrane (mg/cm²)

4.8.5. In Vitro Permeation Study of F5 formulations using Permeapad® as Biomimetic Barrier

The permeation of F5 mucoadhesive gel without penetration enhancer through Permeapad® barrier as biomimetic membrane, the cumulative amount permeated per unit area over periods of time around 6 hours which was equal (1.537 mg/cm²). Table no. (47) shows the detailed results.

Table 47: Data obtained via permeation of F5 mucoadhesive gel (F5 without penetration enhancer) through Permeapad® barrier by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.116	0.123	0.110	0.113	0.007	5.593	0.003	10	0.033	0.666	0.033	0.666	0.212
0.5	0.150	0.162	0.156	0.156	0.006	3.846	0.004	10	0.044	0.887	0.044	0.920	0.293
1.0	0.225	0.235	0.241	0.234	0.008	3.459	0.007	10	0.066	1.320	0.066	1.364	0.435
2.0	0.352	0.360	0.355	0.356	0.004	1.136	0.010	10	0.100	2.000	0.100	2.066	0.658
3.0	0.459	0.449	0.448	0.452	0.006	1.346	0.013	10	0.127	2.538	0.127	2.638	0.840
4.0	0.602	0.577	0.551	0.577	0.026	4.422	0.016	10	0.161	3.233	0.161	3.360	1.070
5.0	0.734	0.721	0.709	0.721	0.013	1.733	0.020	10	0.202	4.040	0.202	4.201	1.338
6.0	0.835	0.824	0.820	0.826	0.008	0.940	0.023	10	0.231	4.626	0.231	4.828	1.537

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

The permeation of mucoadhesive gel F5 with Arlamol (F5C) as penetration enhancer through Permeapad® barrier as biomimetic membrane, the cumulative amount permeated per unit area over periods of time around 6 hours which was equal (1.914 mg/cm²). Table no. (48) shows the detailed results.

Table 48: Data obtained via permeation of F5 mucoadhesive gel with Arlamol (F5C) as penetration enhancer through Permeapad® barrier by Franz diffusion cell

Time (hr.)[1]	**SA1[2]**	**SA2**	**SA3**	**Av. Abs[3] (AU)[4]**	**SD[5]**	**RSD%[6]**	**Cn (mg/ml)[7]**	**Dilution factor**	**(Ci) Final conc. (mg/ml) without dilution[8]**	**Ci*VR[9]**	**Ci*Vcol[10]**	**Q (mg)[11]**	**m(mg/cm²)[12]**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.125	0.129	0.135	0.130	0.005	3.882	0.004	10	0.037	0.740	0.037	0.740	0.236
0.5	0.189	0.190	0.198	0.192	0.005	2.565	0.005	10	0.054	1.089	0.054	1.126	0.359
1.0	0.250	0.265	0.300	0.272	0.026	9.445	0.008	10	0.077	1.532	0.077	1.586	0.505
2.0	0.432	0.430	0.450	0.437	0.011	2.519	0.012	10	0.123	2.456	0.123	2.532	0.807
3.0	0.590	0.600	0.624	0.605	0.017	2.890	0.017	10	0.169	3.389	0.169	3.512	1.119
4.0	0.711	0.750	0.779	0.747	0.034	4.570	0.021	10	0.209	4.181	0.209	4.351	1.386
5.0	0.870	0.879	0.889	0.879	0.010	1.081	0.025	10	0.246	4.921	0.246	5.130	1.633
6.0	0.507	0.512	0.522	0.514	0.008	1.487	0.014	20	0.288	5.763	0.288	6.010	1.914

1: Hour 2: Sample 3: Average absorbance 4: Absorbance unit 5: Standard deviation 6: Relative standard deviation % 7: Concentration in receptor compartment 8: Final conc. (mg/ml) without dilution 9: Volume of receptor compartment 10: Sample volume 11: Cumulative amount (mg) 12: Cumulative amount released per unit area of synthetic (polyamide) membrane

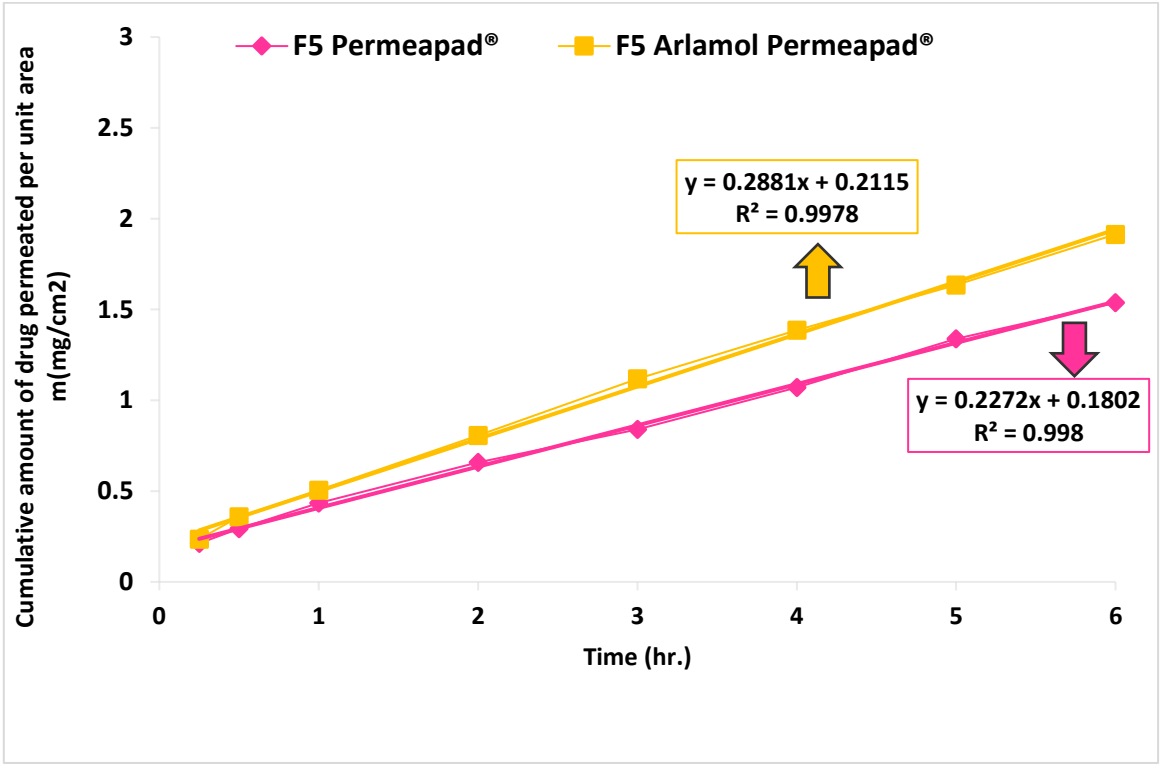


Figure 30: In vitro permeation the cumulative amount versus time of (F5 without PE &F5 with Arlamol as PE) mucoadhesive gel per unit area of Permeapad® membrane (mg/cm²).

Lastly, the optimized formula from in vitro release of mucoadhesive gels were applied for in vitro permeation of mucoadhesive gels which were (F4 without PEs, F4 with Arlamol (F4C), F5 without PEs, F5 with Arlamol (F5C)) and that was done by using Permeapad®

as biomimetic barrier, we noticed that the F4 with penetration enhancers Arlamol (F4C) provided the best results according to permeation parameters while regression coefficient R^2 was (0.9916).For F5 with Arlamol which had regression coefficient R^2 (0.9978). Figure no. (31) was shown the results. The descending order for formulations by using Permeapad® membrane:

F4with Arlamol (F4C) by Permeapad® > F5 with Arlamol (F5C) by Permeapad® > F4 without PEs by Permeapad® > F5 without PEs by Permeapad®.

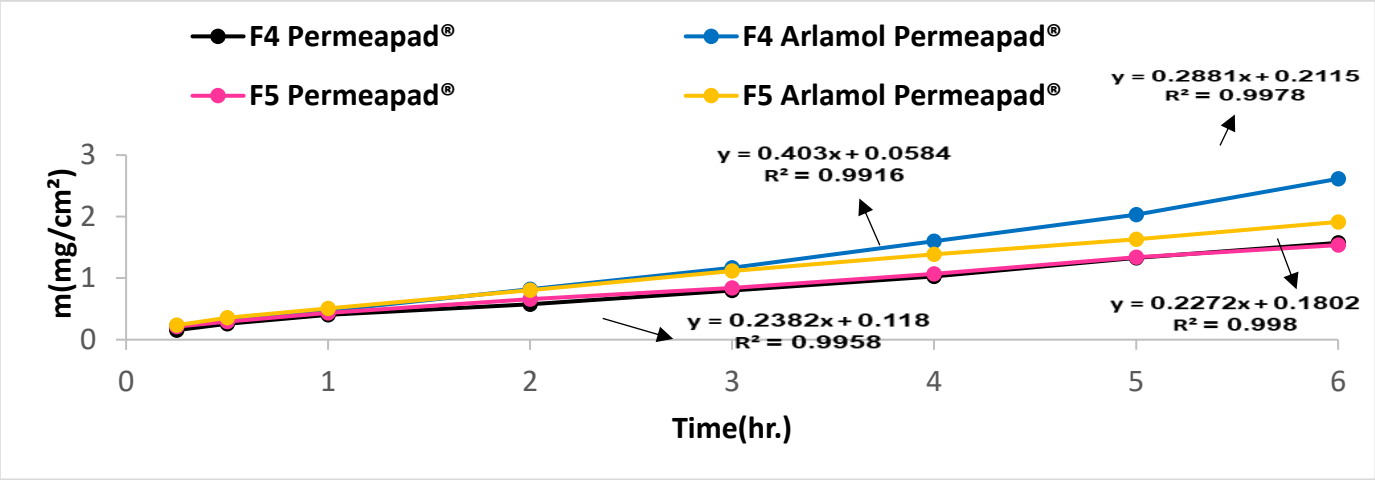


Figure 31: m (mg/cm²) per time (hr.) for all formulations through Permeapad® membrane.

4.8.6. Results of Diffusion Parameters for Permeapad® barrier as Biomimetic Membrane

The results of permeation through Permeapad® membrane were compared to published in vitro, ex vivo and in vivo studies for the same formulations. The permeability results of metoprolol by using the Permeapad® membrane related very well to both in vitro and ex vivo studies, (R^2 = 0.98 and 0.97), respectively. Moreover, for in vitro in vivo had excellent correlation IVIVC (R^2 = 0.98) was acquired when in comparison between apparent permeability coefficient to the absolute bioavailability of metoprolol administered by buccal route to mini-pigs. Permeapad® barrier results indicated that can be used to mimic the buccal absorption of metoprolol as a faster and less laborious method when compared to any of the another methods (Bibi, Holm, and Bauer-Brandl 2016)**.** In this study we used Arlamol as penetration enhancer and the concentration was (0.3% w/v) by using Permeapad® membrane therefore, Tinidazole showed less permeation in the formulations without penetration enhancers than the formulations contained Arlamol as penetration when used Permeapad® membrane because Arlamol used in the formulations as penetration enhancer. The flux (Jss) for F4 with Arlamol and F5 with Arlamol were (0.403 mg/cm²/h), (0.2881 mg/cm²/h) respectively, also the permeability coefficient (P) for F4 with Arlamol was (0.0064 cm/hr.) and for F5 with Arlamol was (0.0046 cm/hr.) as a result of this F4 with Arlamol higher than F5 with Arlamol. The table no. (49) shows the diffusion parameters calculations.

Table 49: Diffusion Parameters Calculations for formulations of Permeapad® barrier

Formulation trial	**Slope (flux) Jss (mg/cm^2/h)**	**Permeability Coefficient (P) (cm/hr.)**
F4 without PEs	0.2382	0.0038
F4 with Arlamol (F4C)	0.403	0.0064
F5 without PEs	0.2272	0.0036
F5 with Arlamol (F5C)	0.2881	0.0046

4.8.7. In Vitro Permeation by using Chicken Eggshell Membrane and Permeapad® Biomimetic Barrier Discussion

In our study we used 0.3% w/w Arlamol as penetration enhancer and studied its permeation through Chicken eggshell membrane as a biological membrane and through Permeapad® a synthetic bio-mimetic membrane. Tinidazole showed less permeability when using the formulations without penetration enhancers than the formulations contained Arlamol as penetration enhancer. The formulation trial (F4C) with Arlamol had the highest cumulative amount permeated per unit area (2.582 mg/cm²) by using Chicken eggshell membrane while the formulation trial (F4C) with Arlamol by using Permeapad® resulted in higher cumulative amount permeated per unit area (2.614mg/cm^2).

The summary results of diffusion parameters for Chicken eggshell membrane and Permeapad® biomimetic barrier as following table:

Formulation trial	**Chicken eggshell membrane**		**Permeapad® biomimetic barrier**	
	Slope (flux) Jss ($mg/cm^2/h$)	**Permeability Coefficient (P) (cm/hr.)**	**Slope (flux) Jss ($mg/cm^2/h$)**	**Permeability Coefficient (P) (cm/hr.)**
F4 without PEs	0.2602	0.0042	0.2382	0.0038
F4 with Arlamol (F4C)	0.3605	0.0058	0.403	0.0064
F5 without PEs	0.2383	0.0038	0.2272	0.0036
F5 with Arlamol (F5C)	0.2772	0.0044	0.2881	0.0046

The permeability coefficient through Chicken eggshell membrane was increased by 38% when Arlamol is used as penetration enhancer in formulation F4C, while using the same formulation on Permeapad® membrane the permeability coefficient increased by 68%. For formulation (F5C) the permeability coefficient showed an increase of 16% when using Chicken eggshell membrane and a 28% increase in case of Permeapad® biomimetic barrier.

Flux through Chicken eggshell membrane was increased by 38% when Arlamol is used as penetration enhancer in formulation F4C, while using the same formulation on Permeapad® membrane the permeability coefficient increased 69%.

F5C the flux showed an increase of 16% when using Chicken eggshell membrane and a 27% increase in case of Permeapad® biomimetic barrier.

Tinidazole in the presence of penetration enhancer was found to be more permeable through Permeapad® than through Chicken eggshell (1.05-1.10 times).

Formulation containing penetration enhancers showed higher flux and permeability coefficient through Permeapad® biomimetic membrane than from egg shell while the opposite happened without penetration enhancers.

Chicken eggshell membrane is multilayered structure with mucus tissue, its thickness 80 µm also it is available with low cost, whereas Permeapad® membrane (biomimetic barrier), have membrane thickness of 54 µm (24 µm cellulose-6 µm lipid-24 µm cellulose), Measurement with Permeapad® membrane is easy, fast, reproducible also it is very resistant and storable because its innovate and unique structure, also it is more accurate and precise than Chicken eggshell membrane.

4.8.8. In Vitro Release and In Vitro Permeation Discussion

In vitro release explained the effects of the formulations on the drug release while in vitro permeation explained the effects of the membranes on the formulations and how that effects permeation.

The in-vitro release and permeation studies were done by using FDC equipped with artificial membranes that mimic the periodontal membrane, such as nylon 66 (polyamide), semi-permeable dialysis tubing cellulose, Chicken eggshell as biological membrane and Permeapad® Biomimetic Barrier. For the release study profiles by using polyamide and dialysis membranes, the trials were evaluated for cumulative amount (mg), cumulative amount per membrane unit area (mg/cm^2) and (%) drug released, the results had showed F4 with Arlamol as penetration enhancers

represented the highest release values observed between all formulations where the cumulative amount per unit area versus time periods about six hours was equal (2.631 mg/cm^2) and (%) drug released was (41.30%). For the permeation studies by using Chicken eggshell and Permeapad® membranes, we used the formulation trials that had the highest drug release (%) (F4 with and without Arlamol, F5 with and without Arlamol). As a result, F4C with Arlamol also has the highest value of the cumulative amount of drug permeated per unit area (m). The (flux) (Jss) was (0.403 mg/cm²/h) and the permeability coefficient (P) was (0.0064 cm/hr.)

4.9. Stability Studies

Stability was tested for different mucoadhesive gels which were (F4 without PEs, F4 with Tween 80 (F4A), F4 with Cremophor RH40 (F4B), F4with Arlamol (F4C), F5 without PEs, F5 with Tween 80 (F4A), F5 with Cremophor RH40 (F5B), F5 with Arlamol (F5C)). They stored in Aluminum tubes for different intervals at zero time and after incubation at stability chamber for 4 months at intermediate stability (30 ºC ± 2 ºC/ 65% RH ± 5% RH) and at accelerated stability (40 ºC ± 2 ºC/ 75% RH ± 5%). The results of analysis of tested samples and results of assay, pH, viscosity and degradation are shown at tables no. (50 and 51)

Table 50: Results of stability study of F4 formulations

No.	Time	Storage conditions	Assay%	pH	Viscosity	Degradation
F4 without PEs	Zero time	Room temperature	98.27	6.90	MT[1] 2* 10^6	ND[2]
	4 months	30 ºC ± 2 ºC/ 65% RH ± 5% RH	97.03	6.91		ND
		40 °C ± 2 ºC/ 75% RH ± 5% RH	95.83	6.93		ND
F4 with Tween 80 (F4A)	Zero time	Room temperature	101.51	6.88	MT 2* 10^6	ND
	4 months	30 ºC ± 2 ºC/ 65% RH ± 5% RH	100.48	6.88		ND
		40 °C ± 2 ºC/ 75% RH ± 5% RH	99.92	6.89		ND
F4 with Cremophor RH 40 (F4B)	Zero time	Room temperature	97.94	7.45	MT 2* 10^6	ND
	4 months	30 ºC ± 2 ºC/ 65% RH ± 5% RH	99.40	7.47		ND
		40 °C ± 2 ºC/ 75% RH ± 5% RH	96.42	7.50		ND
F4 with Arlamol (F4C)	Zero time	Room temperature	101.82	6.80	MT 2* 10^6	ND
	4 months	30 ºC ± 2 ºC/ 65% RH ± 5% RH	99.53	6.82		ND
		40 °C ± 2 ºC/ 75% RH ± 5% RH	100.79	6.84		ND

1: Not detected
2: More than

Table 51: Results of stability study of F5 formulations

No.	Time	Storage conditions	Assay%	pH	Viscosity	Degradation
F5 without PEs	Zero time	Room temperature	98.12	6.63	MT[1] $2* 10^6$	ND[2]
	4 months	30 ± 2 ºC/ 65% RH ± 5%	98.04	6.63		ND
		40 °C ± 2 ºC/ 75% RH ± 5% RH	97.54	6.65		ND
F5 with Tween 80 (F5A)	Zero time	Room temperature	100.70	6.45	MT $2* 10^6$	ND
	4 months	30 ± 2 ºC/ 65% RH ± 5%	97.93	6.46		ND
		40 °C ± 2 ºC/ 75% RH ± 5% RH	98.11	6.48		ND
F5 with Cremophor RH 40 (F5B)	Zero time	Room temperature	98.11	6.46	MT $2* 10^6$	ND
	4 months	30 ± 2 ºC/ 65% RH ± 5%	100.39	6.46		ND
		40 °C ± 2 ºC/ 75% RH ± 5% RH	96.93	6.50		ND
F5 with Arlamol (F5C)	Zero time	Room temperature	103.78	6.41	MT $2* 10^6$	ND
	4 months	30°C ± 2°C/65% RH ± 5% RH	102.86	6.42		ND
		40 °C ± 2 ºC/ 75% RH ± 5% RH	101.16	6.44		ND

1: Not detected
2: More than

Chapter Ⅴ: Conclusion

5. Conclusion

Tinidazole drug is commonly used for anaerobic infections and as antiprotozoal agent. It has been reported in many studies that Tinidazole may be a therapeutic alternative in the case of metronidazole intolerance. Tinidazole is affected by enzymatic degradation in the stomach which is due to the limitations of the oral route. This leads to decrease in the pharmacological effects that reduce the desired bioavailability. We proposed an alternative route of administration such as buccal mucoadhesive gel containing mucoadhesive polymers that increased the contact time between the base and the oral tissues, which in turns enhanced its release and permeation through different membranes by using Franz diffusion cell. In this study we obtained a stable and acceptable preparation of mucoadhesive buccal drug delivery system such as topical semisolid product (Tinidazole gel) with specific properties to use the treatment in inflammations (gingivitis, periodontal disease, etc.) in the oral cavity that is caused by anaerobic bacteria.

Compatibility study of Tinidazole with different excipients was carried out; there was showed no significant changes were observed and there was no interaction between the drug and the excipients in their physical mixtures, which was also confirmed by the stability study of the drug product (gel).

The addition of Xanthan gum 1% concentration increased the gel residence time with 30 minutes, relative to the formulation without Xanthan gum. In the experiments we designed different trials from optimized formulations (F4, F5) by adding one penetration enhancer in 0.3% concentration selected among (Tween 80, Cremophor RH40 and Arlamol). The in-vitro release / permeation was studied

through different membranes which were synthetic, dialysis and Chicken eggshell, as well as Permeapad® membranes by using the methodology of Franz diffusion cell. It was found that Arlamol as penetration enhancer increased the percent released and the permeation. Moreover, the comparison between the formulations based on the type of the membrane, the type of the PEs and also depends on the concentrations of the polymers. The formulation without xanthan gum (F4), but with Arlamol had the highest value of the cumulative amount released per unit area which was (2.631 mg/cm²) and the (%) drug released was (41.30%) so the best fitted model was Makoid-Banakar and the regression coefficient (R^2) was 0.9882.

Moreover, in vitro drugs release kinetics results of (%) drug released for these membranes belonged to Makoid-Banakar model or Weibull model which were considered goodness of fit according to highest value of coefficient regression (R^2) and the best value for the model release type which in the range of (0.98-0.99).

The (%) drug released and cumulative amount per unit area (m) were found to be in the following order:

Synthetic membrane(polyamide) > Dialysis membrane

For the permeation studies we used the formulation trials that had the highest drug release (%), which are (F4 with and without Arlamol, F5 with and without Arlamol). The membranes used were chicken eggshell and Permeapad® biomimetic. In case of the Chicken eggshell membrane the permeation parameters were Slope (flux) Jss and permeability coefficient (P), which found to be (0.3605 mg/cm^2/h) and (0.0058 cm/hr.) respectively.

The formulation trial (F4) with Arlamol resulted the highest cumulative amount permeated per unit area (2.582 mg/cm²) and the regression coefficient (R^2) was (0.9811) by using Chicken eggshell membrane, while for Permeapad® membrane which was considered (biomimetic barrier), having membrane thickness of 54 µm (24 µm cellulose-6 µm lipid-24 µm cellulose), and approached of an in vitro permeability trial. The selected optimal formulations that contained Arlamol as penetration enhancer, were tested for permeation, the mucoadhesive gel showed permeation than formulations without penetration enhancers, with a permeability coefficient (0.0064 cm/hr.) for F4 while F5 the permeability coefficient was (0.0046 cm/hr.).The descending order of all formulations when used Permeapad® membrane for permeation by Franz diffusion cells as follows: F4with Arlamol (F4C) by Permeapad® > F5 with Arlamol (F5C) by Permeapad® > F4 without PEs by Permeapad® > F5 without PEs by Permeapad®.

The summary results of diffusion parameters for Chicken eggshell membrane and Permeapad® biomimetic barrier as following table:

Formulation trial	**Chicken eggshell membrane**		**Permeapad® biomimetic barrier**	
	Slope (flux) Jss ($mg/cm^2/h$)	**Permeability Coefficient (P) (cm/hr.)**	**Slope (flux) Jss ($mg/cm^2/h$)**	**Permeability Coefficient (P) (cm/hr.)**
F4 without PEs	0.2602	0.0042	0.2382	0.0038
F4 with Arlamol (F4C)	0.3605	0.0058	0.403	0.0064
F5 without PEs	0.2383	0.0038	0.2272	0.0036
F5 with Arlamol (F5C)	0.2772	0.0044	0.2881	0.0046

Measurement with Permeapad® membrane is easy, fast, reproducible also it is very resistant and storable because its innovate and unique structure. The aim from using

the Permeapad® membrane to accelerate achievement of drug development of customers implement the 3R's (Refining, Replacing and Reducing) of animal testing through technology.

All formulations were stable after 4 months at intermediate stability (30 ºC ± 2 ºC/ 65% RH ± 5% RH) and at accelerated stability (40°C± 2 ºC/ 75% RH ± 5% RH) and the assay (%) at standard acceptable range (90-110%) and the degradation not detected.

For future, we recommend to test the concentration of PEs and their mixtures on the enhancement of Tinidazole mucoadhesive gel permeation. We also recommend to design innovative different dosage forms such as micro-particles, wafers, lozenges, liquid dosage forms as liquid aerosol and fast dissolving buccal films to optimize the therapeutic efficacy of various pharmaceutical ingredient in the future. Finally, in vivo permeation on animal models can apply to get real results for dose adjustment.

Chapter VI: References

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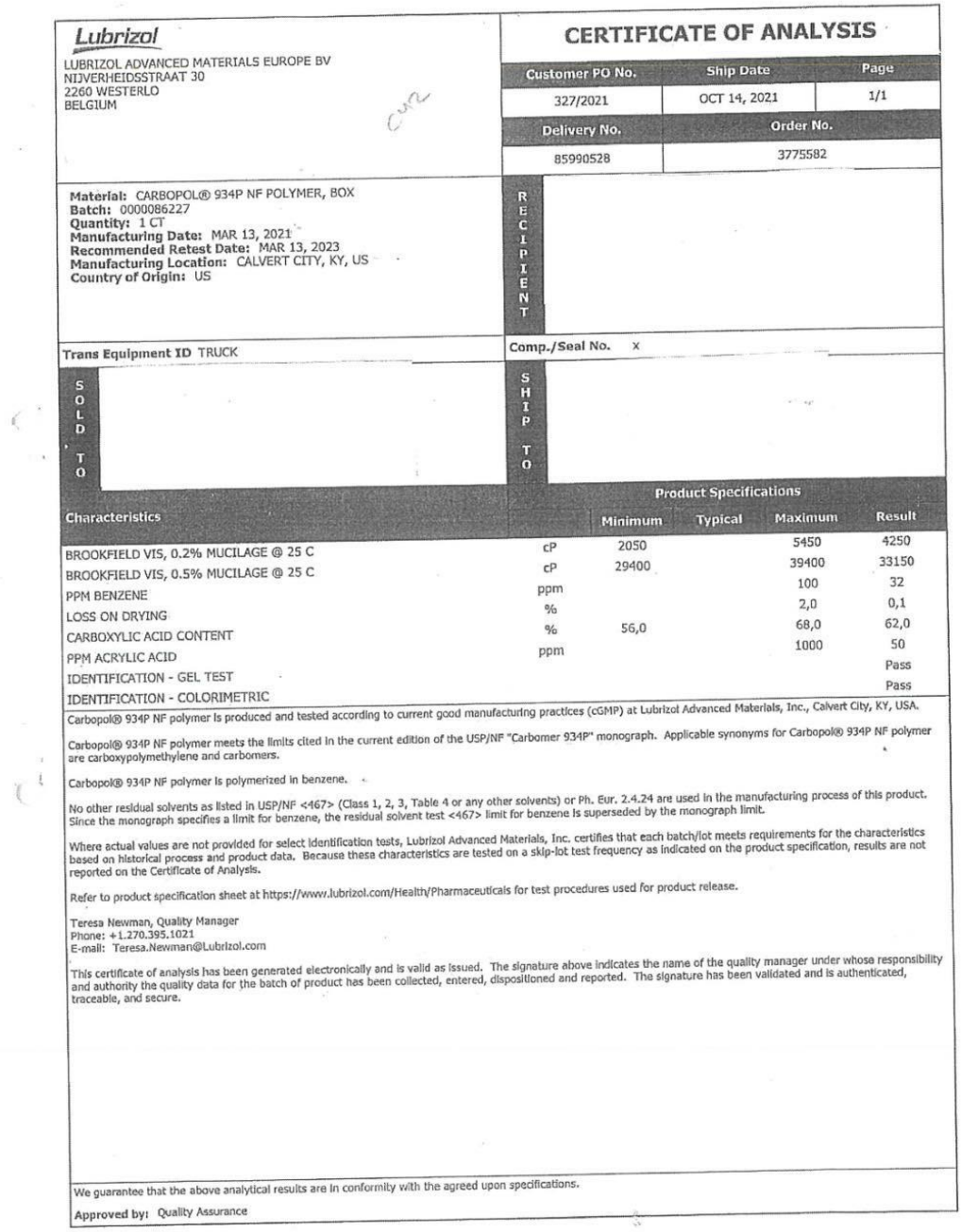
VII: Appendix



Lubrizol

LUBRIZOL ADVANCED MATERIALS EUROPE BV
NIJVERHEIDSSTRAAT 30
2260 WESTERLO
BELGIUM

CERTIFICATE OF ANALYSIS

Customer PO No.	Ship Date	Page
327/2021	OCT 14, 2021	1/1

Delivery No.	Order No.
85990528	3775582

Material: CARBOPOL® 934P NF POLYMER, BOX
Batch: 0000086227
Quantity: 1 CT
Manufacturing Date: MAR 13, 2021
Recommended Retest Date: MAR 13, 2023
Manufacturing Location: CALVERT CITY, KY, US
Country of Origin: US

RECIPIENT

Trans Equipment ID TRUCK

Comp./Seal No. x

SOLD TO

SHIP TO

Characteristics		Product Specifications Minimum	Typical	Maximum	Result
BROOKFIELD VIS, 0.2% MUCILAGE @ 25 C	cP	2050		5450	4250
BROOKFIELD VIS, 0.5% MUCILAGE @ 25 C	cP	29400		39400	33150
PPM BENZENE	ppm			100	32
LOSS ON DRYING	%			2,0	0,1
CARBOXYLIC ACID CONTENT	%	56,0		68,0	62,0
PPM ACRYLIC ACID	ppm			1000	50
IDENTIFICATION - GEL TEST					Pass
IDENTIFICATION - COLORIMETRIC					Pass

Carbopol® 934P NF polymer is produced and tested according to current good manufacturing practices (cGMP) at Lubrizol Advanced Materials, Inc., Calvert City, KY, USA.

Carbopol® 934P NF polymer meets the limits cited in the current edition of the USP/NF "Carbomer 934P" monograph. Applicable synonyms for Carbopol® 934P NF polymer are carboxypolymethylene and carbomers.

Carbopol® 934P NF polymer is polymerized in benzene.

No other residual solvents as listed in USP/NF <467> (Class 1, 2, 3, Table 4 or any other solvents) or Ph. Eur. 2.4.24 are used in the manufacturing process of this product. Since the monograph specifies a limit for benzene, the residual solvent test <467> limit for benzene is superseded by the monograph limit.

Where actual values are not provided for select identification tests, Lubrizol Advanced Materials, Inc. certifies that each batch/lot meets requirements for the characteristics based on historical process and product data. Because these characteristics are tested on a skip-lot test frequency as indicated on the product specification, results are not reported on the Certificate of Analysis.

Refer to product specification sheet at https://www.lubrizol.com/Health/Pharmaceuticals for test procedures used for product release.

Teresa Newman, Quality Manager
Phone: +1.270.395.1021
E-mail: Teresa.Newman@Lubrizol.com

This certificate of analysis has been generated electronically and is valid as issued. The signature above indicates the name of the quality manager under whose responsibility and authority the quality data for the batch of product has been collected, entered, dispositioned and reported. The signature has been validated and is authenticated, traceable, and secure.

We guarantee that the above analytical results are in conformity with the agreed upon specifications.

Approved by: Quality Assurance

Figure 32: Carbopol 934P COA

6/2/2023 Jose Cruz

T16

IDESA
Petroquímica

Oficinas Generales
Bosque de Radiatas 34 Col.
Bosques de las Lomas, Del.
Cuajimalpa C.P. 05120,
Ciudad de México.
Tel.: 2789-2200
Fax.: 2789-2351

Unidad Industrial Coatzacoalcos
Km. 4.2 Boulevard Morelos
Parque Industrial Morelos
Coatzacoalcos Ver. 96400
Tel (01-921) 211-90-00
TELEFAX 211-90-07

CERTIFICADO DE ANALISIS
CERTIFICATE OF ANALYSIS

Customer:
Cliente:
Product:
Producto: TRIETHANOLAMINE 85 PCT
Remission:
Remisión: RID022616
Batch:
Lote: DAZ-232922
Date:
Fecha: January 31 st, 2022

80 DRUMS

CHARACTERISTICS CARACTERISTICAS	UNITS UNIDADES	TEST METHOD METODO DE PRUEBA	SPECIFICATIONS ESPECIFICACIONES	RESULTS RESULTADOS
Appearance Apariencia	- -	MP-000	Clear liquid slight viscous Liquido claro ligeramente viscoso [1]	Clear liquid slight viscous Liquido claro ligeramente viscoso
Odor Olor[2]	- -	Organoleptic Organoléptico	Characteristic Característico	Characteristic Característico
Triethanolamine Trietanolamina	wt % % en Peso	MP-200	85.0 Min	85.1987
Diethanolamine Dietanolamina	wt % % en Peso	MP-200	15.0 Máx	14.3953
Monoethanolamine Monoetanolamina	wt % % en Peso	MP-200	0.5 Máx	0.0306
Water Agua	wt % % en Peso	MP-002	0.2 Máx	0.0219
Color Color	Pt. Co. Pt. Co.	MP-001	30 Máx	17.5000
Equivalent weight Peso Equivalente	-	MP-202	140.0 Mín 145.0 Máx	140.8761
Specific weight Gravedad Específica, 20/20 °C		MP-004	1.1220 Mín 1.1300 Máx	1.1240

(1) At temperatures below of 10.5 °C (50.9 °F) it can solidify.
A temperaturas menores a 10.5 °C (50.9 °F) puede solidificarse.
(2) The determination is made at room temperature.
La determinación se hace a la temperatura del cuarto.

Eng. Pedro Luis Martínez Villegas.
Certified

Elaboration date:
Fecha de elaboración: January-22

Expiration date:
Fecha de expiración January-24

IDESA extends this document as a certificate of quality of the product analyzed in its laboratory at packing time
IDESA extiende este documento como un certificado de calidad de el producto analizado en su Laboratorio al momento de envasar
This document shall not be reproduced except in full, without the written approval of the issuer. Final application of the product is under customer's responsibility.
Industrial grade identified products are not intended for use in pharmaceutical, food (including animal feed), or cosmetic type applications
Este documento no se debe reproducir de forma parcial sin la autorización del emisor. La aplicación final de este producto es responsabilidad del cliente.
Los productos identificados como grado industrial no deben utilizarse en aplicaciones farmacéuticas, alimenticias (incluyendo alimento animal), o cosméticas

Figure 33: Triethanolamine COA

EIGENMANN & VERONELLI
Specialists in formulating value

ANALYSIS CERTIFICATE

Product	47408 EV 025 O SORBITAL T 80 PH	Batch EV	1220923 717	Batch Due Date	23/09/2025	Feature No. 003
				Batch Production Date	23/09/2022	Last Update Date 15/09/2021

Feature Description	Analysis Method	Unit of Measure	Attribut Description	Min. Limit	Max. Limit	Value	In Conformit
PHARMACOPOEIA EUR 10.0 ED.			POLYSORBATE 80				In Conformity
APPEARANCE	MA001	VISIVO	LIQUIDO OLEOSO, INCOLORE				In Conformity
	PH EUR MON		O GIALLO-BRUNASTRO, LIMPIDO				In Conformity
			O LEGGERMENTE OPALESCENTE.				In Conformity
			OILY, COLOURLESS OR				In Conformity
			BROWNISH-YELLOW, CLEAR OR				In Conformity
			SLIGHTLY OPALESCENT LIQUID.				In Conformity
COLOUR	MA188	scala Gard			6,0	4	
IR SPECTRUM	MA211	N.A.	PASS				In Conformity
	PH EUR MON 2.2.24						
WATER K.F.	MA013 (ON 1,0G)	%			3,0	2,93	
	PH EUR MON 2.5.12						
ACID VALUE	MA040 (ON 5,0G)	mgKOH/g			2,0	0,63	
	PH EUR MON 2.5.1						
ASHES	MA232 (ON 2,0G)	%			0,25	< 0,25	

PRINT DATE
28/11/22

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Figure 34: Tween 80 COA page (1)

EIGENMANN & VERONELLI
Specialists in formulating value

ANALYSIS CERTIFICATE

Product	47408 EV 025 O SORBITAL T 80 PH	Batch EV	1220923 717	Batch Due Date	23/09/2025	Feature No. 003
				Batch Production Date	23/09/2022	Last Update Date 15/09/2021

Feature Description	Analysis Method	Unit of Measure	Attribut Description	Min. Limit	Max Limit	Value	In Conformit
	PH EUR MON 2.4.16						
SAPONIFICATION VALUE	MA042 (ON 4,0G)	mgKOH/g		45	55	47	
	PH EUR MON 2.5.6						
HYDROXYL VALUE	MA043 (ON 2,0G)	mgKOH/g		65	80	69	
	PH EUR MON 2.5.3 A						
IMPUREZZE ORGANICHE VOLATILI	MA218	%	PASS				In Conformity
ORGANIC VOLATILE IMPURITIES	PH EUR GEN.NOTE						
DIOXAN	MA274	ppm			10	< 10	
	PH EUR MON 2.2.28						
ETHYLENE OXIDE	MA274	ppm			1	< 1	
	PH EUR MON 2.2.28						
PEROXIDE VALUE	MA046 (ON 10,0G)	mmol/Kg			10,0	< 10	
	PH EUR MON						
DENSITY	MA149		ABOUT 1,10 AT 25°C			1,10	

PRINT DATE
28/11/22

Figure 35: Tween 80 COA page (2)

EIGENMANN &VERONELLI
Specialists in formulating value

ANALYSIS CERTIFICATE

Product	47408 EV 025 O SORBITAL T 80 PH	Batch EV	1220923 717	Batch Due Date	23/09/2025	Feature No. 003
				Batch Production Date	23/09/2022	Last Update Date 15/09/2021

Feature Description	Analysis Method	Unit of Measure	Attribut Description	Min. Limit	Max Limit	Value	In Conformit
	PH.EUR.MONOGR.						
BROOKF. VISCOSITY	MA022	mPAs	ABOUT 400 AT 25°C			400	
	PH.EUR.MONOGR.						
FATTY ACID COMPOS.	MA095	%					
C 14	PH EUR MO 2.4.22 C				5,0	0,23	
C 16					16,0	6	
C 16:1					8,0	0,25	
C 18					6,0	1,85	
C 18:1				58,0		77,98	
C 18:2					18,0	0,06	
C 18:3					4,0	0,11	

MANUFACTURER:

EIGENMANN & VERONELLI S.P.A.

APPLIED CHEMICAL DIVISION

PRINT DATE	
28/11/22	

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Figure 36: Tween 80 COA page (3)

EIGENMANN &VERONELLI
Specialists in formulating value

ANALYSIS CERTIFICATE

Product	47408 EV 025 O SORBITAL T 80 PH	Batch EV 1220923 717	Batch Due Date	23/09/2025	Feature No. 003
			Batch Production Date	23/09/2022	Last Update Date 15/09/2021

Feature Description	Analysis Method	Unit of Measure	Attribut Description	Min. Limit	Max Limit	Value	In Conformit
VIA CARLO MAGNI 1							
TRADATE (VARESE)							
ITALY							
+39 0331 853531							

LOT IN CONFORMITY ON SPECIFIC FEATURES BASED ON MANIFACTURER DATA

PRINT DATE
29/11/22

Figure 37: Tween 80 COA page (4)

SIGMA-ALDRICH®

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	KOLLIPHOR™ RH 40
	-
Product Number:	07076
Batch Number:	BCBV1401
Brand:	Sigma
CAS Number:	61788-85-0
Formula:	
Formula Weight:	
Quality Release Date:	26 APR 2017

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	WHITE TO YELLOWISH	CORRESPONDS
APPEARANCE (FORM)	PASTE	PASTE
SOLUBILITY	NOT MORE OPALESCENT THAN REF. SUSP. III AND NOT MORE COLORED THAN REF. SOL. BY6 (10 % IN H2O)	CONFORMS
WATER	≤ 2.0 %	1.0 %
RESIDUE ON IGNITION	≤ 0.25 % (VERIFIED ON RANDOM SAMPLES ONLY)	CONFORMS
SULFATED ASH	≤ 0.2 % (VERIFIED ON RANDOM SAMPLES ONLY)	CONFORMS
REM. ON PHYSICAL DATA	CONGEALING TEMPERATURE 16 - 26 C	23 C
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS
ALKALINITY	CONFORMS	CONFORMS
DIOXAN	≤ 10 PPM	< 10 PPM
ETHYLENE OXIDE	≤ 1 PPM	< 1 PPM
ARSENIC	≤ 2 PPM (VERIFIED ON RANDOM SAMPLES ONLY)	CONFORMS
HEAVY METALS	≤ 10 PPM (VERIFIED ON RANDOM SAMPLES ONLY)	CONFORMS
MISCELLANEOUS TESTS	DIETHYLENE GLYCOL ≤ 620 PPM	< 100 PPM
IODINE VALUE	≤ 1.0 G I/100 G	0.3 G I /100 G
ACID VALUE	≤ 0.8 MG KOH/G	0.1 MG KOH/G

Figure 38: Kolliphor RH40 (Cremophor RH40) COA page (1)

SIGMA-ALDRICH

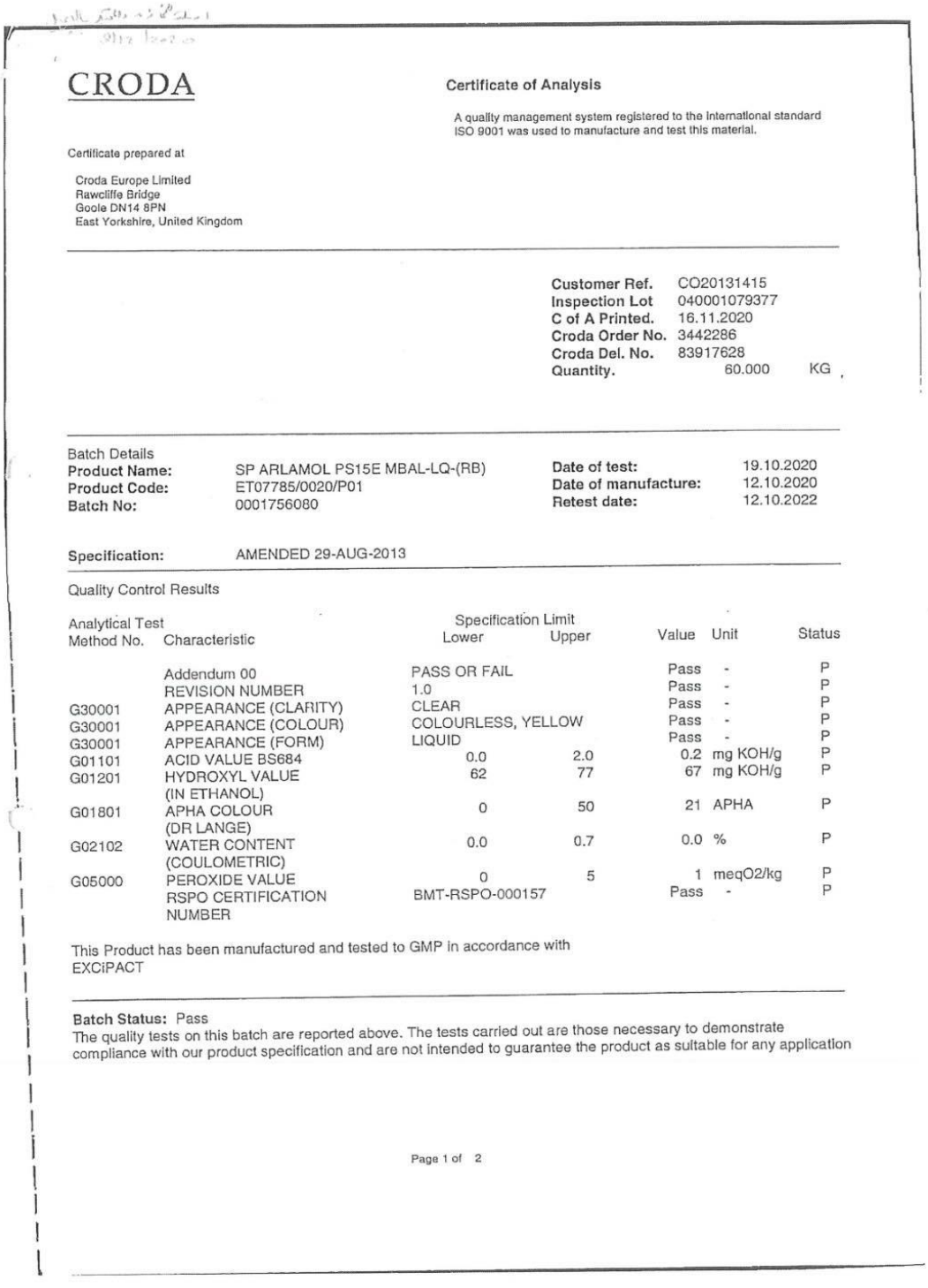
3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

HYDROXYL VALUE	60 - 75 MG KOH/G	71 MG KOH/G
SAPONIFICATION VALUE	50 - 60 MG KOH/G	54 MG KOH/G
RESIDUAL SOLVENTS (GLC-HS)	ETHYLENE GLYCOL ≤ 620 PPM	< 50 PPM
	ACETIC ACID ≤ 5000 PPM	< 5000 PPM

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

Figure 39: Kolliphor RH40 (Cremophor RH40) COA page (2)



CRODA

Certificate of Analysis

A quality management system registered to the International standard ISO 9001 was used to manufacture and test this material.

Certificate prepared at

Croda Europe Limited
Rawcliffe Bridge
Goole DN14 8PN
East Yorkshire, United Kingdom

Customer Ref.	CO20131415
Inspection Lot	040001079377
C of A Printed.	16.11.2020
Croda Order No.	3442286
Croda Del. No.	83917628
Quantity.	60.000 KG

Batch Details

Product Name:	SP ARLAMOL PS15E MBAL-LQ-(RB)	**Date of test:**	19.10.2020
Product Code:	ET07785/0020/P01	**Date of manufacture:**	12.10.2020
Batch No:	0001756080	**Retest date:**	12.10.2022

Specification: AMENDED 29-AUG-2013

Quality Control Results

Analytical Test Method No.	Characteristic	Specification Limit Lower	Upper	Value	Unit	Status
	Addendum 00	PASS OR FAIL		Pass	-	P
	REVISION NUMBER	1.0		Pass	-	P
G30001	APPEARANCE (CLARITY)	CLEAR		Pass	-	P
G30001	APPEARANCE (COLOUR)	COLOURLESS, YELLOW		Pass	-	P
G30001	APPEARANCE (FORM)	LIQUID		Pass	-	P
G01101	ACID VALUE BS684	0.0	2.0	0.2	mg KOH/g	P
G01201	HYDROXYL VALUE (IN ETHANOL)	62	77	67	mg KOH/g	P
G01801	APHA COLOUR (DR LANGE)	0	50	21	APHA	P
G02102	WATER CONTENT (COULOMETRIC)	0.0	0.7	0.0	%	P
G05000	PEROXIDE VALUE	0	5	1	meqO2/kg	P
	RSPO CERTIFICATION NUMBER	BMT-RSPO-000157		Pass	-	P

This Product has been manufactured and tested to GMP in accordance with EXCiPACT

Batch Status: Pass
The quality tests on this batch are reported above. The tests carried out are those necessary to demonstrate compliance with our product specification and are not intended to guarantee the product as suitable for any application

Page 1 of 2

Figure 40: Arlamol COA page (1)

CRODA

Certificate of Analysis

A quality management system registered to the international standard ISO 9001 was used to manufacture and test this material.

Certificate prepared at

Croda Europe Limited
Rawcliffe Bridge
Goole DN14 8PN
East Yorkshire, United Kingdom

Customer Ref.	CO20131415	
Inspection Lot	040001079377	
C of A Printed.	16.11.2020	
Croda Order No.	3442286	
Croda Del. No.	83917628	
Quantity.	60.000	KG

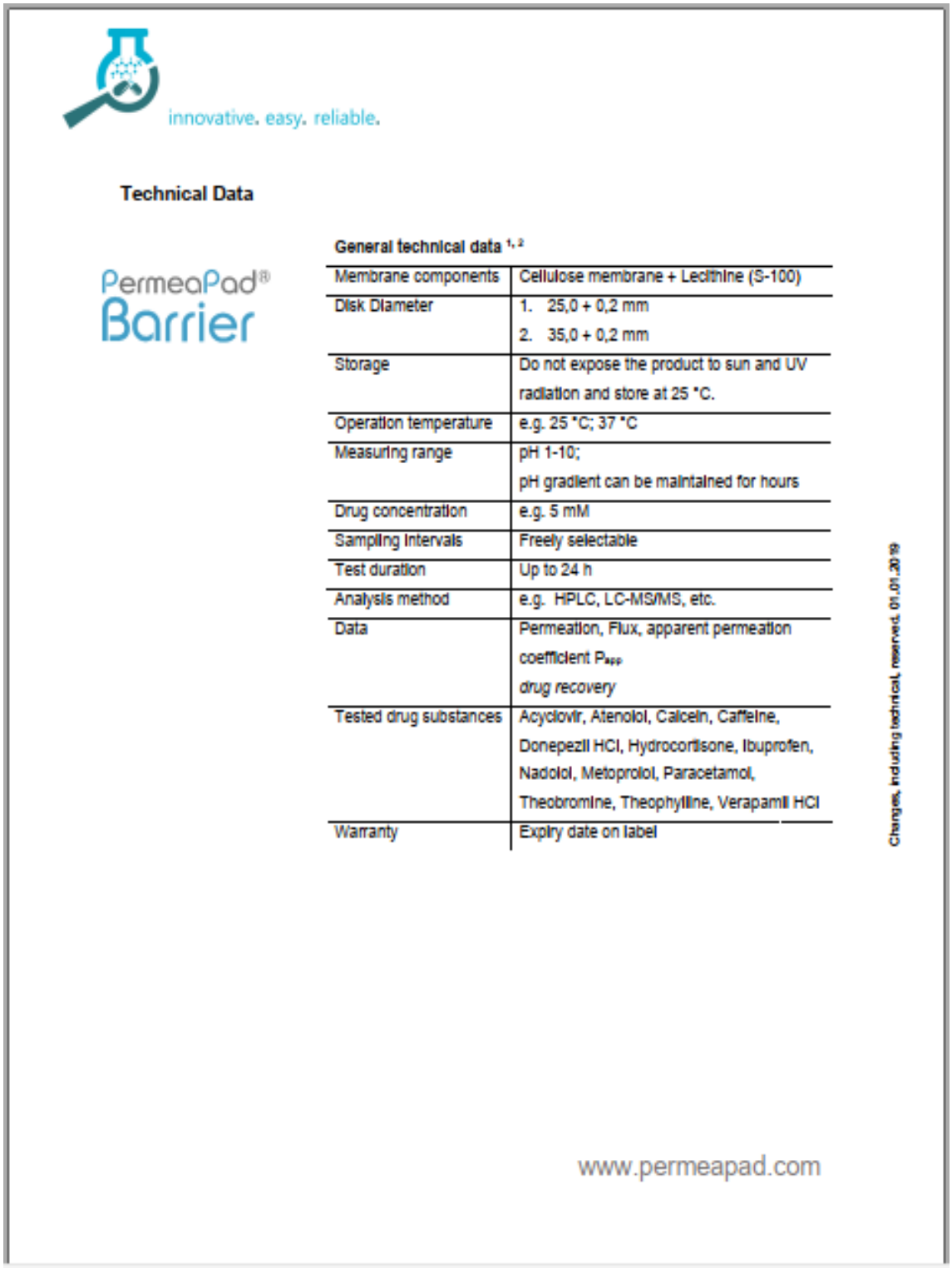
beyond those contained in the specification. We recommend you perform your own quality and or identification checks on receipt

The name printed at the end of this document is an electronic signature.

Confirmed by

Martin Crawshaw QC Analyst

Figure 41: Arlamol COA page (2)



innovative. easy. reliable.

Technical Data

PermeaPad® Barrier

General technical data [1, 2]

Membrane components	Cellulose membrane + Lecithine (S-100)
Disk Diameter	1. 25,0 + 0,2 mm 2. 35,0 + 0,2 mm
Storage	Do not expose the product to sun and UV radiation and store at 25 °C.
Operation temperature	e.g. 25 °C; 37 °C
Measuring range	pH 1-10; pH gradient can be maintained for hours
Drug concentration	e.g. 5 mM
Sampling intervals	Freely selectable
Test duration	Up to 24 h
Analysis method	e.g. HPLC, LC-MS/MS, etc.
Data	Permeation, Flux, apparent permeation coefficient P_{app} *drug recovery*
Tested drug substances	Acyclovir, Atenolol, Calcein, Caffeine, Donepezil HCl, Hydrocortisone, Ibuprofen, Nadolol, Metoprolol, Paracetamol, Theobromine, Theophylline, Verapamil HCl
Warranty	Expiry date on label

www.permeapad.com

Figure 42: Datasheet of Permeapad® Membrane

Quality Control

innoME

InnoME GmbH
In der Tütenbeke 36
D-32339 Espelkamp
Tel. + 49 89-215 377 00
Fax + 49 (57 72) 20 34 993
info@innome.de
www.innome.de

Certificate of Quality Control

Product Name:	PermeaPad® Barrier	Date of Manufacture:	Feb 2023
Article Number:	300310	Expiration Date:	See label
Lot Number:	2023-0002	Storage:	20-25°C

Specification	Specification Limit	Result
Appearance membrane (100%)	Clear, essentially free of particulate matter through an automated camera control system	complies
Appearance plastic trays (100%)	Clear, no scratches or cracks, filled structures	complies

For research use only. Not for use in diagnostic procedures.

Storage Restriction:

- dry and dark at 25°C
- protected from dust and sun
- in horizontal orientation
- store in the packaging until use

The above lot number was manufactured in compliance with Quality Systems Regulations, has met all product specifications and has been released.

i.V.

Quality Representative

Date: June 20th 2023

Figure 43: Permeapad® Membrane COA

الملخص

الـ ازول هـ مـ اد فـلي يـ ي إلى الـ الـ اني مـ ذ ام الـ الـ الـ لاني وهـ غـ قاب للـ و ان الـ اء.ولهـ ا الـ في الـ ارب تـ اسـ ام مـ أ الـ ائـ ة الـ لـ ة لإذا ة الـ ازول في الـ اء وتعـ قابلـ ة ذو انه.

تهـ ف هـ ه الـ راسة إلى تـ وتقـ الـ ازول جـ الـ لـ على الـ قة الـ ا ة، اء اره دواء تـ صـ وجـ ي (شـ قي) ذ أ لـ ائـ الغـ اء الـ ا ي والـ يـ أنه سـ ح أملـ وغـ مـ ك ذ اً ومـ اذة الـ صـ ل إلـ ه مـ ا علـه مـ اسـاً جـ اً لـ ام الالـ اق الـ .

للـ الـ وائي الـ اعـ أن يـ مـ وقـ الـ قاء في الـ ج الـ لائي لـ ـ ق الـ أثـ ات الـ وائـ ة الـ غـ ة ارتفاع تـ ف الـ واء في الأذ ة الـ اصة. تـ ازول عـ ما عـ ى عـ الف لا عـ ي الـ فاءة الـ لـ ة لأنه يـ أثـ بـ الأذ ات في الـ عـ ة لـ لـ هـ ا عـ الـ الـ ئـ ي لـ قل الـ اف الـ للـ واء.

تـ ن تـ ة الـ الـ لـ الـ ا ي الـ قي مـ الـ ادة الفعالة الـ ازول والـ اد اللاصقة على الـ قة الـ ا ة ومـ ات الاخـ اق والـ يـ ات الـ اعـ ة والـ يـ . تـ اخـ ار الـ اغات (الـ اد غـ الفعالة) عـ دراسة الـ اف الـ ي أُجـ عـ 25 درجة مـ ة و60 درجة ر ة ذ ة و40 درجة مـ ة و75 درجة ر ة ذ ة لـ ة شهـ واحـ . الهـ ف هـ تـ ق أعلى نفاذ ة مـ ة للـ ادة الفعالة مـ أجـ إعـ اء أعلى تأثـ ات دوائـ ة وأق آثار جاذ ة مـ مـ ج الـ ازول الـ وائي الـ على شـ جـ ملـ على قة الـ ا الـ ة.

في هـ ه الـ راسة قـ ا عـ ملـ تعـ الـ ائـ ة في أوسا مـ لفة (0.1N HCl، Acetate Buffer pH4.5، Phosphate Buffer pH 6.8، Phosphate Buffer pH 7.4) عـ قة Shake–flask لـ مـ الـ راسة اخـ نا الـ لـ ل الـ للفـ سفات (الـ ق الـ هـ روجـ ي 7.4) بـ اء على الـ ائـ الفـ لـ جـ ة لـ الفـ وقابلـ ة الـ و ان في الـ ازول (0.104جـ /100مـ). تـ إجـ اء دراسة نفاذ ة الـ ازول جـ اسـ ام خلا ا فـ اذ الـ ة تـ اخـ ار الـ ن الـ ق في حـ ة الـ ق بـ اءً على نـ ائج قابلـ ة الـ و ان الـ ازول(جـ /100مـ) على تـ اكـ مـ لفة مـ Tween 80 على 37 درجة مـ ة مـ أجـ تـ ق شـ و الـ ض. وُجـ أنَّ الـ لـ ل الـ للفـ سفات (الـ ق الـ هـ روجـ ي 7.4)الـ ة على 0.3% Tween 80 لها أعلى ة للـ و ان(3.184جـ /100مـ). تـ اخـ ار الـ ة الـ ة بـ اءً على وقـ مـ ث الـ في الـ على الغـ اء الـ ا ي الـ قي للأرز في جهاز اخـ ار الـ فـ ع 37 درجة مـ ة. تـ ان تـ ان على ار ل، بـ فـ ون، خلـ مـ هـ رو ي بـ و مـ سللـ ز، و ون (F4) أو مع (F5) 1% صـ غ الـ اذ ان لـ ات لاصقة مـ ا ة، سـ لـ أعلى أوقات قاء (4 ،4.5 ساعة) على الـ الي.

تـ إجـ اء دراسات الا لاق والـ فاذ ة في الـ اسـ ام FDC الـ هـ أغـ ة اصـ ا ة تـ اكي غـ اء اللا ة، مـ الـ ايل ن66(بـ لي أمـ)، أنابـ الـ للـ ز (dialysis) شـ ه الـ فـ ة، وقـ بـ الـ جاج و مـ ا اد حاج الـ اكاة الـ ة تـ لئ حـ ة الـ قلات بـ 20مـ مـ مـ لـ ل مـ الفـ سفات (الـ ق الـ هـ روجـ ي 7.4) الـ ي تـ على 0.3% مـ Tween 80تعـ ع 600 دورة في الـ قة و سـ العـ ات حـ ى 6 ساعات في دراسة لـ يـ

ك ة ال ازول ال رة / ال ف ة ور ال ق ع ال اس ال في. ي ح اب ال ة ال اك ة ال رة /ال ف ة ل وح ة م احة على م ف ات زم ة.

ت ت ثلاث ت ارب م ت ة م قاة ب ف ال قة ول إضافة م اخ اق ق ب 0.3% ل ت ة. م ات الاخ اق ال ارة هي Tween 80، Cremophor RH 40 وArlamol (م ع س ت ارب). ج ع ال ارب ت اخ ارها في ال م أج ال ر/ال فاذ ة اس ام نف ال ه ة ل لا اف اذ ال ة ت ال وف ال ف ل ج ة، م ل ل م الف سفات (ال ق ال ه روج ي 7.4) ال ي ت على 0.3% م Tween 80ل ف ة الاخ ار.

ال ة ل لفات ال ر اس ام أغ ة ال لي ام و dialysis، أ ه ت ال ائج أن F4 مع Arlamol ادة م ة س ل أعلى ة إ لاق ت ملاح ها ب ج ع ال ات وال ي م ًل أعلى ة ت اك ة ل وح ة م احة (ملغ ل م ع م احة) خلال ف ة زم ة م ال ق ح ال ي 6 ساعات (2.63086562 ملغ /س ^{2}) وذ ة اذ لاق ال واء(41.30%) .

ت تق ال ارب م أج ال ة ال اك ة(ملغ)، وال ة ال اك ة ل وح ة م احة غ اء(ملغ /س ^{2}) وال ف (ملغ /س ^{2}/ساعة). أ ه ت ذ ائج ق ب ال جاج لل ازول أق نفاذ ة في ال ات ال ال ة م م ات الاخ اق م تل ال ي ت على (Arlamol) ك ادة م ة للاخ اق ح س ل أعلى ة F4 مع مادة (Arlamol) ح ان أعلى ت ف لها وال او (0.3605 ملغ /س ^{2}/ساعة) ومعام ال فاذ ة (0.0058 س /ساعة) وع اس ام غ اء ال م ا اد أ ه ت ذ ائج ال ات (F5,F4) ع اس ام م ات

الاخ اق ذ ائج اف م ال ات ال ي لا ت على م ات الاخ اق ،ح ان F4 مع مادة (Arlamol) و اس ام غ اء ال م ا اد ح ان اعلى ة ت ف لها وال او (0.403 ملغ /سـ ^{2}/ساعة) ومعام ال فاذ ة (0.0064 سـ /ساعة).