STUDIES ON TEMPERATURE INDUCED MUCOADHESIVE *IN SITU GEL* FORMULATION OF RIZATRIPTAN BENZOATE FOR NASAL ADMINISTRATION

A Dissertation Submitted to

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Chennai - 600 032

In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutics)

Submitted by

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MELMARUVATHUR - 603 319

APRIL 2013

CERTIFICATE

This is to certify that the dissertation entitled **"STUDIES ON TEMPERATURE INDUCED MUCOADHESIVE *IN SITU GEL* FORMULATION OF RIZATRIPTAN BENZOATE FOR NASAL ADMINISTRATION"** Submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the award of the Degree of the Master of Pharmacy was carried out by **PARAMESWARI.P (Register No.26116010)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2012-2013.

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This is to certify that the dissertation entitled **"STUDIES ON TEMPERATURE INDUCED MUCOADHESIVE *IN SITU GEL* FORMULATION OF RIZATRIPTAN BENZOATE FOR NASAL ADMINISTRATION"** is the bonafide research work carried out by **PARAMESWARI. P (Register No. 26116010)** in the Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under the guidance of **Mr. T. AYYAPPAN,M. Pharm.,** Assistant Professor, Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, during the academic year 2012-2013.

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My Heartfelt Dedication

To

My Beloved Parents

And

My Profession

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PARAMESWARI. P

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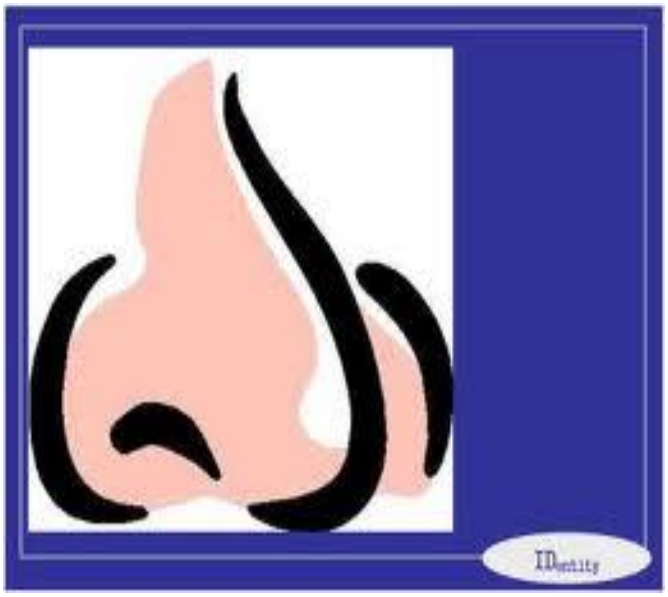
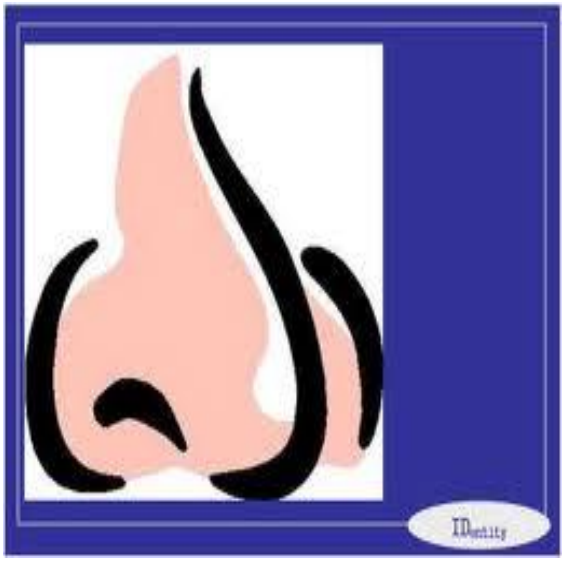
ABBREVIATIONS

%	----	Percentage
°C	----	Degree Celsius
µg	----	Microgram
µg/mL	----	Microgram per milliliter
BBB	----	Blood brain barrier
cm	----	Centimeter
CNS	----	Central nervous system
cPs	----	Centipoise
DSC	----	Differential Scanning Calorimetry
edn	----	Edition
eg	----	Example
Eq	----	Equation
Fig	----	Figure
FTIR	----	Fourier Transform Infra Red Spectroscopy
gm	----	Grams
d/cm^2	----	Dyne per square centimeter
h	----	Hours
HCl	----	Hydrochloric acid
ICH	----	International Conference on Harmonization
IP	----	Indian Pharmacopoeia

I.N.	----	Intranasal
ml	----	Milliliter
mg	----	Milligram
mg/mL	----	Milligram per milliliter
MDDS	----	Mucoadhesive drug delivery system
N	----	Normality
NDDS	----	Novel drug delivery system
nm	----	Nanometer
No.	----	Number
PEG 400	----	Polyethlene glycol 400
PF127	----	Pluronic flake127
pH	----	Negative logarithm of hydrogen ion
RB	----	Rizatriptan Benzoate
rpm	----	Revolutions per Minute
SD	----	Standard Deviation
S.No.	----	Serial Number
t	----	Time
UV	----	Ultra Violet
w/v	----	weight in volume
w/w	----	weight in weight
λmax	----	Absorption maximum



INTRODUCTION



1. INTRODUCTION

(Panchal DR., et al., 2012)

Recently, controlled and sustained drug delivery has become the standard in modern Pharmaceutical design and an intensive research have been undertaken in achieving much better drug product effectiveness, reliability and safety. Over the past 30 years greater attention has been focused on development of controlled and sustained drug delivery systems.

The most desirable and convenient method of drug administration is the oral route because of their ease of administration. However, in many instances oral administration is not desirable when the drug undergoes significant degradation via first pass effect in liver. Hence, lack of systemic absorption through the gastrointestinal tract led to research on alternate routes of drug delivery such as parenteral, intramuscular, subcutaneous, intranasal, transdermal etc.

Intranasal (IN) administration is a needle free and hence an ideal alternative to the parenteral route for systemic drug delivery. Nasal mucosa consists of a rich vasculature and a highly permeable structure for systemic absorption. Drug administration through the nasal cavity is easy and convenient. Avoidance of first pass metabolism is the main advantage of nasal route of drug delivery.

Intranasal delivery is non-invasive, essentially painless, does not require sterile preparation and it is easily and readily administered by the patient or a physician for e.g. in an emergency setting. Given these positive attributes, it is logical to consider intranasal administration extending the life or improving the profile of an existing drug.

1.1. Mucoadhesive Drug Delivery System:

(Flavia Chiva Carvalho, et al., 2010)

Bioadhesive is the term that describes the adhesion of a polymer to a biological substrate. More specifically, when adhesion is restricted to the mucous layer lining of the mucosal surface it is termed as mucoadhesion.

Mucoadhesive controlled release devices can improve the effectiveness of a drug by maintaining the drug concentration between the effective and toxic levels, inhibiting the dilution of drug in the body fluids, and allowing targeting and localization of a drug at a specific site.

Mucoadhesion also increases the intimacy and duration of contact between a drug containing polymer and a mucous surface. The combined effects of the direct drug absorption and decrease in excretion rate (due to prolonged residence time) allow for an increased bioavailability of the drug with a smaller dosage and less frequent administration.

Bioadhesive system can prevent the first pass metabolism of certain protein drugs by the liver through the introduction of the drug via route bypassing the digestive tract. Drugs that are absorbed through the mucosal lining of tissues can enter directly into the blood stream and prevented from enzymatic degradation in the GIT.

1.1.1. Fundamentals of Bioadhesion:

Development of an adhesive bond between a polymer and biological membrane or its coating can be visualized as a two step process:

- Initial contact between the two surfaces.
- Formation of secondary bonds due to non-covalent interaction.

This process of bond formation attributed to surface (or surface coat) of the biological membrane surface of the adhesive and the interfacial layer between the two surfaces. Molecular events that take place in the interfacial layer depend on the properties of the polymer and membrane.

1.1.2. NEED OF MUCOADHESIVE DELIVERY

As compared to oral controlled release systems, mucoadhesive delivery system have several advantages by virtue of prolongation of residence time, drug targeting, intimate contact between dosage form and the absorptive mucosa. In addition, mucoadhesive dosage forms have been used to target local disorders at the mucosal surface to reduce dose and to minimize the side effects. Mucoadhesive formulations use polymers as the adhesive component. These polymers are often water soluble and when used in a dry form, they attract water from the mucosal surface and this water transfer leads to a strong interaction further increasing the retention time over the mucosal surfaces and leads to adhesive interactions. Prolonged contact time of a drug with a body tissue through the use of a bioadhesive polymer can significantly improve the performance of many drugs.

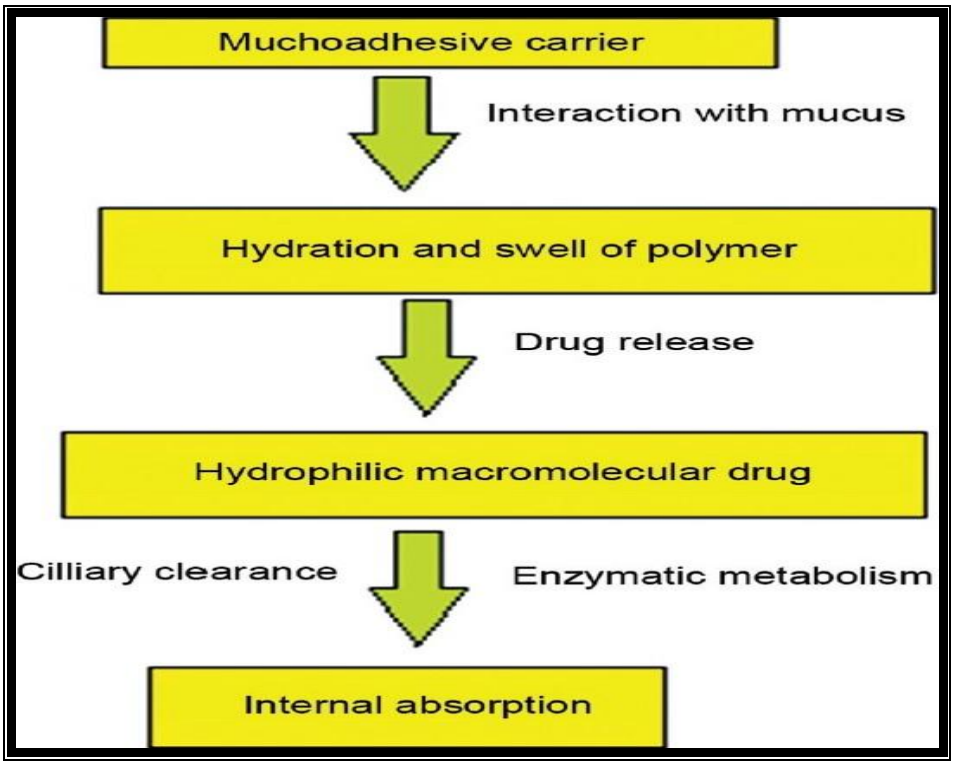


Fig 1.1: Diagramatic representation of mucoadhesive drug delivery system

1.1.3. Mechanism of Mucoadhesion:

The mucoadhesive must spread over the substrate to initiate close contact and increase surface contact, promoting the diffusion of its chains within the mucus. Attraction and repulsion forces arise and, for a mucoadhesive to be successful, the attraction forces must dominate. Each step can be facilitated by the nature of the dosage form and how it is administered. For example, a partially hydrated polymer can be adsorbed by the substrate because of the attraction by the surface water.

As stated, mucoadhesion is the attachement of the drug along with a suitable carrier to the mucous membrane. Mucoadhesion is a complex

phenomenon which involves wetting, adsorption and interpenetration of polymer chains. Mucoadhesion has the following mechanism,

1. Intimate contact between a bioadhesive and a membrane (wetting or swelling phenomenon).

2. Penetration of the bioadhesive into the tissue or into the surface of the mucous membrane (interpenetration)

The mechanism of mucoadhesion is generally divided in two steps,

- **The contact stage**
- **The contact stage**

The contact stage:

The contact stage is characterized by the contact between the mucoadhesive and the mucous membrane, with spreading and swelling of the formulation, initiating its deep contact with the mucus layer. In some cases, such as for ocular or vaginal formulations, the delivery system is mechanically attached over the membrane. In other cases, the deposition is promoted by the aerodynamics of the organ to which the system is administered, such as for the nasal route. On the other hand, in the gastrointestinal tract direct formulation attachment over the mucous membrane is not feasible. Peristaltic motions can contribute to this contact, but there is little evidence in the literature showing appropriate adhesion. Additionally, an undesirable adhesion in the esophagus can occur. In these cases, mucoadhesion can be explained by peristalsis, the motion of organic fluids in the organ cavity, or by Brownian motion. If the particle approaches the mucous surface, it will come into contact with repulsive forces

(osmotic pressure, electrostatic repulsion, etc.) and attractive forces (van der Waals forces and electrostatic attraction). Therefore, the particle must overcome this repulsive barrier).

The consolidation step:

In the consolidation step (Figure 1.2), the mucoadhesive materials are activated by the presence of moisture. Moisture plasticizes the system, allowing the mucoadhesive molecules to break free and to link up by weak van der Waals and hydrogen bonds.

Essentially, there are two theories explaining the consolidation step: the diffusion theory and the dehydration theory.

According to diffusion theory, the mucoadhesive molecules and the glycoprotein of the mucus mutually interact by means of interpenetration of their chains and the building of secondary bonds. For this to take place the mucoadhesive device has features favoring both chemical and mechanical interactions. For example, molecules with hydrogen bonds building groups (–OH, –COOH), with an anionic surface charge, high molecular weight, flexible chains and surface-active properties, which induct its spread throughout the mucus layer, can present mucoadhesive properties.

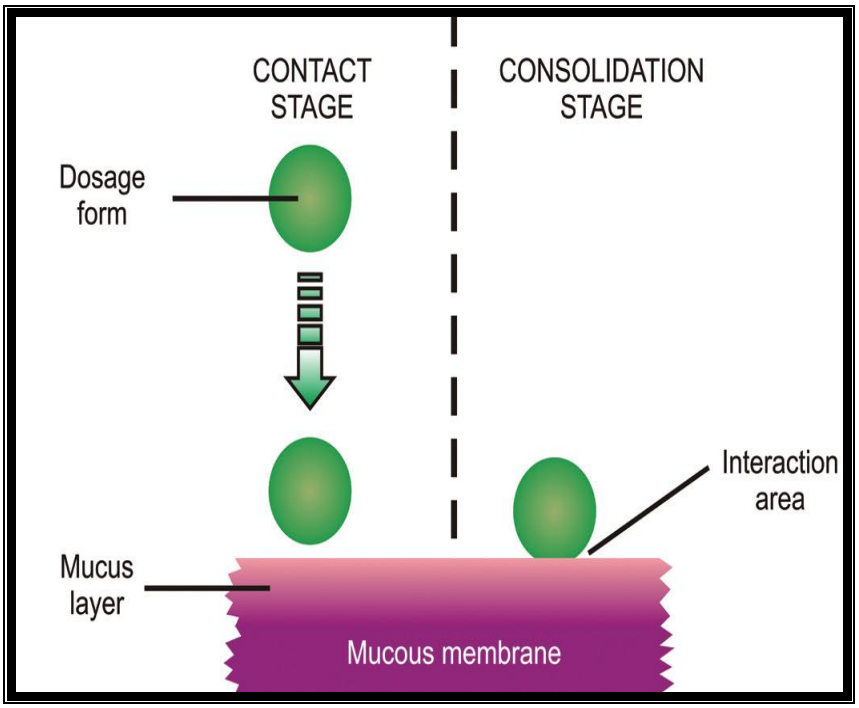


Fig 1.2: Diagramatic representation of two steps of the mucoadhesion process.

According to dehydration theory, materials that are able to readily gelify in an aqueous environment, when placed in contact with the mucus can cause its dehydration due to the difference of osmotic pressure. The difference in concentration gradient draws the water into the formulation until the osmotic balance is reached. This process leads to the mixture of formulation and mucus and can thus increase contact time with the mucous membrane. Therefore, it is the water motion that leads to the consolidation of the adhesive bond, and not the interpenetration of macromolecular chains. However, the dehydration theory is not applicable for solid formulations or highly hydrated forms.

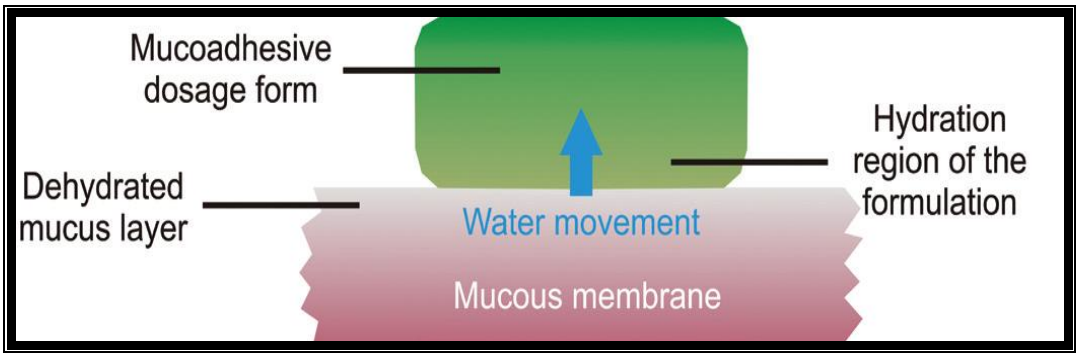


Fig 1.3: A Schematic diagram of dehydration theory of mucoadhesion

Mucoadhesion Theories: *(Flavia Chiva Carvalho1., et al., 2010)*

Although the chemical and physical basis of mucoadhesion are not yet well understood, there are six classical theories adapted from studies on the performance of several materials and polymer-polymer adhesion which explain the phenomenon.

- **Electronic theory**

Electronic theory is based on the premise that both mucoadhesive and biological materials possess opposing electrical charges. Thus, when both materials come into contact, they transfer electrons leading to the building of a double electronic layer at the interface, where the attractive forces within this electronic double layer determines the mucoadhesive strength.

- **Adsorption theory**

According to the adsorption theory, the mucoadhesive device adheres to the mucus by secondary chemical interactions, such as in van der Waals and hydrogen bonds, electrostatic attraction or hydrophobic interactions. For example, hydrogen bonds are the prevalent interfacial forces in polymers containing carboxyl groups. Such forces have been considered the most important in the adhesive interaction

phenomenon because, although they are individually weak, a great number of interactions can result in an intense global adhesion .

- **Wetting theory**

The wetting theory applies to liquid systems which present affinity to the surface in order to spread over it. This affinity can be found by using measuring techniques such as the contact angle. The general rule states that the lower the contact angle then the greater the affinity (Figure 1.4). The contact angle should be equal or close to zero to provide adequate spreadability.

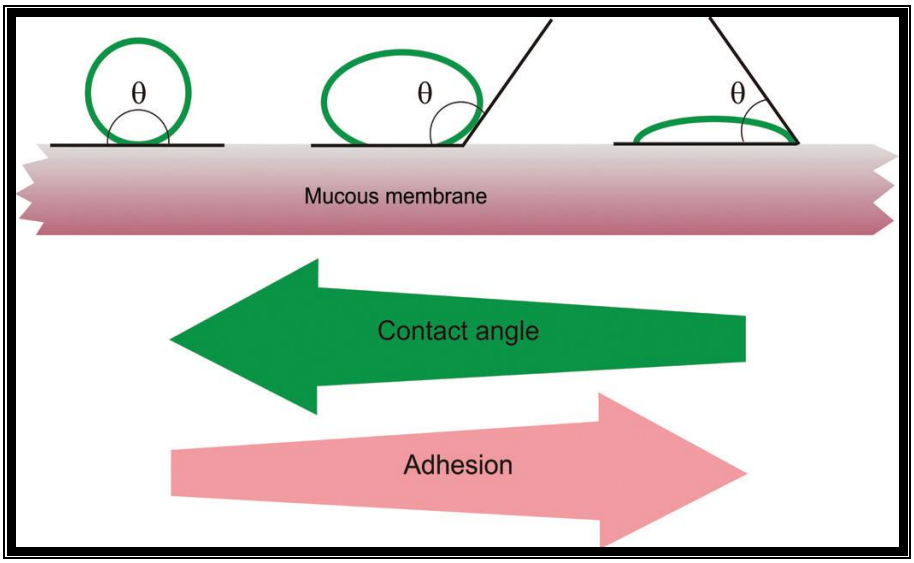


Fig 1.4: Schematic diagram showing influence of contact angle between device and mucous membrane on bioadhesion.

The spreadability coefficient, *SAB*, can be calculated from the difference between the surface energies *γB* and *γA* and the interfacial energy *γAB*, as indicated in equation (1).

$$S_{AB} = \gamma_{B} - \gamma_{A} - \gamma_{AB} \quad (1)$$

The greater the individual surface energy of mucus and device in relation to the interfacial energy, the greater the adhesion work, *WA*, i.e. the greater the energy

needed to separate the two phases.

$$W_A = \gamma_A + \gamma_B - AB \quad (2)$$

- **Diffusion theory**

Diffusion theory describes the interpenetration of both polymer and mucin chains to a sufficient depth to create a semi-permanent adhesive bond (Figure 4). It is believed that the adhesion force increases with the degree of penetration of the polymer chains . This penetration rate depends on the diffusion coefficient, flexibility and nature of the mucoadhesive chains, mobility and contact time. According to the literature, the depth of interpenetration required to produce an efficient bioadhesive bond lies in the range 0.2-0.5 μm. This interpenetration depth of polymer and mucin chains can be estimated by equation 3:

$$l = (tD_b)^{1/2} \quad (3)$$

where *t* is the contact time, and *Db* is the diffusion coefficient of the mucoadhesive material in the mucus. The adhesion strength for a polymer is reached when the depth of penetration is approximately equivalent to the polymer chain size.

In order for diffusion to occur, it is important that the components involved have good mutual solubility, that is, both the bioadhesive and the mucus have similar chemical structures. The greater the structural similarity, the better the mucoadhesive bond.

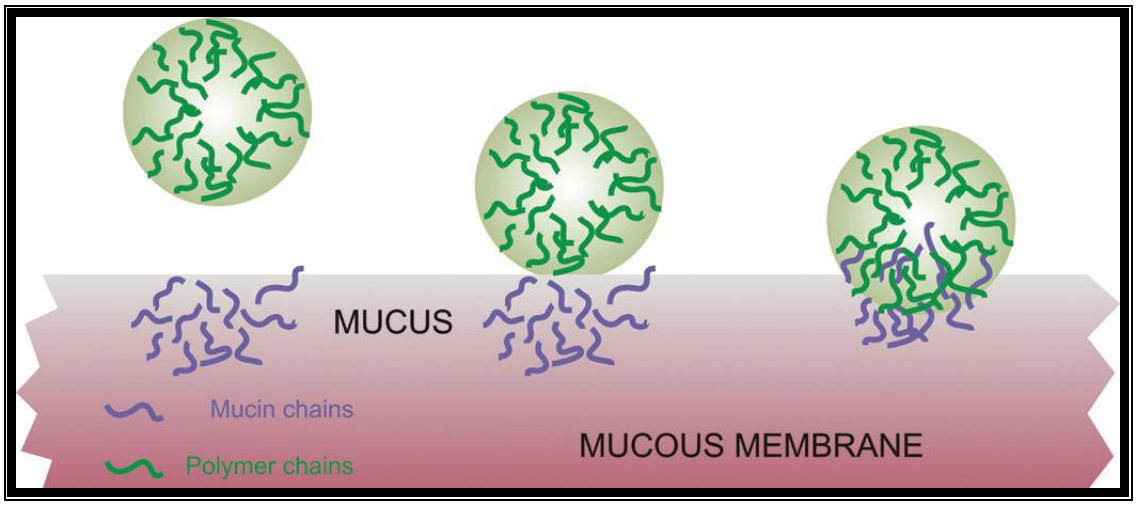


Fig 1.5: A Schematic diagram of Secondary interactions resulting from interdiffusion of polymer chains of bioadhesive device and of mucus

- **Fracture theory**

This is perhaps the most-used theory in studies on the mechanical measurement of mucoadhesion. It analyses the force required to separate two surfaces after adhesion is established. This force, *sm*, is frequently calculated in tests of resistance to rupture by the ratio of the maximal detachment force, *Fm*, and the total surface area, *A0*, involved in the adhesive interaction (equation 4):

$$S_m = Fm/Ao \quad (4)$$

In a single component uniform system, the fracture force, *sj*, which is equivalent to the maximal rupture tensile strength, *sm*, is proportional to the fracture energy (*gc*), for Young's module (*E*) and to the critical breaking length (*c*) for the fracture site, as described in equation 5:

$$S_f \sim [g_c E/c]^{1/2} \quad (5)$$

Fracture energy (*gc*) can be obtained from the reversible adhesion work, *Wr* (energy required to produce new fractured surfaces), and the irreversible adhesion

work, *Wi* (work of plastic deformation provoked by the removal of a proof tip until the disruption of the adhesive bond), and both values are expressed as units of fracture surface (*Af*).

$$g_c = W_r + W_1 \quad (6)$$

The elastic module of the system (*E*) is related to the stress (*s*) and to the shear (*e*) by Hooke's law:

$$E = [\sigma/\varepsilon]\ \varepsilon \text{->} 0 = [F/A_0/\Delta I/l_0]_{\Delta l \text{->} 0} \quad (7)$$

In equation 7, the stress is the ratio between force (*F*) and area (*A0*), and shear is given by the ratio between the variation of system thickness (D*l*) and the original thickness (*l0*).

A criticism of this analysis is that the system under investigation must have known physical dimensions and should be constituted by a single and uniform material. In virtue of this, the relationship obtained cannot be applied to analyze the fracture site of a multiple component bioadhesive. In this case, the equation should be expanded to accommodate elastic dimensions and modules for each component. Besides, it must be considered that a failure of adhesion will occur at the bioadhesive interface. However, it has been demonstrated that the rupture rarely occurs at the surface, but near it or at the weakest point, which can be the interface itself, the mucus layer or the hydrated region of the mucus, as illustrated in Figure 1.5. Since the fracture theory is concerned only with the force required to separate the parts, it does not take into account the interpenetration or diffusion of polymer chains. Consequently, it is appropriate for use in the calculations for rigid or semi-rigid bioadhesive materials, in which the polymer chains do not penetrate into the mucus layer

- **Mechanical theory**

Mechanical theory considers adhesion to be due to the filling of the irregularities on a rough surface by a mucoadhesive liquid. Moreover, such roughness increases the interfacial area available to interactions thereby aiding dissipating energy and can be considered the most important phenomenon of the process.

It is unlikely that the mucoadhesion process is the same for all cases and therefore it cannot be described by a single theory. In fact, all theories are relevant to identify the important process variables.

The mechanisms governing mucoadhesion are also determined by the intrinsic properties of the formulation and by the environment in which it is applied . Intrinsic factors of the polymer are related to its molecular weight, concentration and chain flexibility. For linear polymers, mucoadhesion increases with molecular weight, but the same relationship does not hold for non-linear polymers. It has been shown that more concentrated mucoadhesive dispersions are retained on the mucous membrane for longer periods, as in the case of systems formed by *in situ* gelification. After application, such systems spread easily, since they present rheological properties of a liquid, but gelify as they come into contact the absorption site, thus preventing their rapid removal. Chain flexibility is critical to consolidate the interpenetration between formulation and mucus.

Environment related factors include pH, initial contact time, swelling and physiological variations. The pH can influence the formation of ionizable groups in polymers as well as the formation of charges on the mucus surface. Contact time between mucoadhesive and mucus layer determines the extent of chain

interpenetration. Super-hydration of the system can lead to build up of mucilage without adhesion. The thickness of the mucus layer can vary from 50 to 450 μm in the stomach to less than 1μm in the oral cavity. Other physiological variations can also occur with diseases.

None of these mechanisms or theories alone can explain the mucoadhesion which occurs in an array of different situations. However, the understanding of these mechanisms in each instance can help toward the development of new mucoadhesive products .

1.1.3. Common Sites of Application for Mucoadhesive Drug Delivery Platform:

Mucoadhesive formulations have been widely used for their targeted and controlled release delivery to many mucosal membrane based organelles. Such formulations may deliver active ingredient for local systemic effect, while bioavailability limiting effects such as enzymatic or hepatic degradation can be avoided or minimized.

In each case of these mucosal routes, mucus characteristics and functions are different. By this definition, the mucosal routes for drug delivery are:

- Buccal drug delivery system
- Ophthalmic drug delivery system
- Vaginal drug delivery system
- Nasal drug delivery system

Buccal drug delivery

The buccal cavity offers many advantages for drug delivery application. The most significant advantage offered is high accessibility and low enzymatic activity.

Additionally, buccal drug delivery can be promptly terminated in cases of toxicity through the removal of dosage form thereby offering a safe and easy method of drug utilization. Various polymers such as sodium carboxymethylcellulose, hydroxypropylcellulose and polycarbophil are used for delivery of peptides, protein and polysaccharides by this routes have been examined. Although gel and ointments are the most patient convenient tablets, patches and films have also been examined. Furthermore buccal drug delivery is associated with high patient compliance, low levels of irritation and offers significant ease of administration.

Ophthalmic drug delivery

The delivery of therapeutic agents to the eye may be achieved using various types of dosage forms including liquid drops, gels, ointments and solid ocular inserts (both degradable and nondegradable). Another interesting delivery system is in situ gelling polymer that undergoes a phase transition after application. Mucoadhesive polymers would be expected only to attach to conjunctival mucus in vivo. Additionally limited bioavailability has been experienced in vivo for carbomer and polycarbophil, as a result of the high swelling capacity of such polymers in the neutral pH environment of the eye. Maintenance of a low viscosity in such systems through pH regulation in the range 4–5 is not acceptable as it may result in patient unease and mild lacrimation, both of which will have an effect on treatment success. User acceptance and compliance may subsequently be limited by physical and psychological barriers surrounding such dosage forms.

Vaginal drug delivery systems

Vaginal drug delivery offers many advantages; the avoidance of hepatic first-pass metabolism, a decrease in hepatic side effects and avoidance of pain, tissue

damage, and infection commonly observed for parenteral drug delivery routes of administration. While the vagina provides a promising site for systemic drug delivery because of its large surface area, rich blood supply and high permeability, poor retention due to the self-cleansing action of the vaginal tract is often problematic. However, residence times within the vagina tend to be much higher than at other absorption sites such as the rectum or intestinal mucosa. Another important consideration is the change in the vaginal membrane during the menstrual cycle and post-menopausal period. Typical bioadhesive polymers that have been in vaginal formulations include polycarbophil, hydroxypropylcellulose and polyacrylic acid.

Nasal drug delivery

One of the key advantages provided by intranasal drug delivery is that the nasal cavity provides a large highly vascularised surface area through which first-pass metabolism can be avoided, as blood is drained directly from the nose into the systemic circulation. Successful nasal delivery has been obtained using solutions, powders, gels and microparticles. The most commonly employed intranasal active ingredient are solutions containing sympathomimetic vasoconstrictors for immediate relief of nasal congestion. Local delivery of these alpha adrenergic stimulators is of particular benefit to patients with high blood pressure (or those at heightened risk of cardiovascular incident), as vasoconstriction will occur to the greatest degree within the nose. In addition to local effects, the intranasal route of drug administration has also been used to achieve a distal systemic effect. One such example is the intranasal delivery of the peptide desmopressin that exerts its action on the kidneys, mimicking the action of antidiuretic hormone, used mainly in Diabetes insipidus.

Table 1.1: Comparative properties of gastrointestinal, dermal and transmucosal drug administration:

	Gastrointestinal	**Dermal**	**Nasal**	**Oral mucosal**	**Vaginal**
Accessibility	+	+++	++	++	+
Surface area	+++	+++	+	++	+++
Surface Enviornment	+	++	++	+++	+
Permiability	+++	+	+++	++	+++
Reactivity	++	++	+	+++	++
Vascular Drainage	+++	+	+++	++	+++
First pass clearance	+	+++	+++	+++	+
Patient acceptability	++	+++	++	+++	+++

+ Poor, + + Good, + + + Excellent

1.2. Nasal Drug Delivery System:

The administration of drugs via nose is not a novel approach for drug delivery. In ancient days, nasal drug delivery was used for the systemic administration of psychotherapeutic compounds and other similar substances. But in modern pharmaceutics, nasal drug delivery is considered as a route of choice for local effect rather than systemic effect. Delivery of drugs via nose for maintenance therapy of nasal allergy, sinusitis, nasal congestion, and nasal infections.

In recent years, research has established that the nasal route is a safe and acceptable alternative to the parenteral administration of drugs. The nasal route has also been found to be useful in targeting drugs to the central nervous system (CNS).

The greater permeability of nasal mucosa with large surface area affords a rapid onset of therapeutic effect. The low metabolic environment of nose has potential to overcome the limitations of oral route and duplicate the benefit of intravenous administration.

1.2.1. Anatomy and Physiology of Nose: *(Amol., et al., 2011)*

The nose is divided into two nasal cavities via the septum. The volume of nasal cavity is approximately15 ml with a surface area of around 150 cm^2.

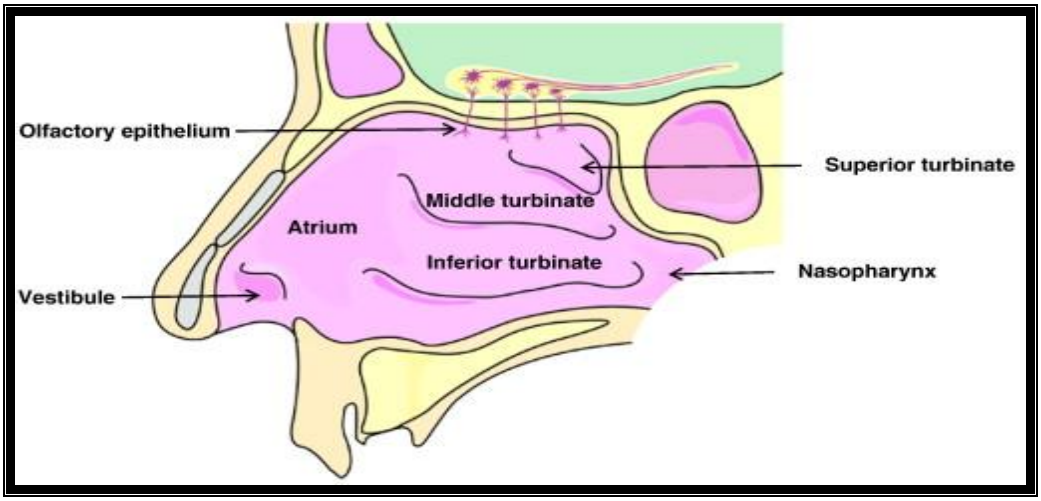


Fig 1.6: A Schematic representation of a sagittal section of human nasal cavity

Schematic representation of a sagittal section of human nasal cavity showing the nasal vestibule , Atrium , respiratory region: inferior turbinate, middle turbinate and the superior turbinate, the olfactory region and nasopharynx .

- ✓ **The Vestibule:**

It consists of the region just inside the nostrils (~0.6 cm^2). The nasal vestibule is covered with stratified sqamous epithelium. This gradually changes in the posterior into a pseudostratified columnar epithelium that covers the respiratory epithelium.

✓ **The Respiratory Region:**

It contains three nasal turbinates, the superior, middle and inferior which project from the lateral wall of each half of the nasal cavity. The presence of these turbinates creates a turbulent airflow through the nasal passages, which ensures better contact between the inhaled air and the mucosal surface. The respiratory epithelial cells are covered with cilia and microvilli, which increase the surface area available for the absorption of drugs.

✓ **The Olfactory Region:**

It is situated in the roof of the nasal cavity (15cm²). The olfactory tissue is often yellow in colour, in contrast to the surrounding pink tissue.

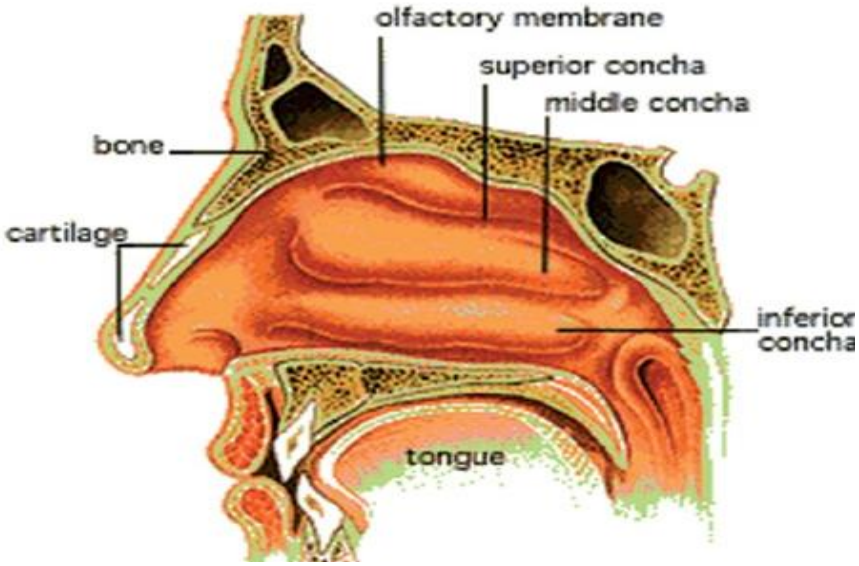


Fig 1.7: A Schematic diagram of olfactory epithelium.

Table 1.2: Structural Feature of Different Sections of Nasal Cavity and their Relative Impact on Permeability.

Region	Structural Features	Structural Features
Nasal vestibule	♦ Nasal hairs (vibrissae) Epithelial cells are stratified, squamous and keratinized Sebaceous glands present	Least permeable because of the presence of Keratinized cells
Atrium	♦ Transepithelial region Stratified squamouscells present anteriorly and pseudo stratified cells with microvilli present posteriorly	Less permeable as it has small surface area and stratified cells are present anteriorly
Respiratory region (inferior turbinate middle turbinate superior turbinate)	♦ Pseudo stratified ciliated columnar cells with microvilli (300 per cell),large surface area ♦ Receives maximum nasal secretions becauseof the presence of seromucus glands, nasolacrimal duct and goblet cells ♦ Richly supplied with blood for heating and humidification of inspired air, presence of paranasal sinuses	Most permeable regionbecause of large surfacearea and rich vasculature
Olfactory region	♦ Specialized ciliated olfactory nerve cells for smell perception ♦ Receives ophthalmic and maxillary divisions of trigeminal nerve	Direct access to cerebrospinal fluid
Nasopharynx	♦ Upper part contains ciliated cells and lower part contains squamous epithelium	Receives nasal cavity Drainage

1.2.2. Advantages of Nasal Drug Delivery System: *(Rahishuddin., et al., 2011)*

- Drug degradation that is observed in the gastrointestinal tract is absent.
- Hepatic first pass metabolism is avoided.
- Rapid drug absorption and quick onset of action can be achieved.
- The bioavailability of larger drug molecules can be improved by means of absorption enhancer or other approach.
- The nasal bioavailability for smaller drug molecules is good.
- Drugs that are orally not absorbed can be delivered to the systemic circulation by nasal drug delivery.
- Drugs possessing poor stability in G.I.T. fluids are given by nasal route.
- Polar compounds exhibiting poor oral absorption may be particularly suited for this route of delivery.
- Convenient for those on long term therapy, when compared with parenteral medication.

1.2.3. Limitations of Nasal Drug Delivery System:

- Absorption surface area is less when compared to GIT.
- Once the drug administered cannot be removed.
- Nasal irritation

1.2.4. Mechanism of Nasal Absorption: *(Rahisuddin., et al., 2010)*

The absorbed drugs from the nasal cavity must pass through the mucus layer, it is the first step in absorption. Small, unchanged drugs easily pass through this layer but large, charged drugs are difficult to cross it. The principle protein of the mucus is mucin, it has the tendency to bind to the solutes, hindering diffusion. Additionally, structural changes in the mucus layer are possible as a result of environmental changes

(i.e. pH, temperature, etc.). So many absorption mechanisms were established earlier but only two mechanisms have been predominantly used, such as:

First mechanism

It involves an aqueous route of transport, which is also known as the paracellular route but slow and passive. There is an inverse log-log correlation between intranasal absorption and the molecular weight of water-soluble compounds. The molecular weight greater than 1000 Daltons having drugs shows poor bioavailability

Second mechanism

It involves transport through a lipoidal route and it is also known as the transcellular process. It is responsible for the transport of lipophilic drugs that show a rate dependency on their lipophilicity. Drug also cross cell membranes by an active transport route via carrier-mediated means or transport through the opening of tight junctions .

For examples: Chitosan, a natural biopolymer from shellfish, opens tight junctions between epithelial cells to facilitate drug transport.

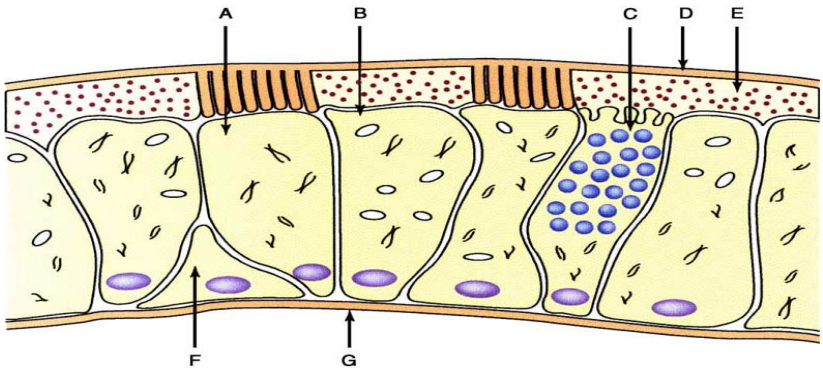


Figure 1.8: Cell types of the nasal epithelium

The cell type of nasal epithelium showing ciliated cell (A), non-ciliated cell(B), goblet cells(C), gel mucus layer (D), sol layer (E), basal cell (F) and basement membrane (G).

1.2.5. Barriers to Nasal Absorption: (*Swamya N.G.N, et al., 2012*)

Nasal drug delivery system is considered has a profitable route for the formulation scientist because it has easy and simple formulation strategies. Intranasally administered drug products therapeutic efficacy and toxicities are influenced by number of factors.

Following factors are the barriers to the absorption of drugs through nasal cavity.

- **Low bioavailability**

Bioavailability of polar drugs is generally low about 10% for low molecular weight drugs and not above 1% for peptides such as calcitonin and insuin.The most important factor limiting the nasal absorption of polar drugs and especially large molecular weight polar drugs such as peptides and proteins is the low membrane permeability. Larger peptides and proteins are able to pass the nasal membrane using an endocytotic transport process but only in low amounts.

- **Mucociliary clearance**

The drugs administered by nasal route are subject to fast clearance from the nasal cavity owing to mucociliary clearance. As a result of this, it leads to decreased transport of drugs across the nasal mucosa. This is especially the case, when the drug is not absorbed rapidly enough across the nasal mucosa. It has been shown that for both liquid and powder formulations, which are not bioadhesive, the half-life for clearance is of the order of 15-30 min. The use of bioadhesive excipients in the

formulations is an approach to overcome the rapid mucociliary clearance. The clearance may also be reduced by depositing the formulation in the anterior and less ciliated part of the nasal cavity thus leading to improved absorption.

- **Enzymatic degradation**

Another contributing, but often less considered factor to the low bioavailability of peptides and proteins across the nasal mucosa is the possibility of an enzymatic degradation of the molecule in the lumen of the nasal cavity or during passage through the epithelial barrier. Both these sites contain exopeptidases such as mono and diamino peptidases that can cleave peptides at their N and C termini and endopeptidases such as serine and cysteine, which can attack internal peptide bonds .

The use of enzyme inhibitors and/or saturation of enzymes may be the approaches to overcome this barrier.

1.2.6. Factors Influencing Nasal Drug Absorption*: (Panchal D. R., et al., 2012)*

Several factors affect the systemic bioavailability of drugs which are administered through the nasal route. The factors influencing nasal drug absorption are described as follows.

- **Physiochemical properties of drug.**
  - Molecular size.
  - Lipophilic-hydrophilic balance.
  - Enzymatic degradation in nasal cavity.
- **Nasal Effect**
  - Membrane permeability.
  - Environmental pH

- Mucociliary clearance
- Cold, rhinitis.

❖ **Delivery Effect**

- Formulation (Concentration, pH, osmolarity)
- Delivery effects
- Drugs distribution and deposition.
- Viscosity

❖ **Physiochemical properties of drug**

➢ **Molecular size**

The molecular size of the drug influence absorption of the drug through the nasal route**.** The lipophilic drugs have direct relationship between the MW and drug permeation whereas water- soluble compounds depict an inverse relationship. The rate of permeation is highly sensitive to molecular size for compounds with MW ≥ 300 Daltons.

➢ **Lipophilic-hydrophilic balance**

The hydrophilic and lipophilic nature of the drug also affects the process of absorption. By increasing lipophilicity, the permeation of the compound normally increases through nasal mucosa. Although the nasal mucosa was found to have some hydrophilic character, it appears that these mucosae are primarily lipophilic in nature and the lipid domain plays an important role in the barrier function of these membranes. Lipophilic drugs like naloxone, buprenorphine, testosterone and 17a-ethinyl- oestradiol are almost completely absorbed when administered intranasal route

➢ **Enzymatic degradation in nasal cavity**

In case of peptides and proteins are having low bioavailability across the nasal cavity, so these drugs may have possibility to undergo enzymatic degradation of the drug molecule in the lumen of the nasal cavity or during passage through the epithelial barrier. These both sites are having exopeptidases and endopeptidases, exopeptidases are monoaminopeptidases and diaminopeptidases. These are having capability to cleave peptides at their N and C termini and endopeptidases such as serine and cysteine, which can attack internal peptide bonds.

❖ **Nasal effect factors**

➢ **Membrane permeability**

Nasal membrane permeability is the most important factor, which affect the absorption of the drug through the nasal route. The water soluble drugs and particularly large molecular weight drugs like peptides and proteins are having the low membrane permeability. So the compounds like peptides and proteins are mainly absorbed through the endocytotic transport process in low amounts. Water-soluble high molecular weight drugs cross the nasal mucosa mainly by passive diffusion through the aqueous pores (i.e. tight junctions).

➢ **Environmental pH**

The environmental pH plays an important role in the efficiency of nasal drug absorption. Small water-soluble compounds such as benzoic acid, salicylic acid, and alkaloid acid show that their nasal absorption in rat occurred to the greatest extent at those pH values where these compounds are in the nonionised form. However, at pH values where these compounds are partially ionized, substantial absorption was found. This means that the nonionised lipophilic form crosses the nasal epithelial barrier via

transcellular route, whereas the more lipophilic ionized form passes through the aqueous paracellular route.

- **Mucociliary clearance**

Mucociliary clearance is a one of the functions of the upper respiratory tract is to prevent noxious substances (allergens, bacteria, viruses, toxins etc.) from reaching the lungs. When such materials adhere to, or dissolve in, the mucus lining of the nasal cavity, they are transported towards the nasopharynx for eventual discharge into the gastrointestinal tract . Clearance of this mucus and the adsorbed/dissolved substances into the GIT is called the MCC. This clearance mechanism influence the absorption process due to the dissolved drugs in the nasal cavity are discharge by the both the mucus and the cilia, which is the motor of the MCC and the mucus transport rate is 6 mm/min. It is of utmost importance that the MCC is not impaired in order to prevent lower respiratory tract infections.

- **Cold, rhinitis**

Rhinitis is a most frequently associated common disease, it influence the bioavailability of the drug. It is mainly classified into allergic rhinitis and common, the symptoms are hyper secretion, itching and sneezing mainly caused by the viruses, bacteria or irritants. Allergic rhinitis is the allergic airway disease, which affects 10% of population. It is caused by chronic or acute inflammation of the mucous membrane of the nose. These conditions affect the absorption of drug through the mucus membrane due the inflammation.

- **Delivery effect factors**

Factors that affect the delivery of drug across nasal mucosa such as surfactants, dose pH, osmolarity, viscosity, particle size and nasal

clearance, drug structure can be used to advantage to improve absorption.

- **Formulation (Concentration, pH, Osmolarity)**

The pH of the formulation and nasal surface, can affect a drugs permeation. To avoid nasal irritation, the pH of the nasal formulation should be adjusted to 4.5-6.5 because lysozyme is found in nasal secretions, which is responsible for destroyin certain bacteria at acidic pH. Under alkaline conditions, lysozyme is inactivated and the tissue is susceptible to microbial infection. In addition to avoiding irritation, it results in obtaining efficient drug permeation and prevents the growth of bacteria.

Concentration gradient plays very important role in the absorption /permeation process of drug through the nasal membrane due to nasal mucosal damage. Examples for this are nasal absorption of L-Tyrosine was shown to increase with drug concentration in nasal perfusion experiments. Another is absorption of salicylic acid was found to decline with concentration. This decline is likely due to nasal mucosa damage by the permanent.

The osmolarity of the dosage form affects the nasal absorption of the drug; it was studied in the rats by using model drug. The sodium chloride concentration of the formulation affects the nasal absorption. The maximum absorption was achieved by 0.462 M sodium chloride concentration; the higher concentration not only causes increased bioavailability but also leads to the toxicity to the nasal epithelium.

- **Drugs distribution and deposition**

The drug distribution in the nasal cavity is one of the important factors, which affect the efficiency of nasal absorption. The mode of drug administration could effect the distribution of drug in nasal cavity, which in turn will determine the absorption efficiency of a drug. The absorption and bioavailability of the nasal dosage forms

mainly depends on the site of disposition. The anterior portion of the nose provides a prolonged nasal residential time for disposition of formulation, it enhances the absorption of the drug. And the posterior chamber of nasal cavity will use for the deposition of dosage form, it is eliminated by the mucociliary clearance process and hence shows low bioavailability. The site of disposition and distribution of the dosage forms are mainly depends on delivery device, mode of administration, physicochemical properties of drug molecule.

- **Viscosity**

A higher viscosity of the formulation increases contact time between the drug and the nasal mucosa thereby increasing the time for permeation. At the same time, highly viscous formulations interfere with the normal functions like ciliary beating or mucociliary clearance and thus alter the permeability of drugs.

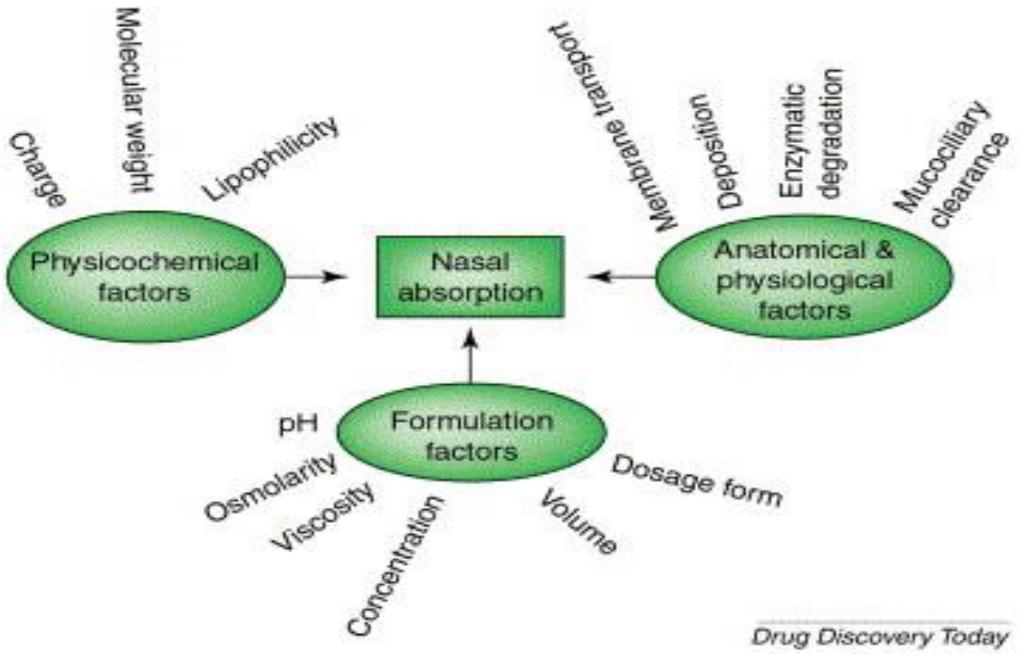


Figure 1.9: Schematic representation of factors affecting nasal drug absorption

1.2.7. Nasal Drug Delivery Formulations:

The nasal drug formulations are,

- ❖ Nasal Drops
- ❖ Nasal Sprays
- ❖ Nasal Gels
- ❖ Nasal Powders
- ❖ Microemulsions
- ❖ Mucoadhesives

Nasal Drops:

Nasal drops are one of the most simple and convenient systems developed for nasal delivery. Nasal drops contain therapeutically active ingredients dissolved in solutions or mixtures of excipients (for example, preservatives, viscosity modifiers, emulsifiers and buffering agents). Usually they are administered to a dropper. The main disadvantage of this system is the lack of dose precision.

Nasal Sprays:

Both solution and suspension formulations can be formulated into nasal sprays. The dose can be metered by a spray pump or it may have been premetered during manufacture. A nasal spray unit can be designed for unit dosing or to discharge up to several hundred metered sprays of formulation containing the drug substance. The particle size and morphology (for suspensions) of the drug and viscosity of the formulation determine the choice of pump and actuator assembly.

Nasal Gels:

Nasal gels are highly viscous, thickened solutions or suspensions. The advantages of the nasal gel include the reduction of postnasal drip due to high viscosity, lowering of the taste impact due reduced swallowing, less anterior leakage of the formulation, reduced irritation by using soothing/emollient excipient and target delivery to the mucosa for better absorption.

Nasal Powders:

This dosage form may be developed if the drug lacks stability in the solution and suspension dosage forms. The other advantages of the nasal powder dosage form are the absence of preservatives and superior stability of the formulation. However, the suitability of the powder formulation is dependent on the solubility, particle size, aerodynamic properties and nasal irritancy of the nasal drug and/or excipients. An intranasal powder form of glucagon was reported to have improved the metabolic status and fatty liver in patients with pancreatectomy.

Microemulsions:

This is thermodynamically stable, isotropically clear product that has a droplet size <0.15µm. It consists of an oil phase, surfactant, co-surfactant and aqueous phase. Oil in water (o/w) microemulsions represents a promising prospect for the development of formulations suitable for the incorporation of poorly water-soluble drugs because of high solubilization capacity as well as the potential for enhanced absorption by the CSF.

Mucoadhesives:

Mucoadhesive formulations using polymers such as carbopol, chitosan and poloxamers have been prolong the duration of contact between the nasal mucosa and the formulation.

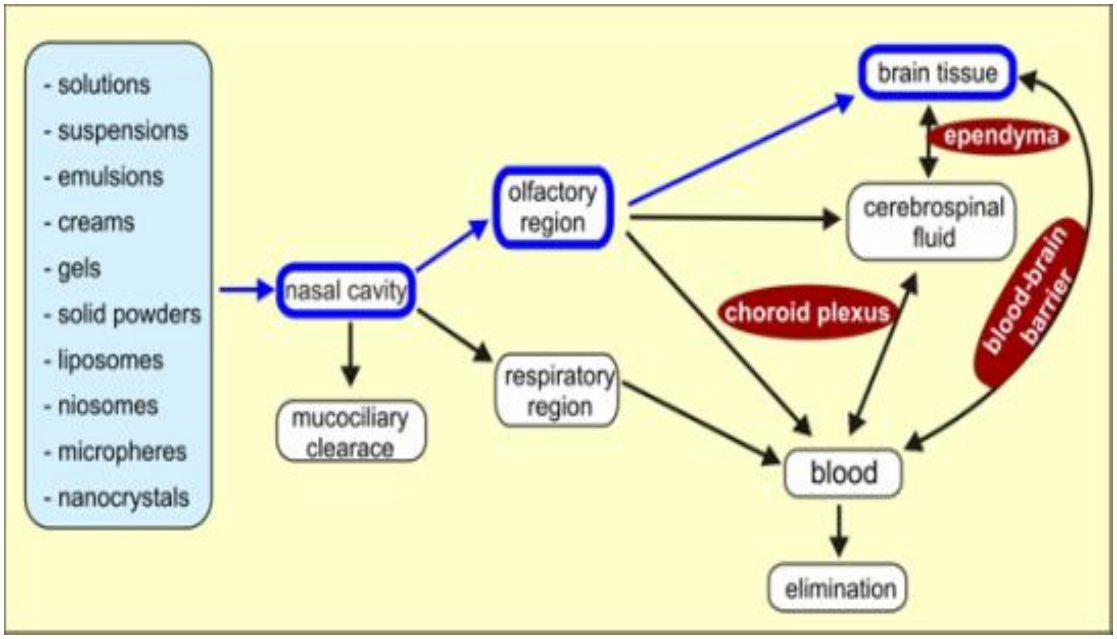


Fig 1.10: A Schematic diagram of Various absorption, distribution, and elimination pathways of Intranasal administration.

1.3. ***In situ*** **Gelling System**: *(Parekh Hejal B., et.al., 2012)*

Innovation is a key driver of growth that in the recent years there has been a continuous effort in the direction of achieving controlled and sustained drug delivery systems. Considerable attention has been received in the *in situ gelling* systems over the past few years. Research and patent in the field of *In situ gels* have increased in the past few years. It has special application in the biomedical field.

Controlled and sustained drug delivery has become the necessity in modern pharmaceutical design and an intensive research have been undertaken in achieving much better drug product effectiveness, reliability and safety. This problem has been solved by In situ drug delivery system.

In situ forming polymeric formulations are drug delivery systems that are in sol form before administration in the body, but once administered, undergo gelation in situ, to form a gel.

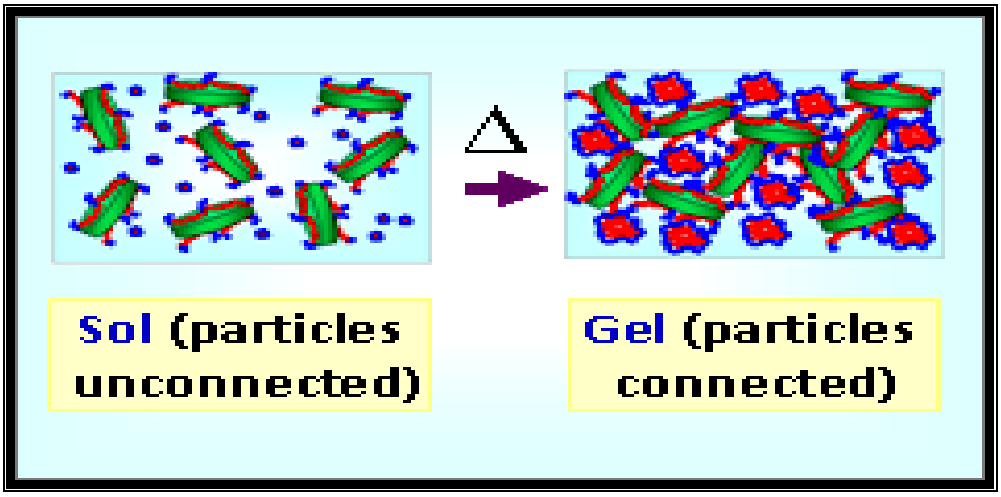


Fig 1.11: A Schematic diagram of Sol-gel mechanism

1.3.1. Advantages of *In situ gels*:

- A drug can prolong the drug contact at the site of administration due to its rheological and mucoadhesive properties as compared to an aqueous solution.
- The gels also possess a broad application spectrum and can be applied in almost every route of administration. Oral, ophthalmic, rectal, transdermal, subcutaneous and vaginal gels are available for different pharmaceutical applications.

- The gel formulations can be used to enhance the local and systemic exposure of potential lead compounds, which ideal to establish animal models for various conditions quickly and cost efficiently.
- Ease of administration and reduced frequency of administration.
- Improved patient compliance and comfort.
- In situ gel formulations offers an interesting alternative for achieving systemic drug effects of parenteral routes, which can be inconvenient of oral route, which can result in unacceptably low bioavailability and passes the hepatic first pass metabolism in particular of proteins and peptides.
- It makes the production less complex and hence lowers the investment and manufacturing cost.

1.3.2. Classification of *In Situ Gel* Formulation:

Based on Route of Administration:

- ♦ In situ polymeric system for oral administration.
- ♦ In situ polymeric system for ocular delivery.
- ♦ In situ polymeric systems for rectal and vaginal delivery.
- ♦ In situ polymeric system forming injectable drug delivery system.
- ♦ In situ polymeric systems forming nasal drug delivery system.

1.3.3. Importance of *In Situ Gelling* System:

- ✓ The major importance is the possibilities of administrating accurate & reproducible quantities compared to already formed gel.
- ✓ In situ forming polymeric delivery system such as ease of administration & reduced frequency of administration improved patient compliance & comfort.

- ✓ Poor bioavailability & therapeutic response exhibited by conventional ophthalmic solution due to rapid precorneal elimination of drug may be overcome by use of gel system that are instilled as drops into eye & undergoes a sol-gel transition from instilled dose.
- ✓ Liquid dosage form that can sustain drug release & remain in contact with cornea of eye for extended period of time is ideal.
- ✓ Reduced systemic absorption of drug drained through the nasolacrimal duct may result in some undesirable side effects.

1.3.4. Ideal Characteristics of Polymer:

A polymer used to *in situ gels* should have following characteristics,

- ✓ It should be biocompatible.
- ✓ It should be capable of adherence to mucus.
- ✓ It should have pseudo plastic behaviour.
- ✓ It should be good tolerance & optical activity.
- ✓ It should influence the tear behavior.
- ✓ The polymer should be capable of decrease the viscosity with increasing shear rate there by offering lowered viscosity during blinking & stability of the tear film during fixation.

1.4. Mucoadhesive *In Situ Gels* as Nasal Drug Delivery Systems:

Conventionally the nasal cavity is used for the treatment of local diseases, such as rhinitis and nasal congestion. However, in the past few decades nasal drug delivery has been paid much more attention as a promising drug administration route for the systemic therapy. This is due to the anatomy and physiology of the nasal passage, such

as the large surface area, highly vascularized epithelium, porous endothelial membrane, and the avoidance of first-pass metabolism.

Nasal drug delivery can also provide a route of entry to the brain that circumvents the blood–brain barrier because the olfactory receptor cells are in direct contact with the central nervous system.

In Situ is a Latin word which means in position.

1.4.1. Advantages of Mucoadhesive *In situ Gels* as Nasal Drug Delivery System:

- Avoids degradation of drug in gastrointestinal tract resulting from acidic or enzymatic degradation.
- Avoids degradation of drug resulting from hepatic first-pass metabolism.
- Results in rapid absorption and onset of action.
- Results in higher bioavailability thus uses lower doses of drug.
- Easily accessible, non-invasive route.
- Self-medication is possible through this route.
- Direct transport into systemic circulation and CNS is possible Offers lower risk of overdose.
- Does not have any complex formulation requirement.

1.4.2. *In situ* forming hydrogels:

There are three broadly defined mechanisms used for triggering the *in situ gel* formation of biomaterials.

- *In situ gel* formation based on physiological stimuli (Eg., temperature and pH)
- *In situ gel* formation based on chemical reactions (Eg., chemical and photo-initiated polymerization).

1.4.2.1. *In situ gel* formation based on physiological stimuli

Thermally triggered systems:

Eg., temperature

Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of biomaterial whose transitions from sol to gel is triggered by increase in temperature provides an attractive way to approach *in situ* gel formation. The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is facilitated and no external source of heat other than that of body is required for triggering gelation.

Three main strategies exist in engineering of thermo responsive sol-gel polymeric system. For convenience, temperature-sensitive hydrogels are classified into negatively thermo sensitive, positively thermo sensitive and thermally reversible gels.

Critical solution temperature (LCST) and contract upon heating above the LCST. Polymers with low critical solution temperature (LCST) transition between ambient and physiologic temperature are used for this purpose. One of the most extensively investigated polymers that exhibit useful LCST transition is poly (N isopropylacrylamide) (PNIPAAm). PNIPAAm is a water soluble polymer at its low LCST, but hydrophobic above LCST, which result on precipitation of PNIPAAm from the solution at the LCST.

Pluronics are poly (ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) triblock co-polymers that are fluid at low temperature, but form thermo reversible gel when heated as a consequence of disorder-order transition in micelle packing which makes these polymers suitable for *in situ* gelation . A

positive temperature sensitive hydrogel has an upper critical solution temperature (UCST) such a hydrogel contracts upon cooling below the UCST. Polymer networks of poly (acrylic acid) (PAA) and polyacrylamide (PAAm) or poly (acrylamide-co-butyl methacrylate) have positive temperature dependence of swelling. The most commonly used thermo reversible gels are those prepared from poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) (Pluronics®, Tetronics®, poloxamer).

pH triggered systems

Another formation of *in situ* gel based on physiologic stimuli is formation of gel induced by pH changes. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The majority of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives.

Likewise, polyvinyl (Acetaldiethylamino Acetate) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition. Mixtures of poly (methacrylic acid) (PMA) and polyethylene glycol (PEG) also have been used as a pH sensitive system to achieve gelation.

1.4.2.2. *In situ gel* formation based on chemical reactions

(Eg., chemical and photo-initiated polymerization).

Chemical reactions that results *in situ* gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes.

Ionic cross linking

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones.While k-carrageenan forms rigid, brittle gels in replacement of small amount of K+, i-carrageenan forms elastic gels mainly in the presence of Ca2+. Gellan gum available commercially as Gelrite® is an anionic polysaccharide that undergoes *in situ* gelling in the presence of mono- and divalent cations, including Ca2+, Mg2+, K+ and Na+. Gelation of the low-methoxypectins can be caused by divalent cations, especially Ca2+. Likewise, alginic acid undergoes gelation in presence of divalent/ polyvalent cations eg. Ca2+ due to the interaction with guluronic acid block in alginate chains.

Photo-polymerization

Photo-polymerization is commonly used for *in situ* gel formation of biomaterials. A solution of monomers or reactive macromers and initiator can be injected into a tissue site and the application of electromagnetic radiation

used to form gel. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and macromers because they rapidly undergo photo-polymerization in the presence of suitable photo initiator.

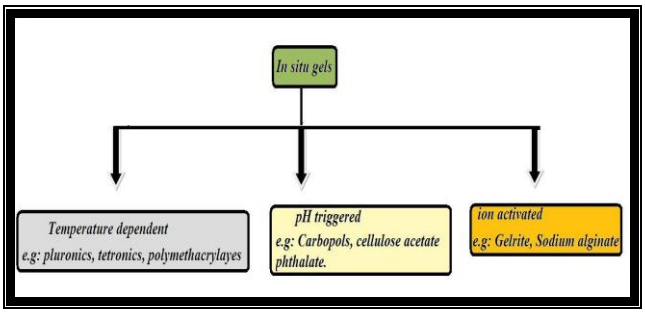


Fig 1.12: A Schematic diagram of In situ gel formulation

1.5. Migraine: *(Viram., et al., 2012)*

Migraine is a physiologic condition, in which a person suffers from tremendous headache. Generally, this headache affects only one side of the head and body. Migraine is a neurological syndrome characterized by altered bodily perceptions, headaches and nausea. Physiologically the migraine headache is a neurological condition more common to women than to men.

In such people, the blood flow in the brain muscles drops, as a result of too much load, squeezing the arteries. When the person suddenly relaxes, these tight brain muscles expand, stretching the blood vessel walls. The blood pumped with each heartbeat, then pushes the vessels further, causes immense pain. Though the exact cause of migraine has not been identified. There are a number of factors that can trigger the severe headache. The typical headache is unilateral and pulsating, lasting from 4 to 72 hours.

In other words migraine is a familial disorder characterized by recurrent attacks of headache widely variable in intensity, frequency and duration. Migraine headaches results from a combination of blood vessel enlargement and the release of chemicals from nerve fibers that coil around these blood vessels. During the headache, an artery

enlarges that is located on the outside of the skull just under the skin of the temple (temporal artery). This causes a release of chemicals that cause inflammation, pain and further enlargement of the artery.

A migraine is a severe painful headache that is often preceded or accompanied by sensory warning signs such as flashes of light, blind spots, tingling in the arms and legs, nausea, vomiting, and increased sensitivity to light and sound. A migraine headache causes the sympathetic nervous system to respond with feelings of nausea, diarrhea, and vomiting. This response also delays the emptying of the stomach in to the small intestine (affecting food absorption), decreases blood circulation (leading to cold hands and feet) and increases sensitivity to light and sound.

1.5.1. Causes of Migraine: *(http://www.medicalnewstoday.com/articles)*

Some people who suffer from migraines can clearly identify triggers or factors that cause the headaches, but many cannot. Potential migraine triggers include:

- Allergies and allergic reactions
- Bright lights, loud noise and certain odors and perfumes
- Physical or emotional stress
- Changes in sleep patterns or irregular sleep
- Smoking or exposure to smoke
- Skipping meals or fasting
- Alcohol
- Menstrual cycle fluctuations, birth control pills hormone fluctuations during menopause onset.

Triggers do not always cause migraines, and avoiding triggers does not always prevent migraines.

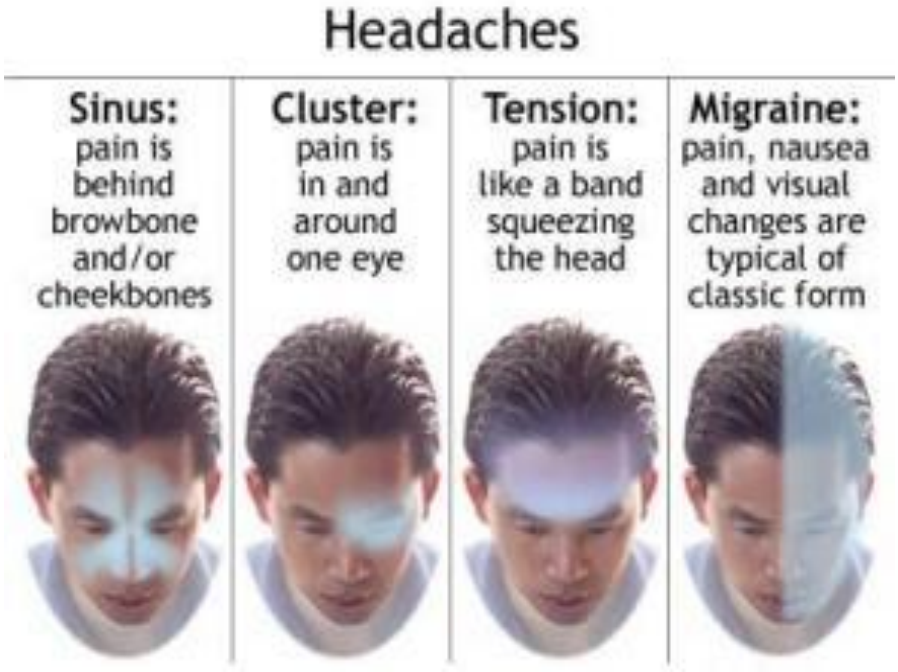


Fig 1.13: A Schematic diagram of causes of migraine

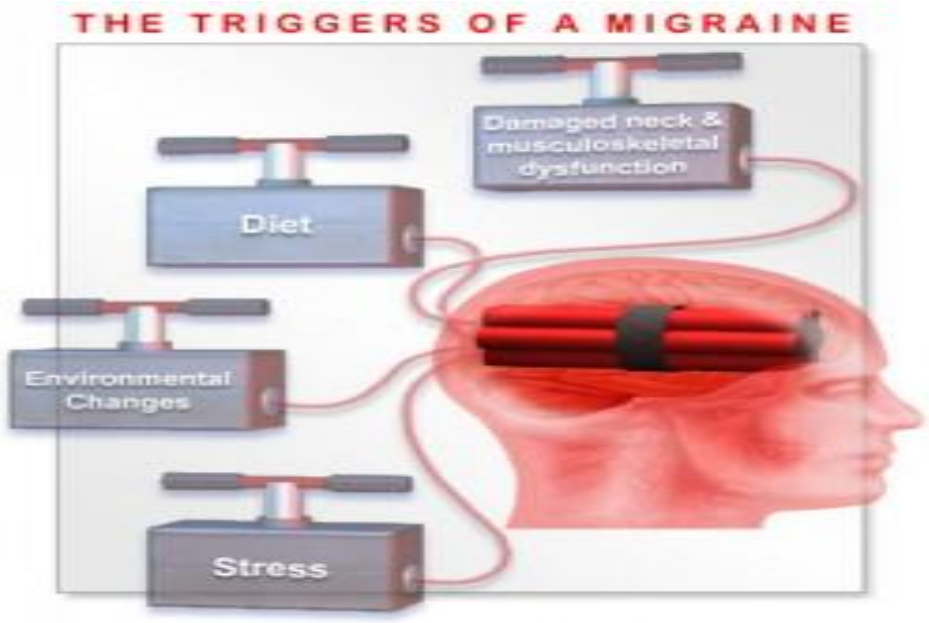


Fig 1.14: A Schematic diagram of triggers of migraine

1.5.2. Symptoms of Migraine:

Symptoms of migraine can occur a while before the headache, immediately before the headache, during the headache, and after the headache. Although not all migraines are same, typical symptoms are include:

- Moderate to severe pain, usually confined to one side of the head, but switching in sucessessive migraines.
- Pulsing and throbbing head pain
- Increasing pain during physical activity
- Inability to perform regular activities due to pain
- Nausea, vomiting and increased sensitivity to light and sound

1.5.3. Stages of migraine: *(http://www.nhs.uk/conditions/migraine)*

There are five distinct stages to a migraine, although not everyone goes through all these stages,

Prodromal (Pre-headache) stage:

Some people experience change in moode, energy levels, behavior and appetite, and sometimes aches and pains several hours or days before an attack. Sometimes also referred to as the **"premonitory stage"** this phase can begin hours or even days prior to the onset of the acute pain phase. It may be characterized by sleepiness, irritability, and other change in mood or any number of diverse symptoms specific to the individual sufferer. About half of all migraine patients report consistent awareness of a prodrome stage. They 'just know' when a migraine is coming. However, it has been found that about 80% of those with migraine can learn to recognize a prodrome stage.

Aura:

Some people experience in a sensation, or aura, just before their migraine starts. Symptoms of aura include flashes of light or blind spots, difficulty focusing, and seeing things as if you are looking through a broken mirror. The aura usually develops over 5-20 minutes and lasts less than 60 minutes. The aura is not harmful and results in no permanent damage. But for someone who has never had a migraine, or never had an aura, the experience can be a very frightening one.

The aura usually disappears before the start of headache pain, which usually follows the aura by a few minutes to an hour. Infrequently the aura may continue during the time of headache pain. Even more infrequently the headache pain may never arrive. When an aura is not followed by a headache, it is called a **migraine equivalent, acephalic migraine or silent migraine.**

Headache Stage or Acute Pain Stage:

This is usually a pulsating or throbbing pain on one side of the head. The characteristic feature of migraine disease is, of course, the migraine headache. But even the term, "migraine headache" is, if not entirely misleading, at least deficient. While the headache pain may be severe, the symptoms of an acute attack are not limited to pain. Migraine headache is accompanied by a host of autonomic symptoms. These may include nausea, vomiting, aversion to bright lights (photophobia), aversion to loud sounds (phonophobia), aversion to strong odors (osmophobia), and any number of other symptoms. This stage lasts for 4 to 72 hours.

The headache of migraine generally exhibits at least some of the following characteristics:

- Throbbing, pounding, or pulsating pain
- Often on just one side of the head, or begins on one side before spreading to both sides
- Pain may alternate sides from one attack to the next
- Made worse with mild activity, such as walking or bending
- Most intense pain is often concentrated around the temple(s) – on the side of the forehead
- Commonly lasts from 4 to 72 hours in adults, 30 minutes to 48 hours in children
- Nausea is reported by almost 90% of migraine sufferers
- Vomiting may occur with up to 35% of migraine attacks

Resolution Stage:

Most attacks gradually fade away. Some people find the headache stops suddenly after they have been sick. Sleep have been relieves the symptoms.

Postdromal or Recovery phase:

There may be a stage of exhaustion and weakness afterwards. Simply stated, the migraine headache is gone but migraine disease remains active. Commonly, patients experience migraine-associated non-headache symptoms for an additional 24 to 48 hours. Given an average prodrome stage lasting 24 hours, an acute pain stage

That averages 24-48 hours and post-headache impairment lasting an additional 24-48 hours, the total duration of migraine impairment, per attack, can be from three to six days. Of course some attacks are more or less severe, and the extent to which migraine impairs functioning is obviously greatest during the acute pain stage. Nonetheless, considering that a person with moderate migraine disease may experience two to four attacks per month, it is often the case that there are more days with migraine impairment than there are without migraine impairment.

There are several types of Migraine:

- **Migraine with Aura** is when there is a warning sign, known as aura, before the migraine begins. Warning sounds may include visual problem (such as flashing lights) and stiffness in the neck, shoulders or limbs.
- **Migraine without Aura**
- **Migraine without Headache** also known as silent migraine is when an aura or other migraine symptoms are experienced but a headache does not develop.

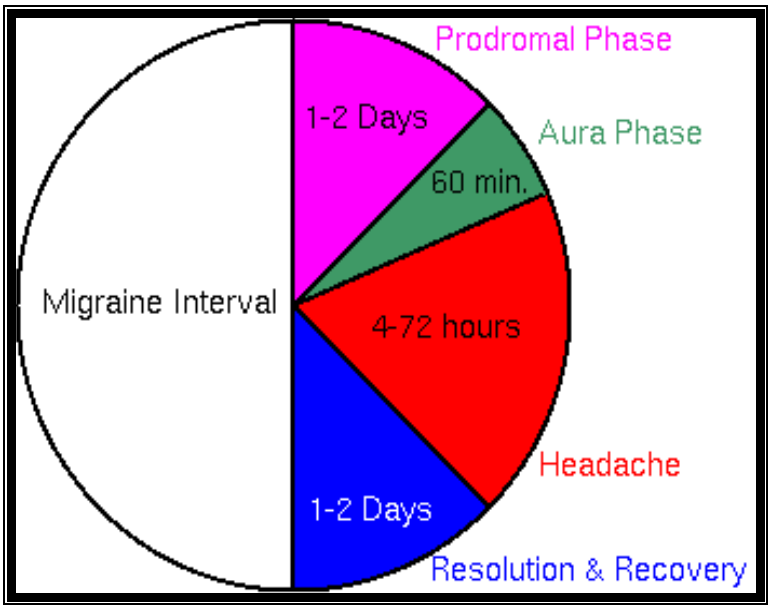


Fig 1.15: A Schematic diagramatic representation of stages of migraine

1.5.4. Diagnosis of Migraine:

The International Headache Society recommends the following criteria to diagnose migraines without aura. This stands for:

- 5 or more attacks
- 4 hours to 3 days in duration
- At least 2 of unilateral location, pulsating quality, moderate to severe pain aggravation by or avoidance of routine physical activity
- At least 1 additional symptoms such as nausea, vomiting, sensitivity to light, sensitivity to sound.
- Tests such as electroencephalography (EEG), computed tomography (CT), magnetic resonance imaging (MRI) and spinal tap may also been performed that check for:
- Bleeding within the skull

- Blood clot within the membrane
- Stroke
- Dilated blood vessels in the brain
- Too much or too little cerebrospinal fluid
- Inflammation of the membranes of the brain or spinal cord
- Nasal sinus blockage
- Postictal headache (after stroke or seizure)
- Tumors

1.5.5. Prevention and treatment of migraine:

(http://www.mayoclinic.com/health/migraine-headache)

Migraine treatment (abortive therapies) and prevention (prophylactic therapies) focus on avoiding triggers, controlling symptoms, and taking medicines.

➢ **Preventive Migraine Medication**

Antimigraine drugs or drugs that prevent migraine or cure migraine are classified as follows:

- **Beta Blockers:** The drugs prevent the widening of the arteries in your head by blocking the beta receptors.
- **Anticonvulsants:** These drugs treat seizures. Like many preventative migraine medications, they increase levels of an amino acid known as GABA, which may play an important in migraine development.
- **Methysergide:** This is one of the more toxic medications, and so is usually reserved for more serious cases.

- **Calcium Channel Blockers:** These medications have the ability to stop the spasm of the arteries, block the release of serotonin and inhibit platelet clumping.
- **Antidepressants:** It's believed that the anti-migraine effect of these medicines is a whole different reaction than what happens when treating depression.
- **Clonidine:** Clonidine is an alpha blocker that also protects the blood vessels.
- **Cyproheptadine**: It's most often prescribed to children, and seems to be only slightly effective for adults.
- **NSAIDs:** Though primarily used to stop headaches once they start, they have also been used as a preventative medication.
- **Combinations:** Sometimes combinations of the above types of drugs will be prescribed.

➢ **Abortive Migraine Medication**

Depending on your physical check up and past history, your doctor may prescribe treatment to stop the headaches once they have started. Medicines to treate migraines are called **"abortive medications"** and include:

- Analgesics with caffeine like Excedrin® Migraine (acetaminophen aspirin and caffeine)
- Analgesics with caffeine and barbiturates lie Fiorinal® and Fioricet®.
- Non steroidal anti-inflammatory drugs (NSAIDs) like Advil® and Aleve®.

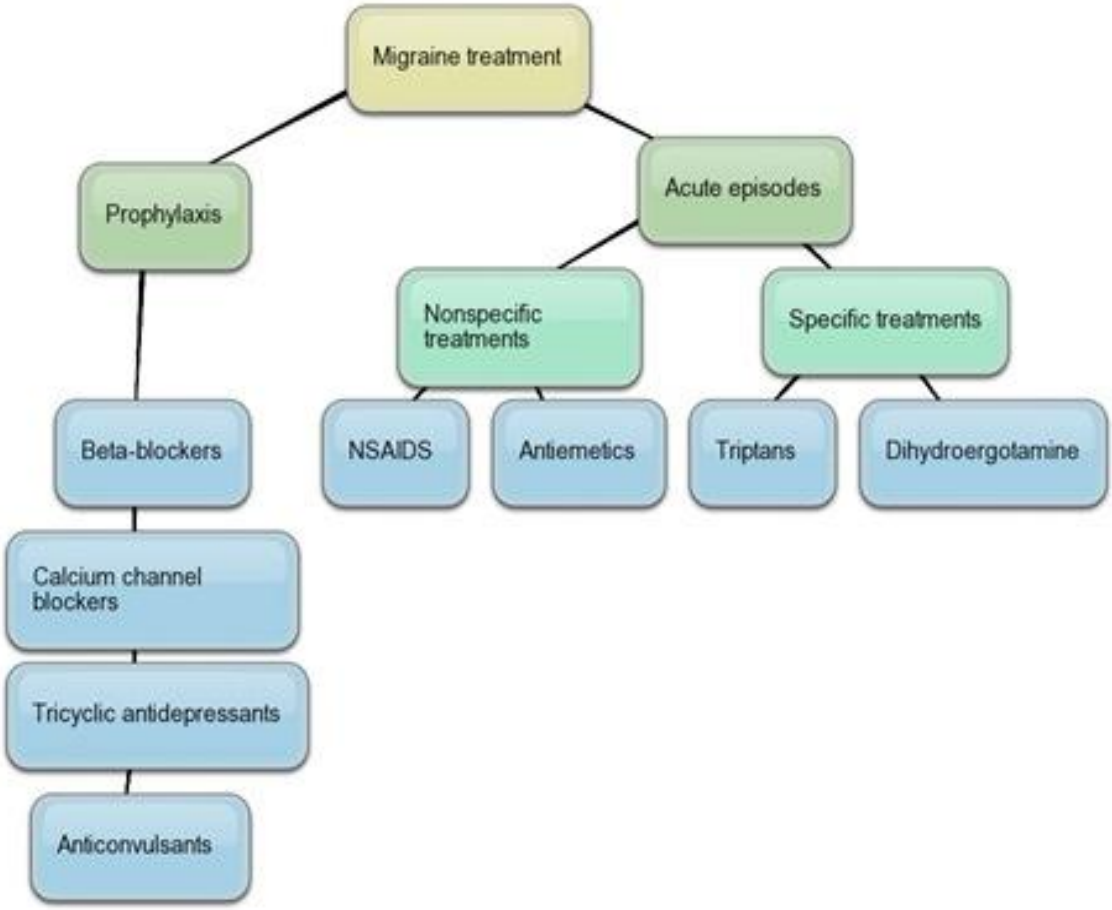


Fig 1.16: A Schematic diagrammatic representation of Treatment of migraine

1.5.6. Popular Migraine Medicines

- Inderal (propranolol)
- Blocadren (timolol)
- Toprol (metoprolol)
- Corzide (nadolol)
- Triptans
- Dihydroergotamine
- Prochlorperazine

- Depakene (sodium valproate)
- Depakote (divalproex sodium)
- Isoptin (Verapamil)
- Nimotop (Nimodipine)
- Elavil
- Sinequan
- Norpramin
- Proza

LITERATURE SURVEY

2. LITERATURE SURVEY

2.1. Literature Review

1) Swamy. N. G. N., *et al*. (2012): had reported that *In situ* forming polymeric formulations are drug delivery systems that are in sol form before administration in the nasal cavity, but once administered, undergo gelation *in situ*, to form a gel. The formation of gel depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, from which the drug gets released in a sustained and controlled manner. Mucoadhesive *in situ* gel formulations have demonstrated increase in the residence time in the nasal cavity as well enhancement of the permeation characteristics of the drug. The *in situ* gel forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems. With a brief introduction to nasal drug delivery, in this paper, the use of novel mucoadhesive *in situ* gels for the intranasal delivery of drugs is reviewed along with methods available for evaluation of *in situ gels*.

2) Swati Pund., *et al*. (2012): had reported that Venlafaxine, a dual acting antidepressant is a new therapeutic option for chronic depression. They analyzed the transport of Venlafaxine through sheep nasal mucosa. Transmucosal permeation kinetics of Venlafaxine was examined using sheep nasal mucosa mounted onto static vertical Franz diffusion cells. Nasal mucosa was treated with Venlafaxine in situ gel (100 µl,1% w/v) for 7 h. Amount of Venlafaxine diffused through mucosa was measured using validated RP-HPLC method. After the completion of the study histopathological investigation of mucosa was carried out. Ex vivo studies through sheep nasal mucosa showed sustained diffusion of Venlafaxine with 66.5%

permeation in 7 h. Histopathological examinations showed no significant adverse effects confirming that the barrier function of nasal mucosa remains unaffected even after treatment with Venlafaxine in situ gel. Permeation through sheep nasal mucosa using in situ gel demonstrated a harmless nasal delivery of Venlafaxine, providing new dimension to the treatment of chronic depression.

3) Yuan Yuana., *et al*. (2012): had reported that Poloxamer 407 has excellent thermo-sensitive gelling properties. The main aim of the present investigation was to develop thermo sensitive and mucoadhesive rectal in situ gel of Nimesulide (NM) by using mucoadhesive polymers such as sodium alginate (Alg–Na) and HPMC. These gels were prepared by addition of mucoadhesive polymers (0.5%) to the formulations of thermosensitive gelling solution containing poloxamer 407 (18%) and Nimesulide (2.0%). Polyethylene glycol (PEG) was used to modify gelation temperature and drug release properties. The gelation temperature and drug release rate of the prepared in situ gels were evaluated. Gelation temperature was significantly increased with incorporation of Nimesulide (2.0%) in the poloxamer solution, while the addition of the mucoadhesive polymers played a reverse role on gelation temperature. The addition of PEG polymers increased the gelation temperature and the drug release rate. Among the formulations examined, the poloxamer 407/Nimesulide/sodium alginate/PEG 4000 (18/2.0/0.5/1.2%) exhibited the appropriate gelation temperature, acceptable drug release rate and rectal retention at the administration site.

4) Bhandwalkar M.J., *et al*. (2012): had reported that in order to improve the bioavailability of the antidepressant drug, Venlafaxine hydrochloride, in situ mucoadhesive thermoreversible gel, was formulated using Lutrol F127 (18%) as a thermo gelling polymer. Mucoadhesion was modulated by trying carbopol 934, PVP K30, HPMC K4M, sodium alginate, tamarind seed gum, and carrageen an as

mucoadhesive polymers. Results revealed that as the concentration of mucoadhesive polymer increased the mucoadhesive strength increased but gelation temperature decreased. Formulation was optimized on the basis of clarity, pH, gelation temperature, mucoadhesive strength, gel strength, viscosity, drug content, diffusion through sheep nasal mucosa, histopathological evaluation of mucosa, and pharmacodynamic study in rats.

5) Jyotivardhah Jaiswal., *et al*. (2012): had reported Metoprolol Succinate undergoes hepatic first pass metabolism and hence it shows poor bioavailability. The objective of present research work is to improve bioavailability by formulating thermo reversible *in-situ* nasal gel. Formulation was developed to reduce the mucociliary clearance by using mucoadhesive polymer in gel, thereby increasing the contact time with nasal mucosa and hence improving the absorption of drug. The *in-situ* gelation was achieved by the use of pluronic F127, which exhibits thermo reversible gelation property and Sodium alginate was used as the mucoadhesive agent. Gels were prepared by cold technique method and characterized by Gelation Temperature, Permeation Studies, Histopathological Evaluation, pH, Drug Content, Rheological studies, Gel strength and drug polymer interaction study.

6) Shital Uttarwar., *et al*. (2012): had reported " Gel" is the state between the liquid and solid which consists of physically cross linked networks of long polymer molecules with liquid molecules trapped within a three dimensional polymeric network swollen by a solvent. The aim of present study was formulation of *in situ* gelling system for nasal administration for an antiemetic drug Ondansetron Hydrochloride by using Pluronics 127P and Pluronics 68. All the formulations showed compliance with pharmacopoeial standards.

7) Pramod K. Kolsure., *et al*. (2012): had Developed the thermo reversible Zolmitriptan nasal gel was aimed to improve absorption and patient compliance. In the present research work, mixture of pluronic F-127 (Poloxamer 407) and pluronic F-68 (Poloxamer 188) were used to confer temperature- sensitive gelation property. To modulate the gel strength and biadhesive force for Zolmitriptan nasal gel, bioadhesive polymers such as sodium alginate, sodium carboxyl methyl cellulose and polyvinyl pyrollidine (PVP K-25) were investigated. Incorporation of 25% w/w Zolmitriptan in the nasal showed no effect on the gelation temperature of the pluronic mixtures, while addition of the bioadhesive polymers reinforced the gel strength and the bioadhesive force of the prepared nasal gel formulation. The effect was most pronounced with sodium alginate. Increasing the concentration of bioadhesive polymers retarded the release of Zolmitriptan from the pluronic gel. PVP has less effect on the drug release. Histopathological examination of sheep nasal mucosa with control and optimized formulation did not show an y histological damage to the nasal tissue.

8) Parmar Viram., *et al.* (2012): had reported that oral Metoclopramide hydrochloride undergoes first-pass metabolism. Nasal delivery could protect drugs from this effect. Mucoadhesive *in situ gels* for Metoclopramide hydrochloride with gellan gum were formulated and evaluated. Metoclopramide Hydrochloride *Insitu* nasal gels (10% w/w) were prepared at concentration of gellan gum 0.2%, 0.4%, 0.6% and 0.8% w/v with xanthan gum 0.1%, 0.15% and 0.2% w/v as bioadhesive polymer and benzalkonium chloride (0.01%) as preservative. The formulation were evaluated by slower release rate during the first hr, Release kinetics followed diffusion model. Microscopic results did not show any mucosal changes after diffusion study of the optimized formulation.

9) Dattatraya J. Yadav., *et al*. (2012): had reported that aimed to formulate and evaluate Nasal drug delivery system containing Salbutamol Sulphate was prepared for improving the bioavailability & sustaining the drug release. Salbutamol sulphate is a selective β2 adrenoreceptor agonist and rapidly absorbed from gastro intestinal tract but it is subjected to first pass metabolism. Thus oral bioavailability is only 50%. The main objective of present work is to enhance the bioavailability; reducing the dose. Thermoreversible, bioadhesive polymers such as poloxamer and Hydroxy Propyl Methyl Cellulose (HPMC) in the form of in situ gel by cold technique. The results revealed that as the increase of bioadhesive polymer HPMC concentration, decrease in gelation temperature (T1) and increase in gel melting temperature (T2). pH of all formulation were found to be within the range between 5.5 to 6. The drug content for all formulation was found to be 96%-100%. The mucoadhesive test indicates that the level of HPMC increases, the mucoadhesive strength also increases. The developed formulations had optimum viscosity. The optimized formulation shows the controlled drug release.

10) Kote Amol P., *et al*. (2011): had reported that nasal drug delivery system offers lucrative way of drug delivery of both topical and systemic therapies. The high permeability, high vasculature and low enzymatic environment of nasal cavity are well suitable for systemic delivery of drug molecules via nose. The noninvasiveness and self administrative nature of nasal delivery also attracts the formulation scientists to deliver protein and peptide compounds. Despite of all the advantages of nasal drug delivery, the bioavailability of nasally administered products, especially for protein and peptide molecules, is affected by many barriers such as physiological barriers, physicochemical barriers, and formulation barriers.

This review will focus on the various bioavailability barriers in nasal drug delivery and the strategies to improve the bioavailability of nasal dosage form.

11) Gowda D.V., *et al*. (2011): had reported that the objective of present investigation was to develop a mucoadhesive in-situ gel; formulation was developed to have a controlled kinetic drug release and to minimize the toxic effects of diltiazem Hydrochloride (DTZ). DTZ was incorporated into the, blends of thermoreversible, bioadhesive polymers such as poloxamer (PLX) and Hydroxy Propyl Methyl cellulose (HPMC) in the form of in-situ gel by cold technique to reduce mucociliary clearance, and thereby it will increase the contact of formulation with nasal mucosa and hence improving the absorption of drug. The drug release performance was greatly affected by bio polymers used and their compositions in the in situ gels preparation, which allows absorption in nasal mucosa.

12) Pranshu Tangri., *et al*. (2011): had reported a brief idea bioadhesive delivery systems based on hydrogels to biological surfaces that are covered by mucus. Techniques that are frequently used to evaluate the mucoadhesive drug delivery systems are discussed.. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains. Mucoadhesive drug delivery systems is one of the most important novel drug delivery systems with its various advantages and it has a lot of potential in formulating dosage forms for various chronic diseases.

13) Nazar H., *et al*. (2011): had reported that the development of a thermosensitive drug-delivery vehicle for nasal delivery, a systematic series of N-trimethyl chitosan chloride polymers, synthesized from chitosans of three different average molecular weights, have been co-formulated into a hydrogel with poly (ethylene glycol) and glycerophosphate. Rheological evaluations have shown that

hydrogels derived from N-trimethyl chitosan with a low degree of quaternisation and high or medium average molecular weight exhibit relatively short sol–gel transition times at physiologically relevant temperatures. Also, the same hydrogels display good water-holding capacity and strong mucoadhesive potential, and their mixtures with mucus exhibit rheological synergy. An aqueous hydrogel formulation, derived from N-trimethyl chitosan of medium average molecular weight and low degree of quaternisation, appears particularly promising in that it exhibits most favorable rheological and mucoadhesive behavior and a sol–gel transition that occurs at 32.5 ^{0} C within 7min.

14) Khan S., *et al.* (2010): had reported that mucoadhesive temperature-mediated in situ gel formulations using chitosan and hydroxyl propyl methyl cellulose were used to enhance intranasal (i.n.) delivery of the dopamine D2 agonist ropinirole to the brain. Formulations were tested for gelation time, thermosensitivity, mucoadhesion, in vitro release and permeation, in- vitro cytotoxicity, nasal clearance, in vivo bioavailability and brain uptake.

15) Alagusundaram. M., *et al*. (2010): had reported the use of the nasal route for the delivery of challenging drugs such as small polar molecules, vaccines, hormones, peptides and proteins has created much interest in nowadays. Due to the high permeability, high vasculature, low enzymatic environment of nasal cavity and avoidance of hepatic first pass metabolism are well suitable for systemic delivery of drug molecule via nose. Many drug delivery devices for nasal application of liquid, semisolid and solid formulation are investigated to deliver the drugs to the treat most crisis CNS diseases (i.e., Parkinson's disease, Alzheimer's disease) because it requires rapid and/or specific targeting of drugs to the brain. It is well suitable for the delivery of biotechnological products like proteins, peptides, hormones, DNA plasmids for

DNA vaccines to give enhanced bioavailability. This review sets out to discuss some factors affecting nasal absorption, bio-availability barriers, strategies to improve nasal absorption, new developments in nasal dosage form design and applications of nasal drug delivery system.

16) Rahisuddin., *et al*. (2010): had reported that the aim of the present investigation is to explain the recent advancement of nasal drug delivery system. Intranasal therapy has been an accepted form of treatment in the Ayurvedic system of Indian Medicine. The interest in intranasal delivery of drugs as a non-invasive is increased. They had also discussed advantages, disadvantages, mechanism of action and application of nasal drug delivery system in local delivery, systematic delivery, and nasal vaccine and CNS delivery of the drug. They were discussed here relevant aspects of biological, physicochemical and pharmaceutical factors of nasal cavity that must be considered during the process of discovery and development of new drugs for nasal delivery as well as in their incorporation into appropriate nasal Pharmaceutical formulations. Nasal route is more suitable for those drugs which cannot be administered orally due to gastric degradation or hepatic first pass metabolism of the drug. Intranasal drug delivery is found much promising route for administration of peptides and protein drugs.

17) Varsha Gaikwad., *et al*. (2010): The objective of present investigation was to develop a mucoadhesive in-situ gel: formulation was developed with aim to reduce the mucociliary clearance by using mucoadhesive polymer in gel, thereby increasing the contact of formulation with nasal mucosa and hence improving the absorption of drug. The in-situ gelation was achieved by the use of pluronic F127, which exhibit thermoreversible geleation property. The formulation additives are having effect on thermodynamic of phase transitions at gelation (TI) and gel melting

(T2) temperature. Result revealed that addition of mucoadhesive polymer carbopol934 has decreased T1 whereas addition of PEG400 increased T1 PEG400 increased in-vitro drug release shows that with increasing carbopol concentration increases viscosity formulation and influences the diffusion of drug particle while addition of PEG 400 enhances in vitro drug release.

18) Shyam D. Badgujar., *et al.* (2010): had reported tha**t** Sumatriptan Succinate is a 5-HT1D (5-hydroxy tryptamine 1d)-receptor agonist, used in the treatment of migraine and cluster headache Sumatriptan Succinate has been shown to have a low oral bioavailability in human volunteers (15%) because of high first pass metabolism. Subcutaneous administration is an alternative: however, dislike of injections or inability to self-administer by this route makes subcutaneous treatment unacceptable to some individuals. These all above things justify a need of nasal drug delivery. To improve the nasal retention time of Sumatriptan Succinate, it has been formulated as in situ mucoadhesive gel by using pluronic PF127 and carbopol 974P. The objective of this work was to improve the nasal bioavailability of Sumatriptan Succinate by increasing its nasal retention time as well as by means of nasal permeation. Nasal permeation of Sumatriptan Succinate was improved by using fulvic acid extracted from shilajit as a novel permeation enhancer.

19) Bhanushali., *et al.* (2009): The objective of this study was to develop intranasal nanoemulsion and gel formulations for Rizatriptan Benzoate for prolonged action. Nanoemulsion formulation were prepared by constructing pseudo-ternary phase diagrams using lipophilic and hydrophilic surfactants and water. Various mucoadhesive agents were tried out to form thermo-triggered mucoadhesive nanoemulsion. Mucoadhesive gel formulation of Rizatriptan were prepared using different ratios of HPMC and carbopol 980.Comparative evaluation of intranasal

nanoemulsions and intranasal mucoadhesive gel indicated that grater brain-targeting could be achieved with nanoemulsions.

20) Shi-lei Caoa., *et al*. (2009): had explain the main purpose of this study was to prepare a novel in situ gel system for nasal delivery of MF and study its efficacy on allergic rhinitis model. An ion-activated in situ gel was developed and characterized with gellan gum as a carrier. The system was stable kept at 40±2 ◦C for 6 months, and the micrographic results showed that in situ gel was safety without mucosa irritation when given at 20μg once daily for 1 month to rats with allergic rhinitis. MF in gellan gum produced obviously effect on allergic rhinitis at the doses of 20μg/body following intranasal administration, and the efficacy was significantly superior to that of the common suspension ($P < 0.01$). The in situ gel system is a promising approach for the intranasal delivery of MF for the therapeutic effects improvement.

21) Noha M. Zai., *et al*. (2007): had reported the objective of the present investigation was to develop a mucoadhesive *in situ* gel with reduced nasal mucociliary clearance in order to improve the bioavailability of the antiemetic drug, Metoclopramide Hydrochloride (MCP HCl). The *in situ* gelation upon contact with nasal mucosa was conferred via the use of the thermogelling poloxamer 407 whereas mucoadhesion and drug release enhancement were modulated via the use of mucoadhesive and polyethylene glycol (PEG) polymers respectively. The study point to the potential of mucoadhesive nasal *in situ* gel in terms of ease of administration, accuracy of dosing, prolonged nasal residence and improved drug bioavailability.

22) Michael I., *et al*. (2005): had reported that nasal drug delivery has now been recognized as a very promising route for delivery of therapeutic compounds including biopharmaceuticals. It has been demonstrated that low absorption of drugs

can be countered by using absorption enhancers or increasing the drug residence time in the nasal cavity, and that some mucoadhesive polymers can serve both functions. This article reviews the background of nasal mucoadhesive drug delivery with special references to the biological and pharmaceutical considerations for nasal mucoadhesive drug administration.

23) Ketousetuo Keotsu., *et al*. (2005): had developed using a natural mucoadhesive agent obtained from the fruit of Delinia Indica L.The mucoadhesive strength and viscosity of this natural mucoadhesive agent was found to be higher in comparison to the synthetic polymers, namely hydroxyl propyl methyl cellulose (HPMC) and carbopol 934, which are conventionally used for a similar purpose. Invitro drug release characteristics using a Franz-diffusion cell and excised bovine nasal membrane was also found to be better in comparison to the above synthetic polymers. This patient friendly, needle free dosage form may replace the Oxytocin injections in the future.

24) Sudipta Ganguly., *et al*. (2004): had reported the objective of this study was to develop a novel chitosan-glyceryl monooleate (GMO) in situ gel system for sustained drug delivery and targeting. The delivery system consisted of 3% (w/v) chitosan and 3% (w/v) GMO in 0.33M citric acid. In situ gel was formed at a biological pH. In vitro release studies were conducted in Sorensen's phosphate buffer (pH 7.4) and drugs were analyzed either by HPLC or spectrophotometry. Characterization of the gel included the effect of cross-linker, determination of diffusion coefficient and water uptake by thermogravimetric analysis (TGA). Mucoadhesive property of the gel was evaluated in vitro using an EZ-Tester. Incorporation of a cross-linker (glutaraldehyde) retarded the rate and extent of drug release. The in-vitro release can further be sustained by replacing the free drug with

drug-encapsulated microspheres. Drug release from the gel followed a matrix diffusion controlled mechanism. Inclusion of GMO enhanced the mucoadhesive property of chitosan by three to seven fold. This novel in situ gel system can be useful in the sustained delivery of drugs via oral as well as parenteral routes.

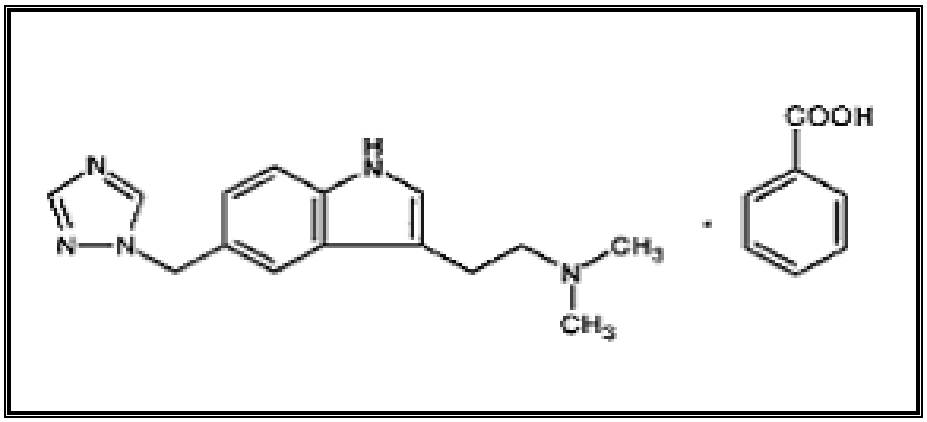
2.2. DRUG PROFILE

(http:// www.google.com, drug bank; http://en.wikipedia.org/wiki/Rizatriptan; http://www.rxlist.com/cgi/generic2/rizatrip.htm; http://www.drugs.com/cdi/rizatriptan.html)

Rizatriptan Benzoate:

Rizatriptan Benzoate is a class of Serotonin $5HT_1$ receptor antagontst.

1. **Synonyms** **:** MK 462 Free Base Rizatriptan, Rizatriptan benzoat, Rizatriptan benzoate.
2. **Molecular formula** **:** $C_{15}H_{19}N_5 \bullet C_7H_6O_2$
3. **Molecular weight** **:** 391.47
4. **IUPAC Name** **:** dimethyl({2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethyl})amine
5. **Chemical name** **:** N,N-Dimethyl-5-(1H-1,2,4-triazol-1-yl-methyl)-1H-indole-3- ethanamine monobenzoate
6. **CAS number** **:** 145202-66-0
7. **Category** **:** Anti-migraine drug.
8. **Melting point** **:** 178-180°C
9. **Structural formula:**



10. Solubility:

- Soluble in water 42 mg/mL (for free base).

11. Description:

- Off white crystalline solid
- characteristic odour

12. Pharmacokinetic data:

- **Bioavailability** **:** 45%
- **Protein binding** **:** 14%
- **Metabolism** **:** Extensive First pass metabolism (MAO-A involved)
- **Half-life** **:** Approximately 2-3 h.
- **Volume of distribution :** 140L in men, 110L in women
- **Excretion** **:** Renal
- **Pka** **:** Strongest acedic: 17.24 **,** Strongest basic: 9.56
- **logP** **:** 1.4
- **Nature** **:** Hydrophilic

13. Dosage and Administration:

Table 2.1: Table indicating dosage Forms & Strengths

S.NO	DOSAGE FORMS	STRENGTHS
1	Tablets	5mg 10mg
2	Tablets orally disintegrating	5mg

⦿ **Adults**

PO 5 or 10 mg per migraine attack. A second dose may be repeated after a minimum of 2 h as needed (max, 30 mg per 24 hours).

⦿ **Children (6 to 17 y of age)**

PO For weight 40 kg or more, 10 mg per migraine attack; for weight 39 kg or less, 5 mg per migraine attack.

Concomitant therapy with propranolol

⦿ **Adults**

PO Only the 5 mg dose of rizatriptan is recommended, up to a maximum of 3 doses per 24 h (15 mg).

⦿ **Children (6 to 17 y of age)**

PO For weight 40 kg or more, only a single 5 mg dose of rizatriptan is recommended (max, 5 mg per 24 h). Do not coadminister rizatriptan and propranolol to patients who weigh less than 40 kg.

General Advice

- The safety of treating, on average, more than 4 headaches in a 30-day period has not been established.
- If a patient has no response to the first dose of rizatriptan, the diagnosis of migraine should be reconsidered before administration of a second dose.
- Administration of the disintegrating tablets with liquid is not necessary.

14. Mechanism of action:

Three distinct pharmacological actions have been implicated in the antimigraine effect of the triptans: (1) stimulation of presynaptic 5-HT1D receptors, which serves to inhibit both dural vasodilation and inflammation; (2) direct inhibition of trigeminal nuclei cell excitability via 5-HT1B/1D receptor agonism in the brainstem and (3) vasoconstriction of meningeal, dural, cerebral or pial vessels as a result of vascular 5-HT1B receptor agonism.

15. Therapeutic uses

- Vasoconstrictor Agents
- Anti-inflammatory Agents
- Anti-migraine Agents
- Selective Serotonin Agonists

16. Toxicities:

Symptoms of overdose include dizziness, fainting, heart and blood vessel problems, high blood pressure, loss of bowel and bladder control, slow heartbeat, and vomiting.

17. Drug interactions

5-HT $_1$ agonists (eg, sumatriptan)

Increased risk of vasospastic reactions; therefore, coadministration of two 5-HT $_1$ agonists within 24 h of each other is contraindicated.

Ergot-containing drugs (eg, ergotamine)

Additive and prolonged vasospasm. Coadministration within 24 h is contraindicated.

MAOIs (eg, phenelzine)

Use of rizatriptan with MAOIs or within 14 days following discontinuation of an MAOI is contraindicated.

Propranolol

Increased rizatriptan plasma concentrations. The dose of Rizatriptan should be adjusted.

Serotonin syndrome has been reported during coadministration of SSRIs, SNRIs, or TCAs and selective 5-HT $_1$ agonists. The onset of serotonin syndrome can occur within minutes to hours of receiving a new or a greater dose of a serotonergic

medication. Rizatriptan treatment should be discontinued if serotonin syndrome is suspected.

18. Adverse Reactions:

Cardiovascular

Palpitation (at least 1%); coronary artery vasospasm; hypertension; MI; stroke; transient myocardial ischemia; ventricular tachycardia; ventricular fibrillation.

CNS

Dizziness (9%); somnolence (8%); asthenia/fatigue (7%); paresthesia (4%); headache (2%); euphoria, hypoesthesia, tremor (at least 1%); seizure (postmarketing).

GI

Nausea (6%); dry mouth (3%); abdominal discomfort, diarrhea, vomiting (at least 1%); dysgeusia (postmarketing).

Hypersensitivity

Allergic conditions, including anaphylaxis/anaphylactoid reaction, angioedema, wheezing, and TEN (postmarketing).

Miscellaneous

Pain and other pressure sensations (9%); atypical sensations (5%); localized pain, pain, tightness, pressure, and/or heaviness of chest (3%); regional tightness, pressure, or heaviness, tightness, pain, or pressure of neck, throat, or jaw (2%); dyspnea, flushing, warm sensations (at least 1%).

19. Precautions:

General Precautions

- **Ocular Effects**
- Possible accumulation of rizatriptan in melanin-rich tissues (e.g., eye) over time, resulting in potential toxicity in these tissues with extended use.

- **Phenylketonuria**
- Advise individuals who must restrict phenylalanine intake that each 5- or 10-mg Maxalt-MLT orally disintegrating tablet contains aspartame, which is metabolized in GI tract to provide 1.05 or 2.1 mg of phenylalanine, respectively. Conventional tablets do not contain aspartame.

20. Brand names :

Table 2.2: Table indicating brand names& manufactures

Brand names	**Manufactures**
Maxalt RPD	Merck Sharp & Dohme, Chile
Maxalt	Merck Sharp & Dohme, Austria; MSD, South Africa
Maxalt-Rapidisc	Merck Sharp & Dohme, Turkey
Rizact	Cipla, India

21. Storage/Stability

Store between 59° and 86°F.

2.3.POLYMERS PROFILE

2.3.1. CARBOPOL 974 P:

(Raymond C. R., 2003; www.pharma.lubrizol.com)

1. Nonproprietary Names:

- **BP:** Carbomers
- **PhEur:** Carbomer
- **USP-NF:** Carbomer

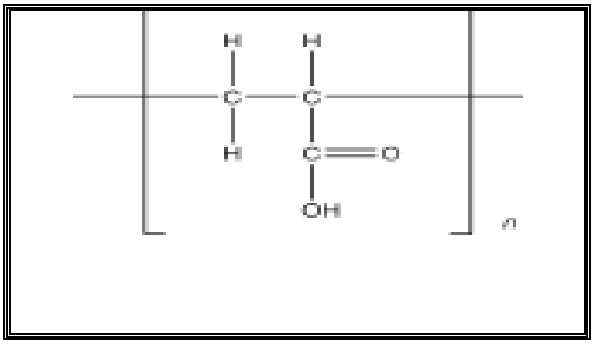
2. Synonyms **:** carboxypolymethylene; carbomers.

3. Chemical Name and CAS Registry Number : Carbomer [9003-01-4]

4. Empirical Formula **:** $C_5H_{10}O_2$

5. Molecular Weight **:** 86,000

6. Structural Formula :



7. Functional Category:

Stabilizing agent, Bioadhesive material, Controlled-release agent, Emulsifying agent, Emulsion stabilizer, Rheology suspending agent, Tablet binder.

8. Applications in Pharmaceutical Formulation or Technology:

Carbomers are used in liquid or semisolid pharmaceutical formulations as rheology modifiers. Formulations include creams, gels, lotions and ointments for use in ophthalmic, rectal, topical and vaginal preparations. Carbomer polymers have also been investigated in the preparation of sustained-release matrix beads, as enzyme inhibitors of intestinal proteases in peptide-containing dosage forms, as a bioadhesive for a cervical patch and for intranasally administered microspheres, in magnetic granules for site-specific drug delivery to the esophagus, and in oral mucoadhesive controlled drug delivery systems.

➢ **Use**	**Concentration (%)**
❖ Emulsifying agent	0.1–0.5
❖ Gelling agent	0.5–2.0
❖ Suspending agent	0.5–1.0
❖ Tablet binder	0.75–3.0
❖ Controlled-release agent	5.0–30.0

9. Description:

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

10. Typical properties:

- **Acidity/alkalinity:** pH = 2.7–3.5 for a 0.5% w/v aqueous dispersion;

  pH = 2.5–3.0 for a 1% w/v aqueous dispersion.

- **Density (bulk):** 1.76–2.08 g/cm3
- **Density (tapped):** 1.4 g/cm3

- **Melting point:** Decomposition occurs within 50 minutes at 260°C.
- **Dissociation constant:** pKa = 6.5-0.5
- **Glass transition temperature:** 100–105.8°C
- **Moisture content:** Typical water content is up to 2% w/w. However, carbomers are hygroscopic and a typical equilibrium moisture content at 258C and 50% relative humidity is 8–10% w/w. The moisture content of a carbomer does not affect its thickening efficiency, but an increase in the moisture content makes the carbomer more difficult to handle because it is less readily dispersed.
- **Solubility:** Swellable in water and glycerin and, after neutralization, in ethanol (95%). Carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally crosslinked microgels.
- **Viscosity (dynamic):** Carbomers disperse in water to form acidic colloidal dispersions of low viscosity that, when neutralized, produce highly viscous gels Carbomer powders should first be dispersed into vigorously stirred water, taking care to avoid the formation of indispersible agglomerates, then neutralized by the addition of a base.

10. Method of Manufacture:

Carbomers are synthetic, high-molecular-weight, crosslinked polymersof acrylic acid. These acrylic acid polymers are crosslinkedwith allyl sucrose or allyl pentaerythritol. The polymerization solvent used previously was benzene; however, some of the newer commercially available grades of carbomer are manufactured using either ethyl acetate or a cyclohexane–ethyl acetate cosolvent mixture. The Carbopol and Carbopol Ultrez polymers areproduced in the cosolvent mixture with a proprietary polymerization aid.

11. Stability and Storage Conditions:

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104°C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability.

2.3.2. Polyethylene glycol 400: *(Raymond C. R., 2003)*

1. Nonproprietary Names:

- **BP:** Macrogols
- **JP:** Macrogol 400
- **PhEur:** Macrogols
- **USP-NF:** Polyethylene Glycol

2. Synonyms:

Carbowax; Carbowax Sentry; Lipoxol; Lutrol E; macrogola; PEG; Pluriol E; polyoxyethylene glycol.

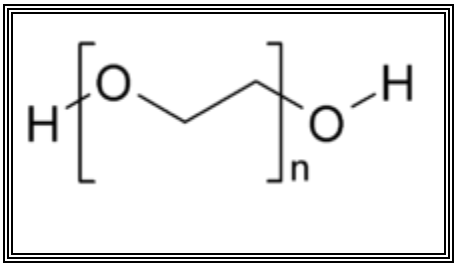
3. Chemical Name and CAS Registry Number:

a-Hydro-o-hydroxypoly(oxy- 1,2-ethanediyl) [25322-68-3]

4. Empirical Formula **:** H(OCH2CH2)nOH

5. Molecular Weight **:** 380–420

6. Structural formula:



7. Functional Category:

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

8. Description:

- **Appearance:** Clear, colorless or slightly yellow-colored, viscous liquids.tic odor and a warm.
- **Odor:** Slight but characteristic odor
- **Taste:** Bitter, slightly burning taste.

9. Typical properties:

- **Freezing point** **:** 4–8 ºC
- **Flash point** **:** 238 ºC
- **Density** **:** 1.11–1.14 g/cm3 at 25 ºC
- **Moisture content** **:** Liquid polyethylene glycols are very hygroscopic, although hygroscopicity decreases with increasing molecular weight.
- **Viscosity (mPas)** **:** 90.0 (25 ºC); 7.4 (99 ºC)
- **Solubility:**

  All grades of polyethylene glycol are soluble in water and miscible in all proportions with other polyethylene glycols (after melting, if necessary). Aqueous solutions of highermolecular-weight grades may form gels. Liquid polyethylene glycols are soluble in acetone, alcohols, benzene, glycerin, and glycols.

10. Method of Manufacture:

Polyethylene glycol polymers are formed by the reaction of ethylene oxide and water under pressure in the presence of a catalyst.

11. Applications in Pharmaceutical Formulation or Technology:

Polyethylene glycols (PEGs) are widely used in a variety of pharmaceutical formulations, including parenteral, topical, ophthalmic, oral, and rectal preparations. Polyethylene glycol has been used experimentally in biodegradable polymeric matrices used in controlled-release systems. Aqueous polyethylene glycol solutions can be used either as suspending agents or to adjust the viscosity and consistency of other suspending vehicles. When used in conjunction with other emulsifiers, polyethylene glycols can act as emulsion stabilizers.

12. Stability and Storage Conditions:

Polyethylene glycols are chemically stable in air and in solution, although grades with a molecular weight less than 2000 are hygroscopic. Polyethylene glycols do not support microbial growth, and they do not become rancid.

Polyethylene glycols and aqueous polyethylene glycol solution can be sterilized by autoclaving, filtration, or gamma irradiation.

2.3.3. PLURONIC F-127 (Poloxamer 407): (*Raymond C Rowe., 2003)*

1. Nonproprietary Names:

- **BP:** Poloxamers
- **PhEur:** Poloxamers
- **USP-NF:** Poloxa

2. Synonyms :

Lutrol; Monolan; Pluronic; poloxalkol; poloxamera; polyethylene-propylene glycol copolymer; polyoxyethylene-polyoxypropylene copolymer; Supronic; Synperonic.

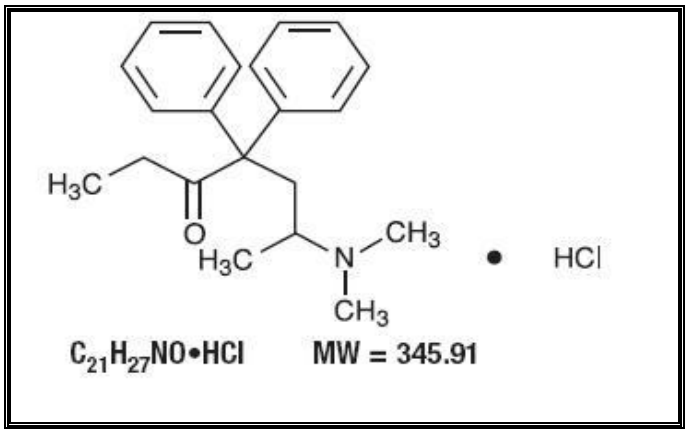
3. Chemical Name and CAS Registry Number :

a-Hydro-o-hydroxypoly(oxyethylene)poly(oxypropylene) poly-(oxyethylene) block copolymer [9003-11-6].

4. Empirical Formula **:** $C_{21}H_{27}NO.HCl$

5. Molecular Weight **:** 345.91

6. Structural Formula :



7. Functional Category:

Dispersing agent; emulsifying agent; solubilizing agent; tablet lubricant; wetting agent.

8. Applications in Pharmaceutical Formulation or Technology:

Poloxamers are nonionic polyoxyethylene–polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents. Poloxamers are used as emulsifying agents in intravenous fat emulsions, and as solubilizing and stabilizing agents to maintain the clarity of elixirs and syrups.

Poloxamers may also be used as wetting agents; in ointments, suppository bases, gels and as tablet binders and coatings.

9. Description:

- **Color:** White, waxy, free-flowing prilled granules, or as cast solids.
- **Odor:** odorless.
- **Taste:** tasteless.

10. Typical properties:

- **Acidity/alkalinity:** pH = 5.0–7.4 for a 2.5% w/v aqueous solution
- **Flash point:** 260 ºC
- **Flowability:** Solid poloxamers are free flowing.
- **Melting point:** 52–57 ºC
- **Moisture content:** Poloxamers generally contain less than 0.5% w/w water and are hygroscopic only at relative humidity greater than 80%.
- **Solubility of Pluronic F127:**

Table 2.3: Table indicating solubility of pluronic F127

S.No	Solvent	Solubility
1	Ethanol (95%)	Freely soluble
2	Propan-2-ol	Freely soluble
3	Water	Freely soluble

10. Method of Manufacture:

Poloxamer polymers are prepared by reacting propylene oxide with propylene glycol to form polyoxypropylene glycol. Ethylene oxide is then added to form the block copolymer.

11. Stability and Storage Conditions:

Poloxamers are stable materials. Aqueous solutions are stable in the presence of acids, alkalis, and metal ions. However, aqueous solutions support mold growth.

The bulk material should be stored in a well-closed container in a cool, dry place.

Aim

&

Objective

3. AIM AND OBJECTIVE

The nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption. This is due to the large surface area, porous endothelial membrane, and high total blood flow. Recently focus has been made on the nasal mucosa as an alternate route to achieve faster and higher absorption.

Rizatriptan Benzoate is $5HT_{1B/1D}$ receptor agonist with an oral bioavailability of 40% and half life of 2-3 h. To increase the bioavailability and brain tissue deposition of antimigraine therapy, Intranasal delivery system were developed. Intranasal administration offers a practical noninvasive alternative mode for drug targeting to the brain.

Nasal delivery of drugs offers many advantages over other delivery routes still posses some drawbacks like nasal mucociliary clearance which results in reduced absorption of drug. Several strategies were tested to improve nasal absorption of drug. To improve the nasal absorption of drug it is necessary to increase the nasal residence time. One method to lengthen nasal residence time has been to include a bioadhesive in the formulation. The use of bioadhesive polymers can increase nasal absorption of drugs with several ways such as increasing the residence time of drug in the nasal cavity. Carbopol derivatives are bioadhesive polymers used in attempt to formulate mucoadhesive drug delivery system for application to nasal mucosa.

Poloxamers or pluronics are triblock copolymers which form micelles at low concentration and clear, thermoreversible gel at high concentrations.The concentrated solutions (20-30%) are transformed from low viscosity transparent solutions at 5^0 C to solid gel on heating to body temperature. By modulating the gelation temperature of different PF 127 solutions, liquid bases for nasal nasal use can be formulated that form a gel in nasal cavity at body temperature resulting in enhancement of residence time in the nasal cavity.

Possible strategy used to decrease the mucociliary clearance by use of gel/mucoadhesive formulation to prolong nasal residence time. Neverthless, the most prominent advantage of the *in situ gel* over the silent gel is that is fluid like prior to contact with nasal mucosa, a feature that offers the convenience of administration for patients since it can be easily instilled as a drop allowing accurate drug dosing.

Hence, the objective of this study was to develop temperature induced mucoadhesive *in situ gel* formulation for Rizatriptan Benzoate by using 3^2 full factorial design in order to achieve prolonged action and controlled release and direct delivery by nasal route. This may give patient friendly, needle free dosage form

PLAN OF WORK

4. PLAN OF WORK

1. **LITERATURE REVIEW**
2. **SELECTION OF DRUG AND POLYMERS**
3. **PROCUREMENT OF DRUG AND POLYMERS**
4. **EXPERIMENTAL WORK**

- **PREFORMULATION STUDIES**
  - **Identification of Drug**
    - Organoleptic Properties
    - Determination of Melting Point
    - Solubility Study
    - FTIR Spectroscopy
    - UV Spectrophotometric Study
      - Determination of λ_{max}
      - Development of Standard Curve of Rizatriptan Benzoate
    - Quantification of Rizatriptan Benzoate
  - **Drug - Polymers Interaction Studies.**

    Fourier Transforms Infra-Red (FTIR) Spectroscopy Study.
    - Differential Scanning Calorimetry (DSC) Analysis

- **FORMULATION DESIGNING BY 3^2 FULL FACTORIAL DESIGN**
- **FORMULATION OF MUCOADHESIVE *NASAL IN SITU GEL***
- **EVALUATION OF MUCOADHESIVE *NASAL INSITU GEL***
  - Clarity
  - Measurement of Gelation Temperature ($T1^0$ C)
  - Measurement of Gel Melting Temperature ($T2^0$ C)
  - Determination of pH
  - Drug Content
  - Determination of Mucoadhesive Strength
  - Measurement of Viscosity
  - *In-vitro* Drug Permeation Studies
  - Histopathological Study of Optimized Formulation
  - Kinetic Modeling of Drug Permeation.
  - Stability Studies

5. **RESULTS AND DISCUSSION**
6. **SUMMARY AND CONCLUSION**
7. **FUTURE PROSPECTS**
8. **BIBLIOGRAPHY**

Materials And Equipments

5. MATERIALS AND EQUIPMENTS

5.1. MATERIALS USED:

Table 5.1: List of Drug and Polymers with source

S.No.	Ingredients	Supplier
1	Rizatriptan Benzoate	Strides Arco lab, Bangalore.
2	Carbopol 974P	Loba Chemie Pvt. Ltd., Mumbai.
3	Poly ethylene glycol 400	S D fine-chem limited, Mumbai.
4	Pluronic F127	S D fine-chem limited, Mumbai.
5	Potassium dihydrogen phosphate	Qualigens fine chemicals, Mumbai
6	Sodium hydroxide	S D fine-chem limited, Mumbai
8	Dialysis bag	Himedia labaratories, Mumbai.

5.2. EQUIPMENTS USED:

Table 5.2: List of Equipments with model/make

S.No	Equipments	Model/ Make
1.	Electronic Balance	Shimadzu BL-220H
2.	Clarity Test Apparatus	Labtech
2.	Chemical Balance	Shimadzu BL-220H
3.	pH Meter	LI120 pHmeter, ELICO LTD
4.	Humidity Chamber	Labtech
5.	UV Visible Spectrophotometer	Shimadzu-1700 Pharmaspec UV-visible spectrophotometer, Elico
6.	FTIR Spectrophotometer	Shimadzu FTIR-801 spectrophotometer
7.	Differential Scanning Calorimeter	Shimadzu DSC W70
8.	Melting Point Apparatus	Guna enterprises, Chennai
9.	Viscometer	Brookfield DV-11 + PRO Viscometer
10.	Franz Diffusion Cell Apparatus	Scientific glass, Coimbatore

PREFORMULATION STUDIES

6. PREFORMULATION STUDY

6.1. Identification of Drug:

The preliminary studies were carried out by testing of different physical and chemical properties of drug as follows.

6.1.1. Organoleptic properties: *(Lachmann L., et al., 1991)*

The Organoleptic properties like physical state, color, odor etc., of the drug was reported with help of the descriptive terminology. It helps to identify the drug.

6.1.2. Determination of Melting point: *(IP., 2007)*

It is the easy way to identify the drug. The melting point of Rizatriptan Benzoate was tested by use of a laboratory melting point apparatus with a procedure given in the Indian Pharmacopeia 2007.

6.1.3. Solubility study: *(IP., 2007)*

The solubility of Rizatriptan Benzoate was determined by micropipette method in various solvents in order to meet the official standards. The solubility of drug was recorded by using various descriptive terminology specified in Indian pharmacopoeia, 2007.

The general description of solubility as per Indian Pharmacopoeia was listed out in the Table 6.1.

Table 6.1: Description of solubility

Descriptive term	Parts of solvent required for 1 part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble	Greater than or equal to 10,000

6.1.4. FTIR spectroscopy:

(Skoog D.A., et al. (1996); Robert M. Silverstein., et al., 2003)

The infrared spectrum was generally used as an identification parameter to know the chemical structure of drugs. For the FTIR spectrum of Rizatripan Benzoate FTIR spectrophotometer was used.

A small quantity of sample was mixed with sufficient potassium bromide and compressed into a pellet by applying a 10 tons pressure with help of a hand operated press. This pellet was kept in a sample holder and scanned from 4000 to 400 cm^{-1}. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum.

6.1.5. UV Spectrophotometric Study:

6.1.5.1. Determination of λ_{max}: **(***Prashant S.Khairnar. et al., 2011)*

The absorption maximum of the standard solution was scanned between 200-400 nm regions on Shimadzu-1700 Pharmaspec UV-visible spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum.

6.1.5.2. Development of standard curve of Rizatriptan Benzoate in Ditilled water:

- **Preparation of stock solution:**

Accurately weighed 10 mg of Rizatriptan Benzoate was dissolved in 100 mL of distilled water to get the concentration of 100 µg/mL.

- **Procedure:**

From the stock solution, aliquots of 1, 2, 3, 4 and 5 mL were transferred to 50 mL volumetric flasks and final volume was made to 50 mL with distilled water to get 2 to 10 µg/mL. Absorbance values of these solutions were measured against blank (methanol) at 224.4 nm using UV-visible spectrophotometer.

6.1.5.3. Development of standard curve of Rizatriptan Benzoate:

- **Preparation of phosphate buffer pH 6.4:**

Phosphate buffer pH 6.4 was prepared according to I.P. 2007. A quantity of 50 mL of 0.2 M potassium dihydrogen phosphate in a 200 mL volumetric flask and added 11.6 mL of 0.2 M sodium hydroxide was diluted with fresh distilled water to produce 200 mL.

- **Preparation of stock solution of Rizatriptan Benzoate Buffer pH 6.4 solution:**

  Accurately weighed 10 mg of Rizatriptan Benzoate was dissolved in little quantity of phosphate buffer solution pH 6.4 and volume was adjusted to 100 mL with the same to prepare standard solution having concentration of 100 µg/ mL.

- **Procedure:**

  From the stock solution, aliquots of 2, 4, 6, 8 and 10 mL were transferred to 50 mL volumetric flasks and final volume was made to 10 mL with pH 6.4 phosphate buffer to get 2 to 10 µg/mL. Absorbance values of these solutions were measured against blank (phosphate buffer pH 6.4) at 224.4 nm using UV-visible spectrophotometer.

6.1.6. Quantification of Rizatriptan Benzoate:

(Achariya Sasmita Kumari. et al., (2010)

Accurately weighed 10 mg of Rizatriptan Benzoate was dissolved in little quantity of phosphate buffer 6.4 and volume was adjusted to 100 mL with the same to prepare standard solution having concentration of 100 µg/mL. From the above solution, aliquots of 5 mL were transferred to 50 mL volumetric flasks and final volume was made to 50 mL with phosphate buffer 6.4. Absorbance values of these solutions were measured against blank (phosphate buffer 6.4) at 225.5 nm using Shimadzu-1700 Pharmaspec UV-visible spectrophotometer. The percentage purity of drug was calculated by using calibration graph method (least square method).

6.2. Drug – Polymers Intraction Study:

6.2.1. Fourier Transforms Infra-Red (FTIR) Spectroscopy:

(Skoog D.A., et al. (1996); Robert M. Silverstein., et al., 2003)

FTIR study was carried out to check identity of drug. Infrared spectrum of Metoprolol tartrate was determined on Fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug by using FTIR spectrophotometer. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum.

6.2.2. Differential Scanning Calorimetry (DSC) Analysis:

(IP., 2007)

The proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of all drug substances and excipients to be used in the fabricating the product. Each polymer used in the formulations was blended with the drug levels that are realistic with respect to the final dosage form. Each polymer was thoroughly blended with drug to increase drug-polymer molecular contacts to accelerate the reactions if possible.

FORMULATION OF MUCOADHESIVE

NASAL *IN SITU GELS*

7. FORMULATION OF MUCOADHESIVE *NASAL IN SITU GELS*

7.1. Designing the formula: *(Varsha gaikwad., et al., 2010)*

In situ gels were designed by 3^2 full factorial design to study the interaction variables of formulation on characterization of *In situ gels*. Amount of carbopol 974P and PEG 400 were selected as independent variable. Amount of Rizatriptan Benzoate (100mg) were kept constant. The variables and the level of each factor were shown in table 7.1.

Table 7.1: Variables in 3^2 full factorial design

Independent variable factors	**Levels used**		
	Lower (-1)	**Middle (0)**	**Upper (+1)**
Carbopol 974P (X1) %	0.25	0.5	0.75
PEG 400 (X2) %	6	8	10
Amount of drug used was 100mg in all formulation			

Each row identifies an experiment and each row provides a result (response). The levels of factors studied were chosen that the irrelative difference was adequate to have a measurable effect on response, along with the information that the selected levels are within practical use. The formulation of the factorial design is represented in table 7.2.

Table 7.2: Designing the formulation with a 3^2 full factorial design

S.NO	Formulation code	Carbopol 974P (X1) %	PEG 400 (X2)%
1	MG1	-1 (0.25)	-1 (6)
2	MG2	-1 (0.25)	0 (8)
3	MG3	-1 (0.25)	+1 (10)
4	MG4	0 (0.5)	-1 (6)
5	MG5	0 (0.5)	0 (8)
6	MG6	0 (0.5)	+1 (10)
7	MG7	+1 (0.75)	-1 (6)
8	MG8	+1 (0.75)	0 (8)
9	MG9	+1 (0.75)	+1 (10)

Table 7.3: Composition of Mucoadhesive *Nasal In situ Gels*:

INGREDIENTS (%)	MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8	MG9
Drug(Rizatriptan Benzoate)	1	1	1	1	1	1	1	1	1
Carbopol 974P	0.25	0.25	0.25	0.5	0.5	0.5	0.75	0.75	0.75
Poly Ethylene Glycol 400	6	8	10	6	8	10	6	8	10
Pluronic F127	18	18	18	18	18	18	18	18	18
Distilled water(Q.S.)ml	100	100	100	100	100	100	100	100	100

PREPARATION OF MUCOADHESIVE *NASAL IN SITU GELS*:

(Varsha Gaikwad., et al., 2010)

Thermoreversible pluronic *insitu gels* were prepared by **cold technique**.

To the 1%, solution of drug in distilled water, PEG 400 was added in the quantity of 6, 8, and 10% w/v. To this Carbopol 974 P was added in qty i.e. 0.5% w/v.

This solution was then stirred until Carbopol 974P completely dissolves in it. After the complete dissolving of this Carbopol 974P, Pluronic F127 (Poloxamer 407) was added to it.

Pluronic F127, 18% w/v used as base throughout preparations. This resulting formulation was then kept at 4^0 C overnight until clear gel obtained.

EVALUATION

OF

MUCOADHESIVE NASAL *IN SITU* GELS

8. EVALUATION OF MUCOADHESIVE *NASAL IN SITU GELS*

8.1. Clarity: *(Jitendra V. Shinde., et al.,2008)*

The clarity of various formulations was determined by visual inspection under black and white background by using clarity test apparatus and it was graded as follows;

Turbid +, Clear ++, Very clear (glassy):+++.

8.2. Measurement of gelation temperature (T1) :

*(Jyothivarthan Jaiswal, et al., 2012; Varsha gaikwad., et al., 2010***)**

It was determined by using method described by Miller and Donovan technique. A 2 mL aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly and left to equilibrate for 5 min at each new setting. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer moves upon tilting through 90^{o} C.

8.3 Measurement of gel meting temperature (T2): *(Varsha gaikwad., et al., 2010)*

After attaining the temperature T1, further heating of gel causes liquification of gel and form viscous liquid and it starts flowing, this temperature is noted as T2 i.e. gel melting temperature.It is a critical temperature when the gel starts flowing upon tilting test tube through 90^{o}C.

8.4. Determination of pH: *(Jitendra V. Shind., et al., 2008)*

1 mL quantity of each formulation was transferred to the 10 mL volumetric flask and diluted by using distlled water to make 10 mL. pH of resulting solution was determined by using digital pH meter (LI120 pH meter, ELICO LTD)

8.5. Drug content: *(Jyothivarthan Jaiswa., et al., 2012)*

1 mL of formulation was taken in 10 mL volumetric flask, diluted with distilled water and volume adjusted to 10 mL. 1 mL quantity from this solution was again diluted with 10 mL of distilled water. Finally the absorbance of prepared solution was measured at 225.5 nm by using UV visible spectrophotometer.

8.6. Determination of Mucoadhesive strength: *(Jitendra V. Shinde., et al., 2008)*

The mucoadhesive strength of each formulation was determined by measuring the force required to detach the formulation from goat nasal mucosal tissue by using a modified chemical balance.

A section of nasal mucosa was cut from the goat's nasal cavity and mucosal side was instantly fixed into each glass vial using a rubber band. The vials with nasal mucosa were stored at 37°C for 5 minutes. Then next vial with a section of mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. Fixed amount of sample of each formulation were placed onto the nasal mucosa of first vial. Then the height of second vial was adjusted so that mucosal surfaces of both vials come in intimate contact. Two minutes contact time was given to ensure intimate contact between tissues and the sample. Then weight was increased in the pan until vials got detached.

Fig 8.1: Mucoadhesion test assembly

The bioadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the tissues from the surface for each formulation using the following equation.

Detachment stress (dyne/cm^2) = m x g /A

Where, m =Weight required for detachment of two vials in gm

g = Acceleration due to gravity [980cm/s^2]

A = Area of tissue exposed

The nasal mucosa was changed for each measurement. Measurements were repeated three times for each of the gel preparations.

8.7. Measurement of Viscosity: *(Varsha gaikwad., et al., 2010)*

The viscosity measurements were carried out by using Brookfield DV-11 Pro viscometer. The gel sample was placed in small sample adaptor. Temperature was increased in the range of 20 ^{0}C – 34 ^{0}C, using a water circulation jacket. The temperature sensing probe was lowered in gel and temperature of gel was recorded. Viscosity at various temperatures was recorded.

8.8. *In-vitro* Drug Permeation Studies: *(Vijaya Kumar., et al., 2012)*

Drug release from gel was tested with Franz diffusion cell, using dialysis membrane (mol.wt.12000-14000) with permeation area of 2.545 cm^2. 25 mL of phosphate buffer 6.4 was added to the acceptor chamber. Gel containing drug equivalent to 10 mg was placed in donor compartment. At predetermined time points, 1 mL sample were withdrawn from the acceptor compartment, replacing the sample volume with phosphate buffer buffer pH 6.4 after each sampling for a period of 5 h. The samples were suitably diluted and measured spectrophotometrically at 225.5 nm.

Fig 8.2: *In-vitro* permeation assembly

8.9. Histopathological Studies Of Optimized Formulation: (*Jaiswal., et al., 2012)*

Fresh nasal tissue was removed from the nasal cavity of goat. The tissue was inserted in the Franz diffusion cell. Phosphate buffer (pH6.4) was then added to the acceptor chamber. Then gel was applied to the mucosa and left for the 12 h. Another control mucosa was also setup without using the drug. After 12 h each piece of mucosa was carefully removed from the diffusion chamber, rinsed with phosphate buffer. The mucosa sample was then fixed in the 1% formaldehyde solution for 6h. The samples were then incubated in methyl benzoate for 24 h in order to soften the material. The samples were immersed first into benzene:paraplast (1:1) mixture, and then into pure paraplast for 6 h in a vacuum oven and embedded in paraplast. The

blocks were cut in sections of 5-6μm in thickness with a rotary microtom. The section was stained for the light microscope examination.

8.10. Kinetic Modeling of Drug Permeation:

(Brahmankar D.M. and Jaiswal S.B., 2006)

To study the release kinetics of *in-vitro* drug release, data was applied to kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas.

- **Zero order:**

$$\mathbf{C = K_0 t}$$

Where K_0 - is the zero-order rate constant expressed in units of concentration/time

t -is the time in hrs.

- **First order:**

$$\mathbf{LogC = LogC_0 - Kt / 2.303}$$

Where C_0 - is the initial concentration of drug,

K - is the first order constant

t - is the time in hrs.

- **Higuchi:**

$$\mathbf{Qt = Kt^{1/2}}$$

Where Q_t - is the amount of the release drug in time t,

K- is the kinetic constant and

t- is time in hrs.

- **Korsmeyer Peppas:**

Mt / M∞ = Kt n

Where M_t - represents amount of the released drug at time t,

M_∞- is the overall amount of the drug (whole dose) released after 12 hrs

K- is the diffusional characteristic of drug/ polymer system constant

n- is a diffusional exponent that characterizes the mechanism of release of drug.

The value of n indicates the drug release mechanism related to the geometrical shape of the delivery system, if the exponent n = 0.5, then the drug release mechanism is Fickian diffusion. If n < 0.5 the mechanism is quasi-Fickian diffusion, and 0.5 < n < 1.0, then it is non-Fickian or anomalous diffusion and when n = 1.0 mechanism is non Fickian case II diffusion, n> 1.0 mechanism is non Fickian super case II.

8.11. Stability studies: *(Manavalan R and Ramasamy S., 1991)*

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods (FDA, 1998).

Stability of pharmaceutical preparation can be defined as the capability of particular formulation (dosage form) in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicology specifications throughout its shelf life.

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted. The International Conference on Harmonization (ICH) Guidelines titled "Stability testing of New Drug Substances and Products" describes the stability test requirements for drug registration application in the European Union, Japan and the States of America.

ICH specifies the length of study and storage conditions

- **Long-Term Testing**: 25°C ± 2°C at 60% RH ± 5% for 12 Months
- **Accelerated Testing:** 40°C ±2°C at 75% RH ± 5% for 6 Months

Procedure: *(Zheng Cai., et al., 2011)*

The study was carried out to observe the effect of temperature on optimized formulation (MG4). Stability studies were carried out at 40°C ±2°C at 75% RH ± 5% for the formulation MG4 for 3 months. A quantity of Rizatriptan Benzoate *in situ gel* in cillin bottles were stored in a dessicator containing a saturated solution of sodium chloride, which provided a relative humidity of 75 ±5%. The dessicator was plaed in a hot air oven maintained at 40^0 C ± 2^0 C, and the samples were withdrawn at 1, 2 and 3 months .

The samples were analyzed for its parameters such as,

- Clarity
- Gelatiom Temperature (T1)
- Gel melting Temperature (T2)
- pH
- Drug content
- *In-vitro* drug permeation study

.

Results

And

Discussion

9. RESULTS AND DISCUSSION

9.1. Identification of Drug:

9.1.1. Organoleptic Properties:

Colour : Off white crystalline solid

Odour : Characteristic odour

9.1.2. Determination of Melting Point:

Melting point of Rizatriptan Benzoate was found to be 179 ºC. The official melting point range for Rizatriptan Benzoate is between 178-180°C. Hence, results were complied the limits specified in official Book.

9.1.3. Solubility Study:

Table 9.1: The solubility of Rizatriptan Benzoate in various solvents

S.No.	Name of solvent	Solubility	Parts of solvent required for 1 part of solute
1	0.1 N sodium hydroxide	Freely soluble	10
2	Distilled water	Soluble	20
3	Ehanol	Slightly soluble	100
4	Methanol	Soluble	20
5	0.1 N HCl	Soluble	30
6	Phosphate buffer pH 6.4	Soluble	10
7	PEG 400	Soluble	30

9.1.4. FTIR spectroscopy:

The FTIR spectrum of Rizatriptan Benzoate was shown in Figure 9.1 and the interpretations of IR frequencies were showed in Table 9.2.

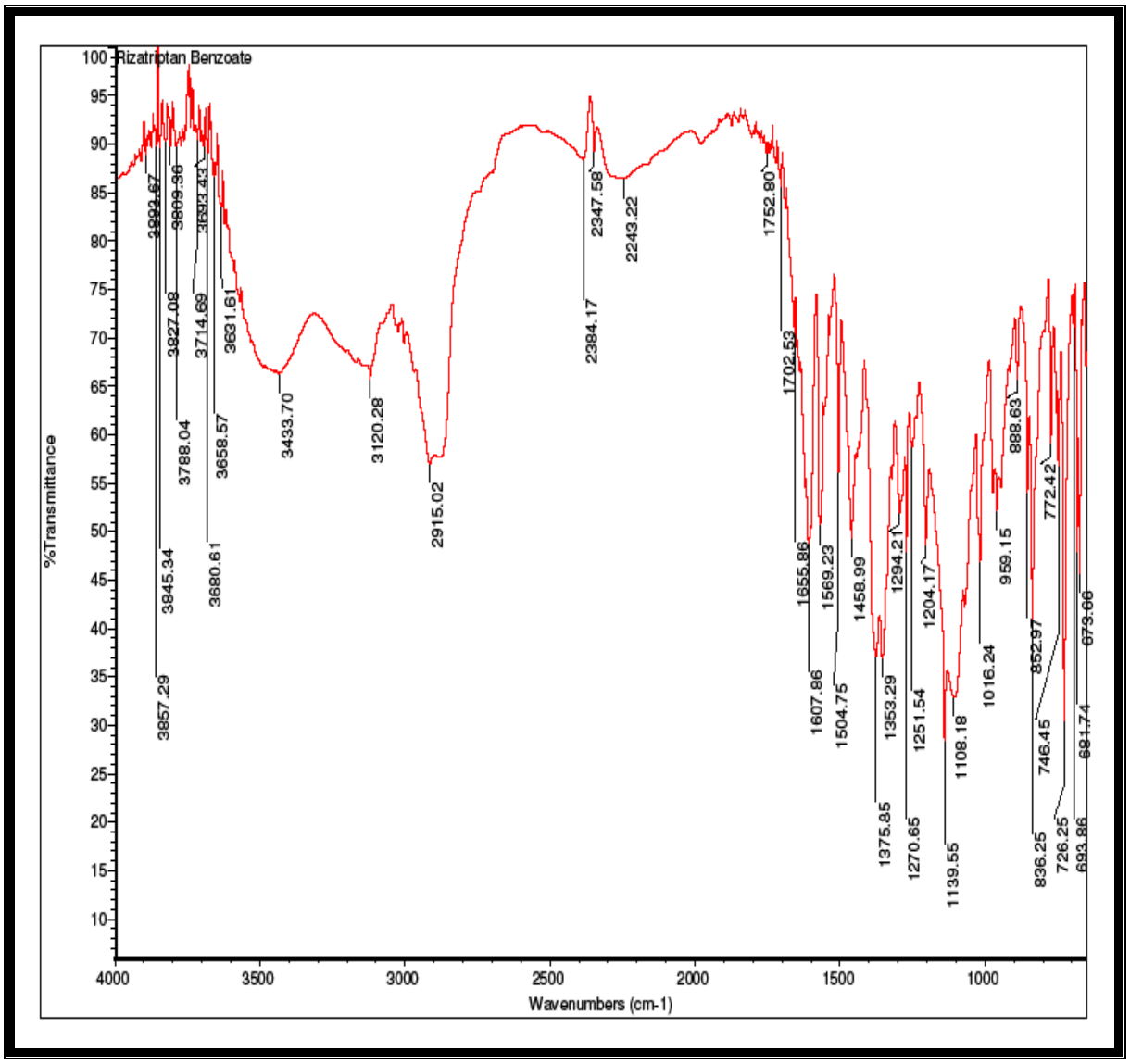


Fig.9.1: FTIR spectrum of Rizatriptan Benzoate

Table 9.2: Characteristic Frequencies in FTIR spectrum of Rizatriptan Benzoate

S.No.	Transition	IR Range (cm^{-1})	Absorption wave number of Rizatriptan Benzoate (cm^{-1})
1	C-H bending	4000-3980	3857.29
2	O-H stretching	3840-2920	3714.69
3	C=C bending	2400-2100	2243.22
4	C=O stretching	1730-1600	1702.53
5	C=C stretching	1680-1500	1655.86
6	C-H bending	1480-1300	1458.99
7	C-O stretching	1350-1200	1294.21
8	C-C stretching	995-985	959.15
9	C-H bending	900-860	888.63
10	C-C bending	760-685	673.66

Interpretation of FTIR Spectrum:

Major functional groups present in Rizatriptan Benzoate show characteristic peaks in IR spectrum. Table 9.2 shows peaks observed at different wave numbers and the functional group associated with these peaks. The major peaks are identical to functional group of Rizatriptan Benzoate. Hence, the sample was confirmed as Rizatriptan benzoate.

9.1.5. UV Spectrophotometric Study:

- The absorption maximum for Rizatriptan Benzoate in distilled water was found to be 225.5 nm.

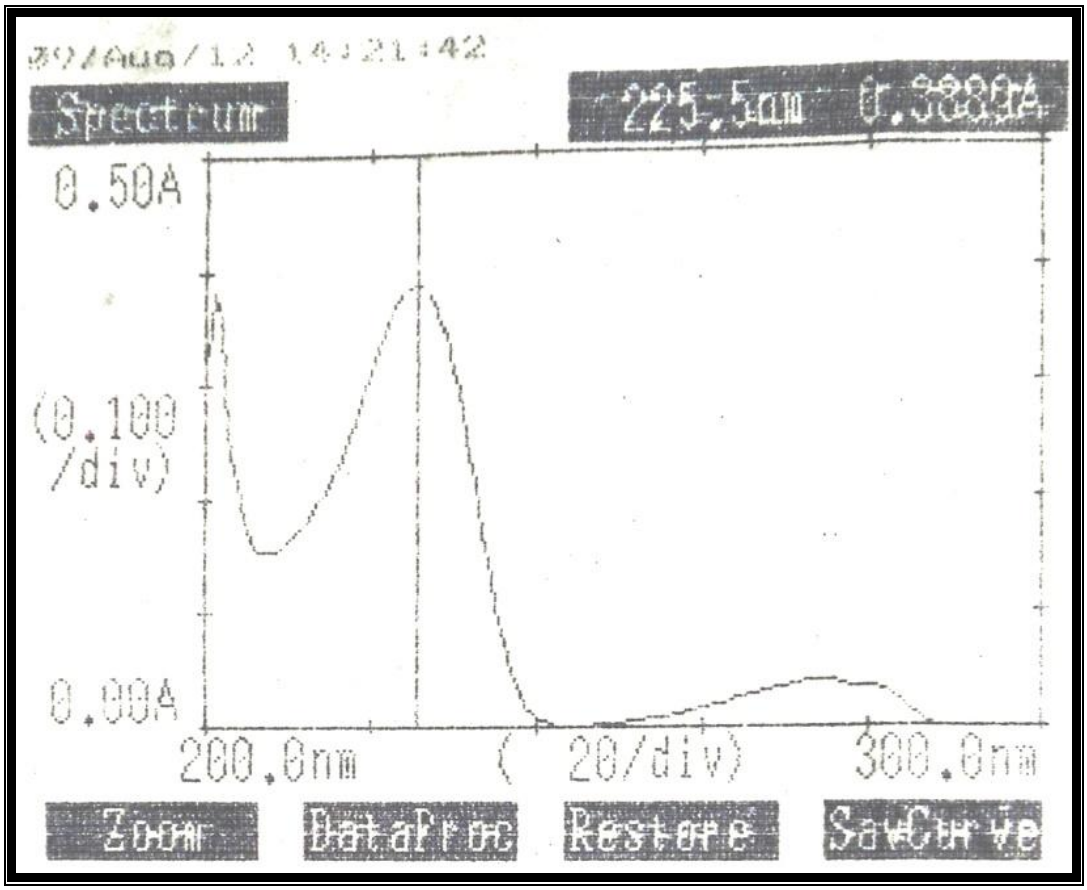


Fig. 9.2: λ_{max} of Rizatriptan Benzoate in distilled water

❖ The absorption maximum for Rizatriptan Benzoate in phosphate buffer pH 6.4 was found to be 225.5 nm

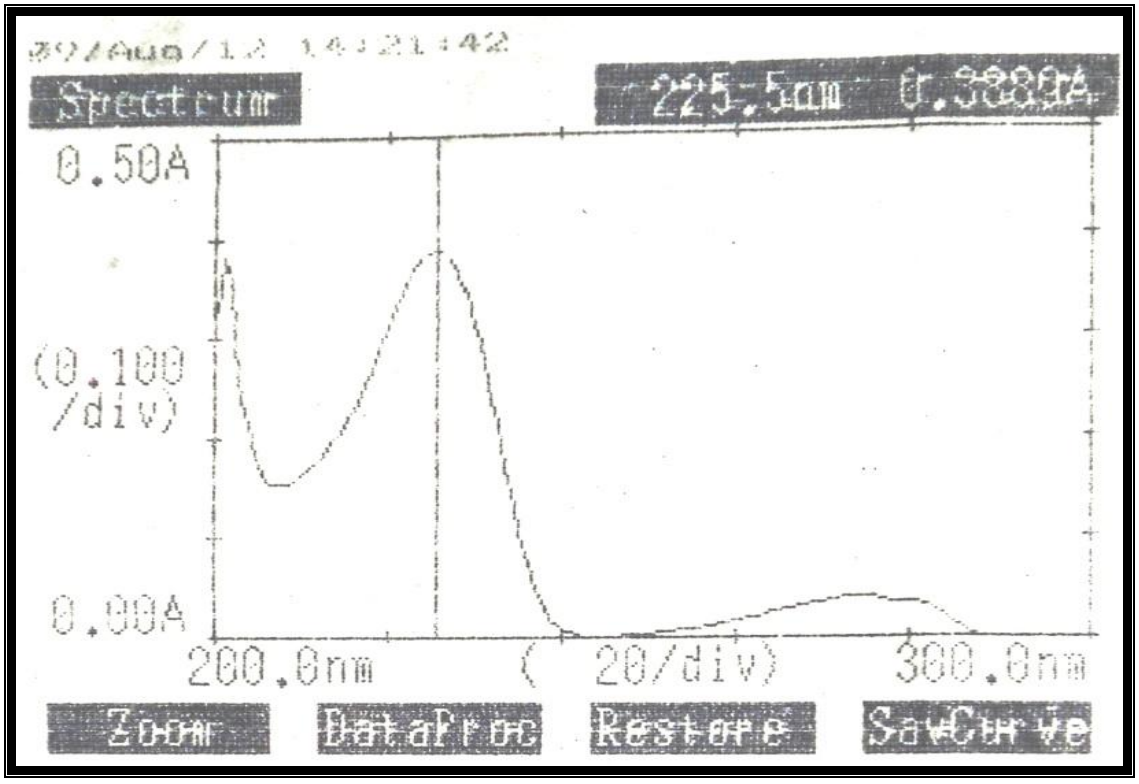


Fig. 9.3: λ_{max} of Rizatriptan Benzoate in Phosphate buffer pH 6.4

9.1.6. Calibration curve of Rizatriptan Benzoate in distilled water:

UV absorption spectrum of Rizatriptan benzoate in distilled water showed λ_{max} at 225.5 nm. Absorbance obtained for various concentrations of Rizatriptan Benzoate in distilled water were given in Table 9.3. The graph of absorbance vs concentration for Rizatriptan Benzoate was found to be linear in the concentration range of 2-10 µg /mL. The drug obeys Beer- Lambert's law in the range of 2-10 µg /mL.

Table 9.3: Data of concentration and absorbance for Rizatriptan Benzoate in distilled water.

S.No.	Concentration (µg/mL)	Absorbance
1	0	0.0000
2	2	0.195
3	4	0.377
4	6	0.567
5	8	0.745
6	10	0.926

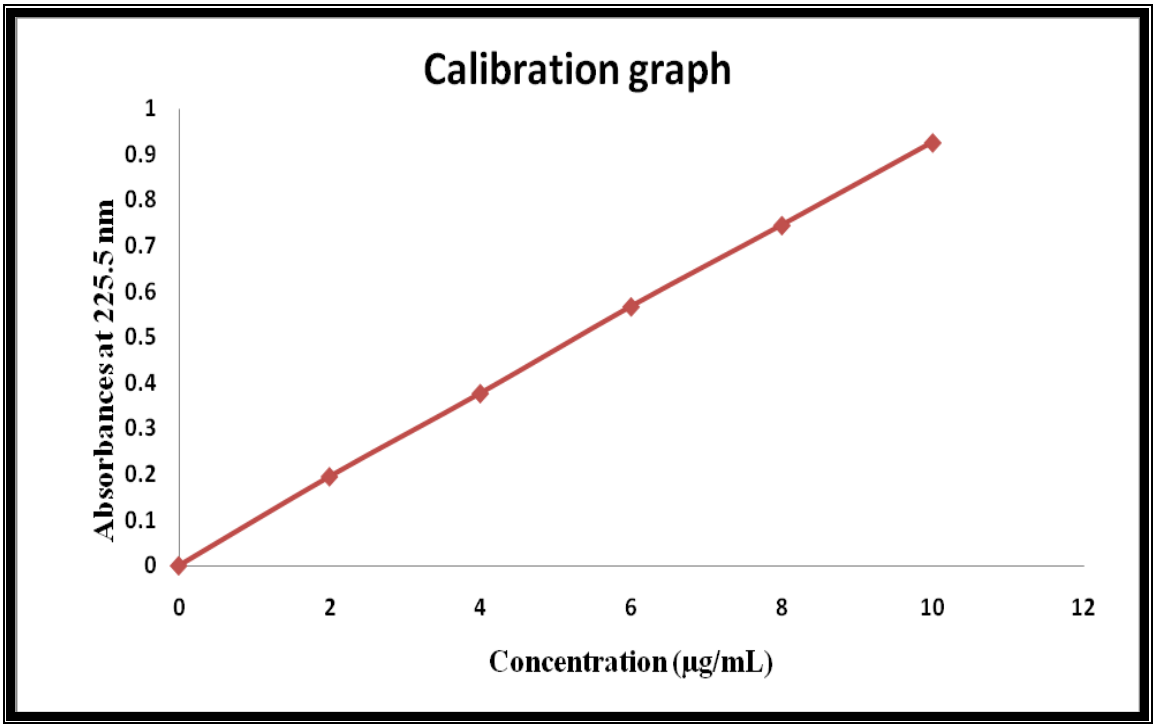


Fig. 9.4: Standard graph of Rizatriptan Benzoate in Distilled Water

Table 9.4: Data for Calibration Curve Parameters

S.No.	Parameters	Values
1	Correlation coefficient (r)	0.9999
2	Slope (m)	0.0915
3	Intercept (c)	0.013

9.1.7. Calibration Curve of Rizatriptan Benzoate in Phosphate buffer pH 6.4:

UV absorption spectrum of Rizatriptan Benzoate in phosphate buffer pH 6.4 showed λ_{max} at 225.5 nm. Absorbance obtained for various concentrations of Rizatriptan Benzoate in phosphate buffer pH 6.4 were given in Table 9.5.

The graph of absorbance Vs concentration for Rizatriptan Benzoate was found to be linear in the concentration range of 2-10 µg /mL. The drug obeys Beer- Lambert's law in the range of 2-10 µg /mL.

Table 9.5: Data of concentration and absorbance for Rizatriptan Benzoate in Phosphate buffer pH 6.4.

S.No.	Concentration (µg/mL)	Absorbance
1	0	0.0000
2	2	0.189
3	4	0.386
4	6	0.571
5	8	0.755
6	10	0.936

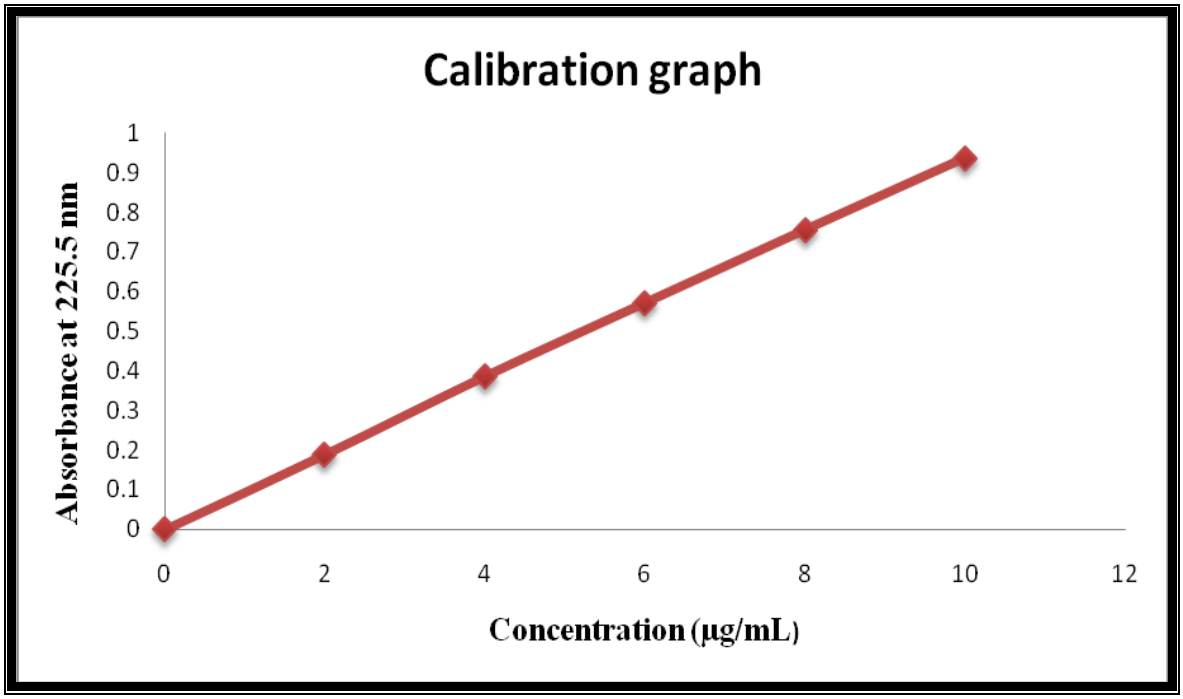


Fig. 9.5: Standard graph of Rizatriptan Benzoate in Phosphate Buffer pH 6.4.

Table 9.6: Data for Calibration Curve Parameters

S.No.	Parameters	Values
1	Correlation coefficient (r)	0.9997
2	Slope (m)	0.0931
3	Intercept (c)	0.0085

9.1.8. Quantification of Rizatriptan Benzoate

The percentage purity of drug was calculated by using calibration graph method. The values were recorded in the table 9.7.

Table 9.7: Quantification of Rizatriptan Benzoate

S.No.	Percentage purity (%)	Average percentage purity (%)
1	99.78	100.13±0.38
2	100.01	
3	100.53	

The official percentage purity of Rizatriptan Benzoate is not less than 98% and not more than 102%. So, it can be declared as pure drug. The percentage purity of raw material Rizatriptan Benzoate was found to be 100.13±0.38. Hence, the sample declared as pure.

9.2. **Drug - Polymers Interaction Studies.**

9.2.1. Fourier Transforms Infra-Red (FTIR) Spectroscopy Study.

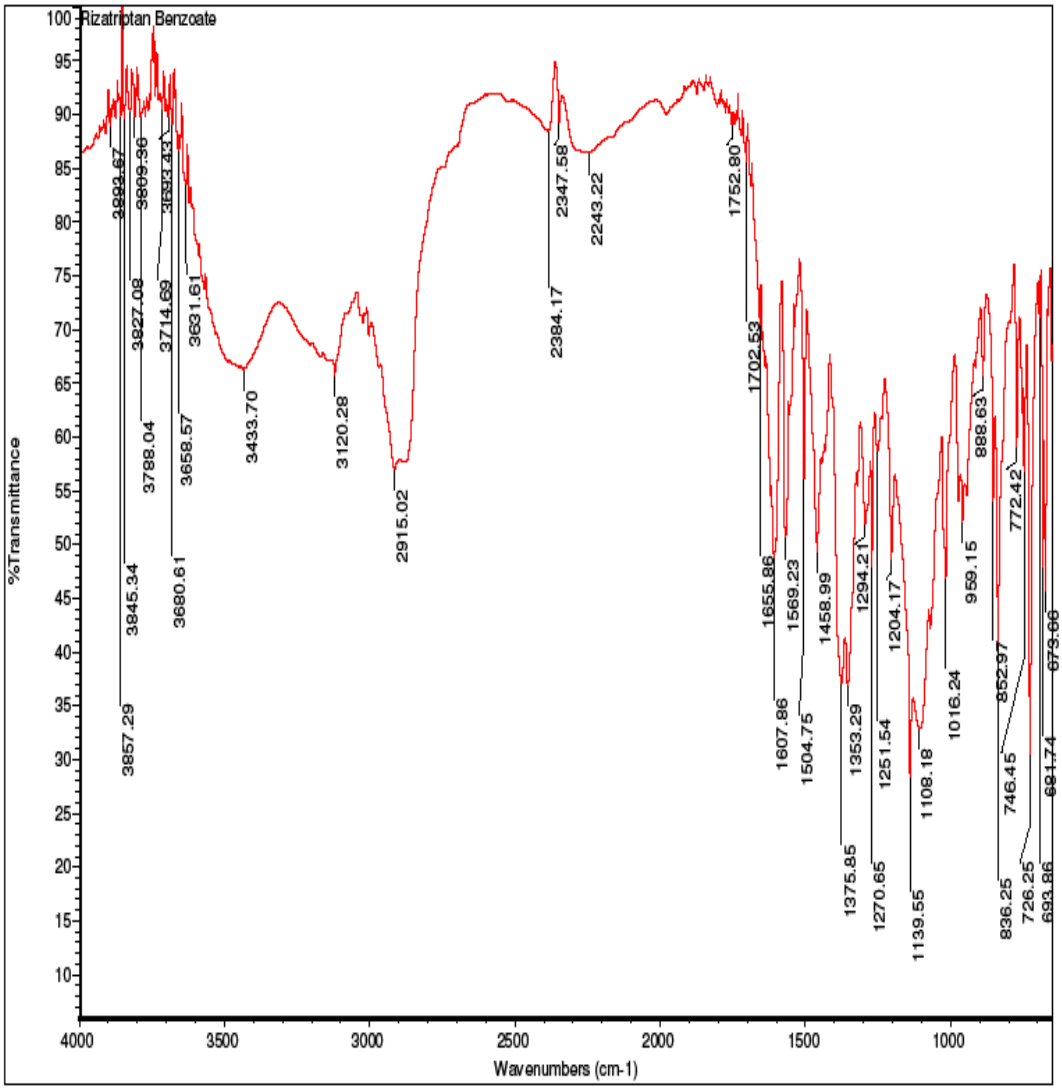


Fig. 9.6: FTIR spectrum of Rizatriptan Benzoate

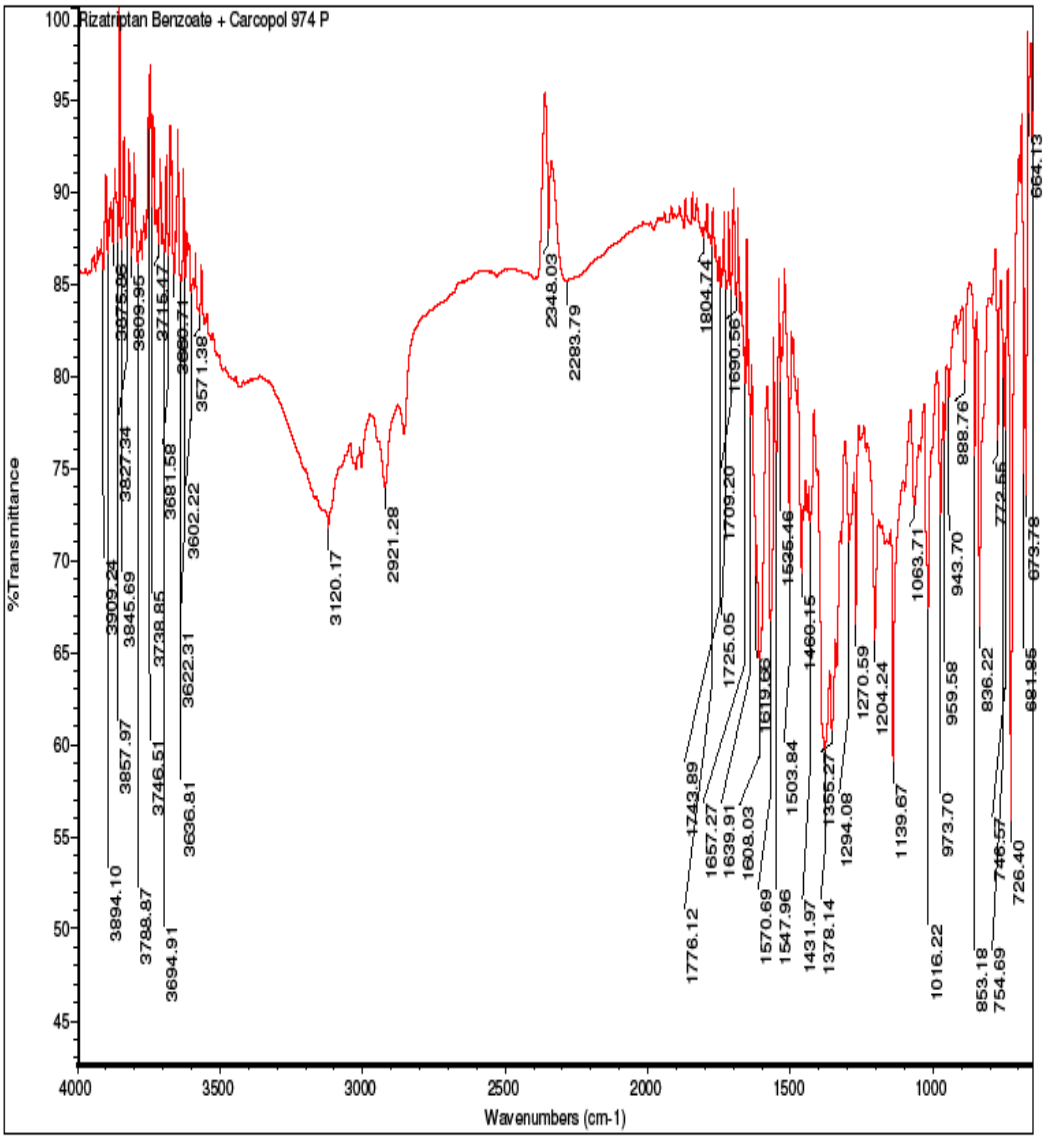


Fig. 9.7: FTIR spectrum of Rizatriptan Benzoate and carbopol 974P

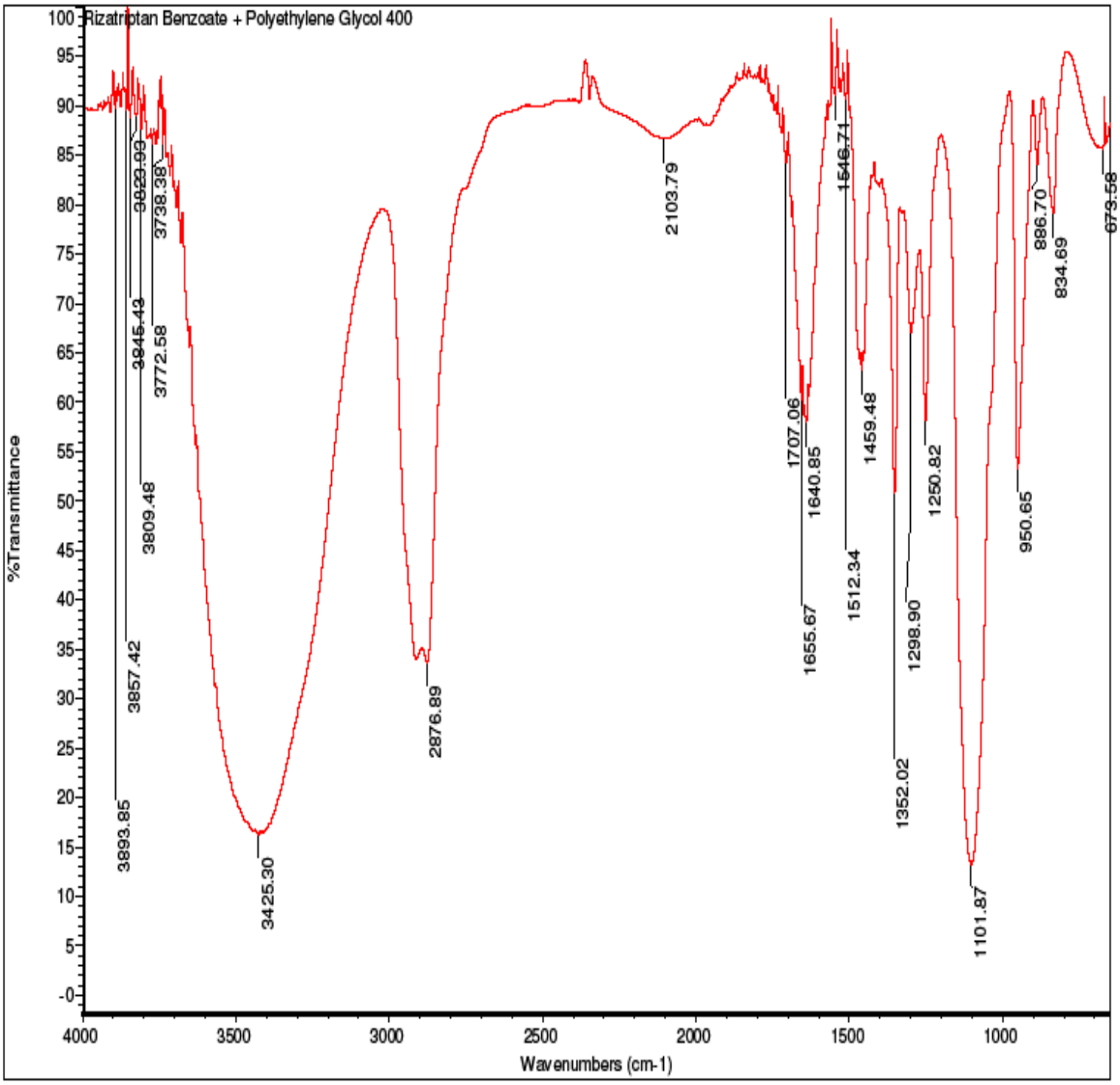


Fig. 9.8: FTIR spectrum of Rizatriptan Benzoate and PEG 400

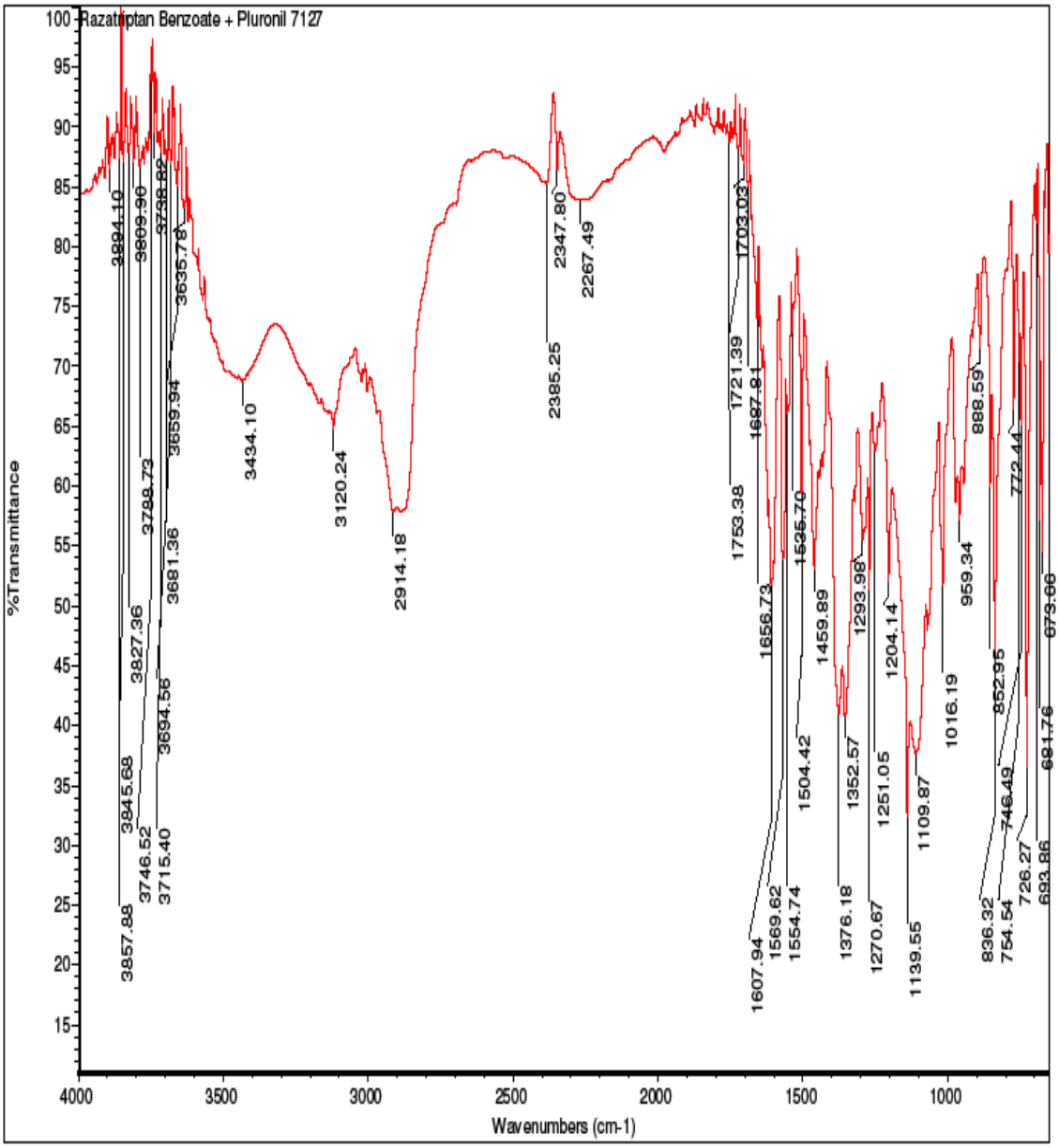


Fig. 9.9: FTIR spectrum of Rizatriptan Benzoate and Pluronic F127

Table 9.8: Major peak observed in FTIR spectrum of pure Rizatriptan Benzoate and Rizatriptan Benzoate with polymers carbopol 974P, PEG 400, Pluronic F127.

S.NO	Wave No. (cm^{-1})	Functional group	Peak observed (Yes/No)			
			Drug	Carbopol 974P	PEG 400	Pluronic F127
1	3857.29	C-H bending	**Yes**	**Yes**	**Yes**	**Yes**
2	3714.69	O-H stretching	**Yes**	**Yes**	**Yes**	**Yes**
3	2243.22	C=C bending	**Yes**	**Yes**	**Yes**	**Yes**
4	1702.53	C=O stretching	**Yes**	**Yes**	**Yes**	**Yes**
5	1655.86	C=C stretching	**Yes**	**Yes**	**Yes**	**Yes**
6	1458.99	C-H bending	**Yes**	**Yes**	**Yes**	**Yes**
7	1294.21	C-O stretching	**Yes**	**Yes**	**Yes**	**Yes**
8	959.15	C-C stretching	**Yes**	**Yes**	**Yes**	**Yes**
9	888.63	C-H bending	**Yes**	**Yes**	**Yes**	**Yes**
10	673.66	C-C bending	**Yes**	**Yes**	**Yes**	**Yes**

From the spectral analysis it was found that FTIR spectrum of Rizatriptan Benzoate with Polymers Carbopol 974P, PEG 400, Pluronic F127 showed all charecteristics peaks in combination with no significant changes as shown in fig.9.6 to 9.9. Therefore, it could indicate that there was no incompatability between drug and polymers.

9.2.2. Differential Scanning Calorimetry (DSC) Analysis:

The compatability and interactions between drug and polymers were cheked using DSC; results obtained were shown in Fig. 9.10 and 9.11.

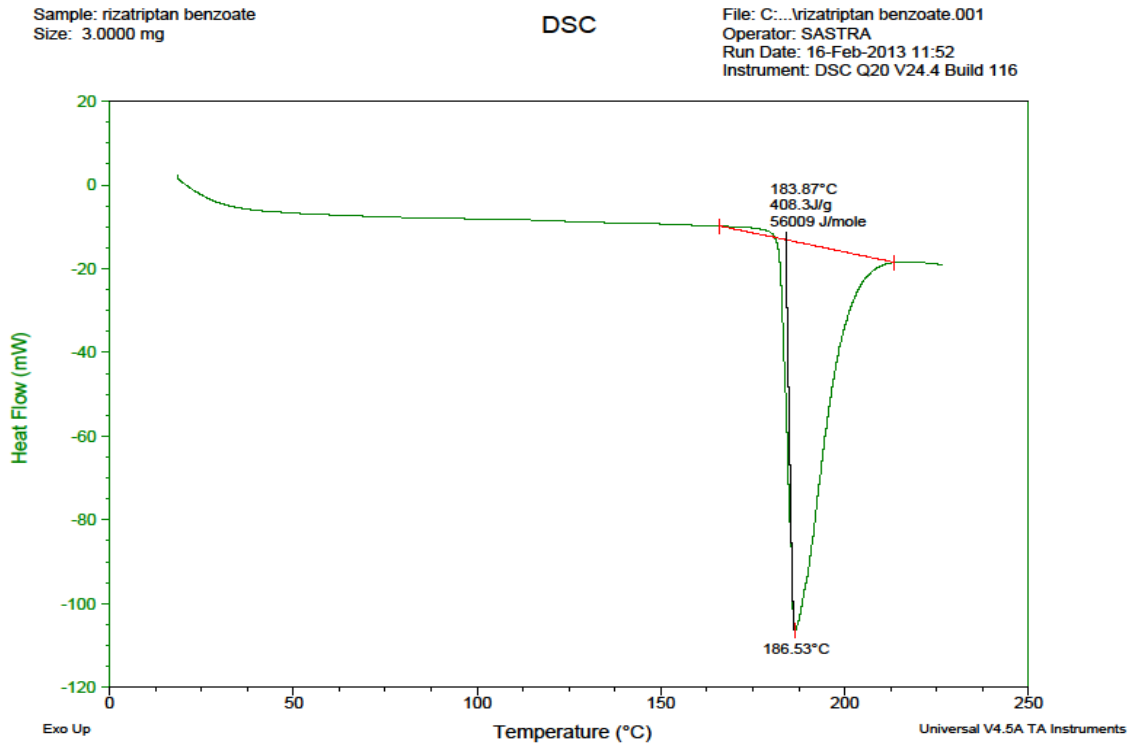


Fig. 9.10: DSC Thermogram of Rizatriptan Benzoate

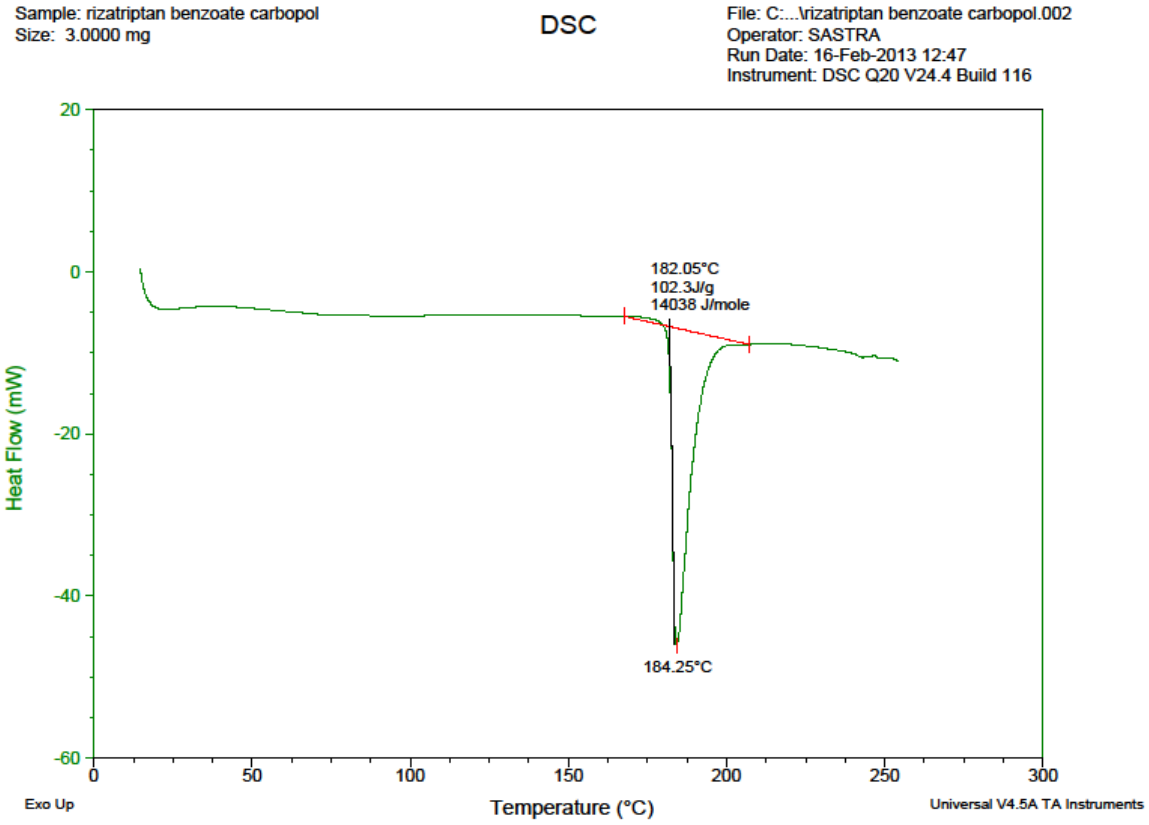


Fig. 9.11: DSC Thermogram of Rizatriptan Benzoate and Carbopol 974P + PEG 400 + Pluronic F127

Table 9.9: Data for DSC Thermogram Parameters

S. No.	DSC thermogram sample	Onset temperature (°C)	Peak temperature (°C)
1	Rizatriptan Benzoate	183.87	186.53
2	Rizatriptan Benzoate + Carbopol974P + PEG 400 + Pluronic F127	182.05	184.25

According to Table 9.9, DSC thermogram showed that there was no major difference in onset temperature and peak temperature when compared with pure drug's thermogram. No interaction was found between drug and polymers. The DSC studies suggest that polymers carbopol 974P, PEG 400, Pluronic F127 and the drug Rizatriptan Benzoate do not interact to form any chemical entity but remain as a mixture. Therefore, it could indicate that there was no incompatability between drug and polymers.

9.3. Evaluation of Mucoadhesive N*asal In Situ Gels*:

9.3.1 Clarity:

The clarity of nine formulations of Rizatriptan Benzoate Mucoadhesive Nasal *In Situ Gels* were recorded in Table 9.10.

The clarity of various formulations were determined by visual inspection under black and white background. All the prepared formulations were found to be clear (+) and very clear (+++ glassy). The very clear transparent solution was found to be MG4 with the grade of +++ under the visual inspection of both black and white background.

Table 9.10: Clarity of Mucoadhesive Nasal *In Situ Gels*

S.NO	FORMULATION CODE	VISUAL APPEARANCE	
		BLACK BACKGROUND	WHITE BACKGROUND
1	MG1	++	++
2	MG2	++	++
3	MG3	++	+++
4	MG4	+++	+++
5	MG5	++	+++
6	MG6	++	++
7	MG7	++	++
8	MG8	++	++
9	MG9	++	++

where +++ indicates Very Clear solution, ++Clear solutions, + indicates Turbid solutions

9.3.2. Measurement of gelation temperature (T1):

The physiological range of the nasal mucosal temperature lies between 32-34ºC. Pluronic F127 undergo thermal gelation or sol-gel transition at a temperature of about 25-37 ºC.

The gelation temperature study shows that loading of drug Rizatriptan Benzoate and polymers like carboplol 974P and PEG 400 alters the T1 of pluronic gel formulation that MG1,MG2, MG3 having gelation temperature of 27.53, 30.53, 28.53 having low level (0.25%) of carbopol whereas MG4,MG5, MG6 having gelation temperature of 33.86, 36.73, 33.00 having middle level (0.5%) of carbopol where as MG7, MG8, MG9 having the gelation temperature of 37.93, 36.00, 38.53. It indicates that mucoadhesive polymer carbopol 974 P has increased T1 whereas the addition of water soluble PEG 400 increased the T1. The phenomenon may be mediated through modification of miceller association of the PF 127 molecule. In addition the PEG molecules may form mixed micelles with PF127. The hydrophilic end chains of PF127 the same PEO chains that are present in PEG. It is suggested that esters binds to these chains, promoting dehydration and causing an increase in entanglement of adjacent micelle. In the presence of PEG, association of Pluronic molecules were hindered and mixed miceller system with different physicochemical properties found.

9.3.3. Measurement of gel melting temperature (T2):

The gel-sol effect depends on the addition of water soluble polymer PEG 400. The mucoadhesive polymer carbopol 974 P increases the T2 of the formulations. The gelmelting temperature of the formulations MG1,MG2, MG3 having 57.4, 50.6, 47.56 whereas MG4, MG5, MG6 having 44.00, 47.8, 53.13 where as MG7, MG8, MG9 having 53.26, 53.26, 59.2. It indicates that addition of water soluble PEG 400 produces increase in T1 while decrease in T2.

The gelation temperature (T1) and gel melting temperature T2 of nine formulations of Rizatriptan Benzoate mucoadhesive nasal *in situ gels* were recorded in Table 9.11.

Table 9.11: Gelation Temperature (T1) and Gel Melting Temperature (T2) of Mucoadhesive Nasal *In Situ Gels*

S.N0	**FORMULATION CODE**	**GELATION TEMPERATURE (T1°C) ± SD**	**GEL MELTING TEMPERATURE (T2°C) ± SD**
1	MG1	27.53±0.94	57.40±1.03
2	MG2	30.53±1.66	50.60±1.01
3	MG3	28.53±1.13	47.56±1.35
4	MG4	33.86±0.30	44.00±0.52
5	MG5	36.73±0.30	47.80±1.38
6	MG6	33.00±1.38	53.13±1.44
7	MG7	37.93±0.46	53.26±1.17
8	MG8	36.00±0.34	53.26±1.33
9	MG9	38.53±1.15	59.20±0.91

All the values are expressed as mean± S.D., n=3.

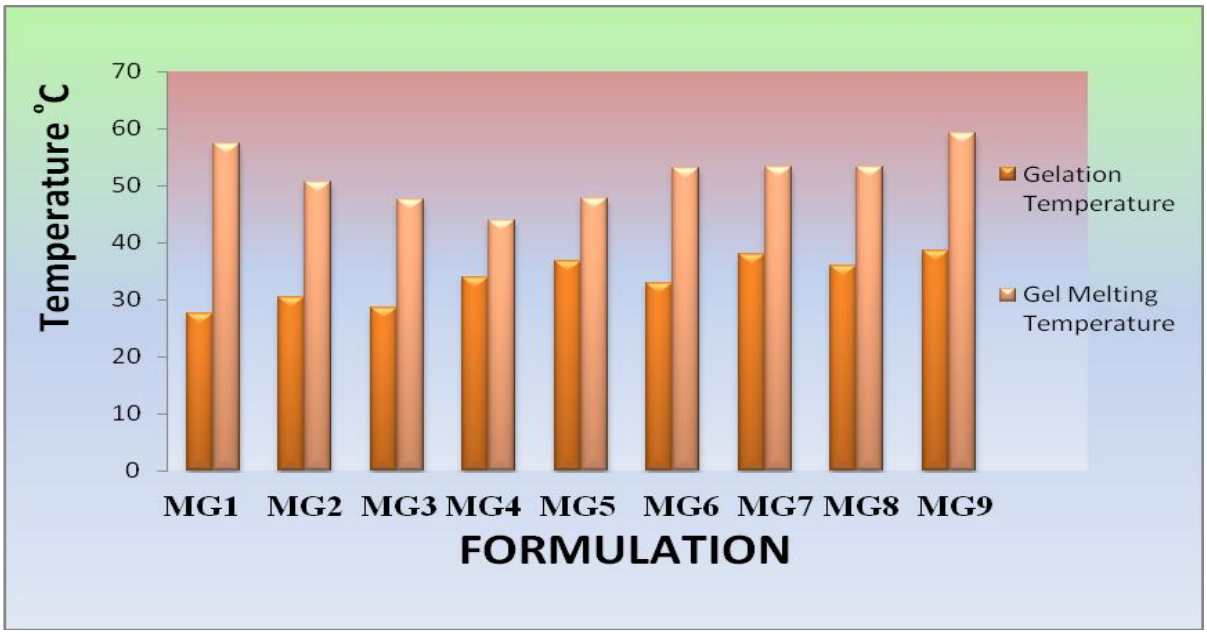


Fig. 9.12: Comparison of Gelation Temperature (T1) and Gel Melting Temperature (T2)

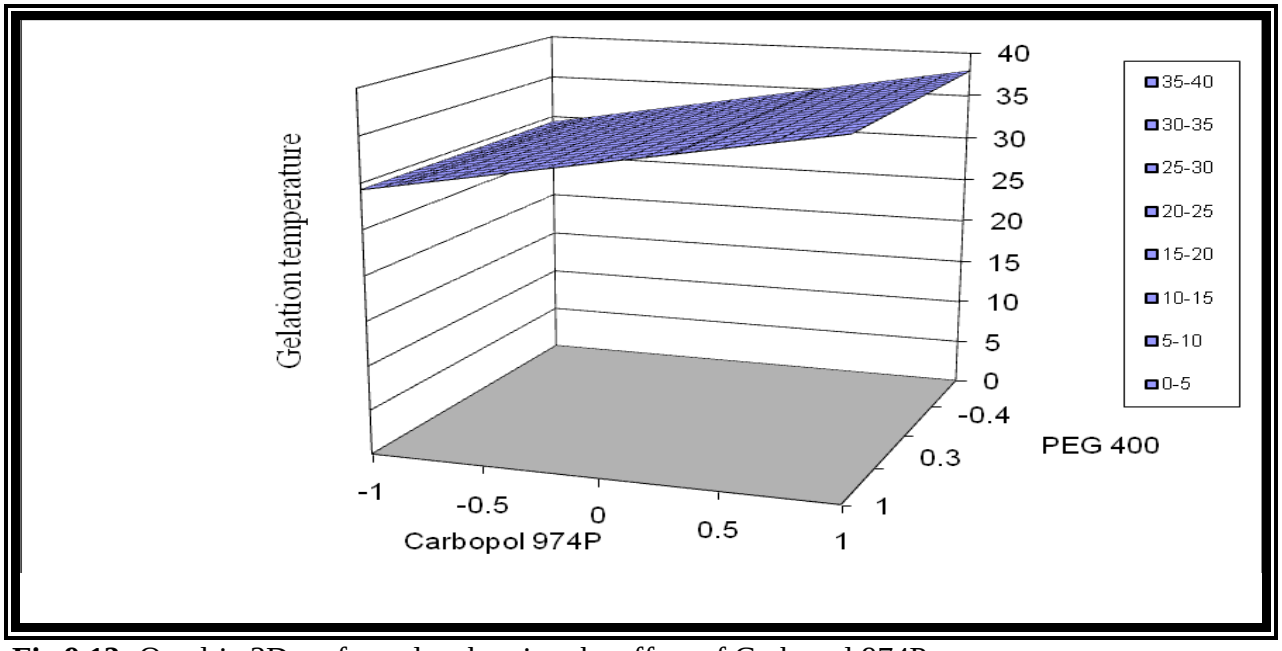


Fig.9.13: Quadric 3D surface plot showing the effect of Carbopol 974P and PEG 400 on Gelation temperature (T1)

The above figure (9.13) indicates, that the mucoadhesive polymer use i.e. Carbopol 974P has significant T1 (gelation temperature) lowering effect. Also the addition of water soluble polymer PEG 400 produces increase in T1.

9.3.3. Determination of pH:

The pH of nine formulations of Rizatriptan Benzoate mucoadhesive nasal *in situ gels* were recorded in Table 9.12.

Table 9.12: pH of Mucoadhesive Nasal *In Situ Gels*

S.NO	FORMULATION CODE	pH ±S.D.
1	MG1	6.17±0.06
2	MG2	5.70±0.28
3	MG3	6.22±0.02
4	MG4	6.40±0.02
5	MG5	6.51±0.09
6	MG6	6.28±0.21
7	MG7	6.51±0.08
8	MG8	6.78±0.12
9	MG9	5.98±0.01

All the values are expressed as mean± S.D., n=3.

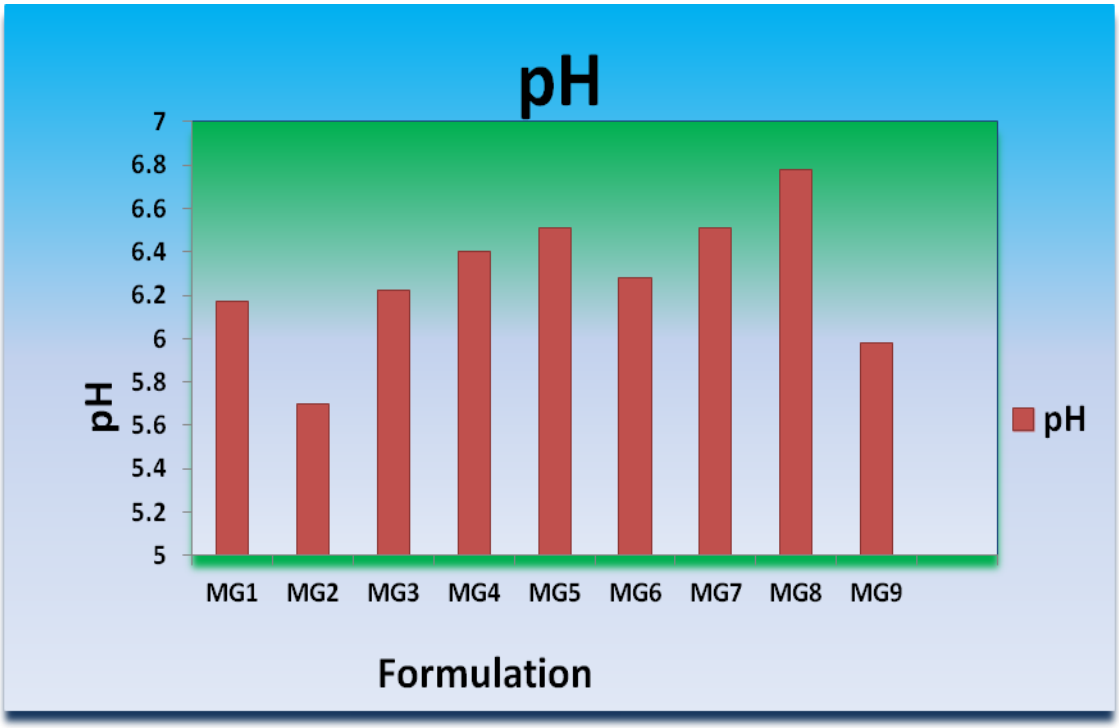


Fig. 9.14: Comparison of pH of all Formulations

It is known that the normal physiological pH of nasal mucosa is 4.5-6.5. However the nasal mucosa can tolerate solutions within pH range of 3-10. pH of all the nine formulations were found to be within 5.70-6.78 that is between physiological range of pH of nasal mucosa.

9.3.4. Determination of Drug Content:

The drug content of nine formulations of Rizatriptan Benzoate mucoadhesive nasal in situ gels were recorded in Table 9.13.

Table 9.13: Drug Content of Mucoadhesive Nasal *In Situ Gels*

S.NO	FORMULATION CODE	DRUG CONTENT±S.D. (%)
1	MG1	85.60±0.27
2	MG2	87.64±0.43
3	MG3	83.23±0.27
4	MG4	98.97±0.16
5	MG5	97.11±0.27
6	MG6	96.56±0.26
7	MG7	92.42±0.99
8	MG8	88.52±0.33
9	MG9	90.92±0.21

All the values are expressed as mean± S.D., n=3.

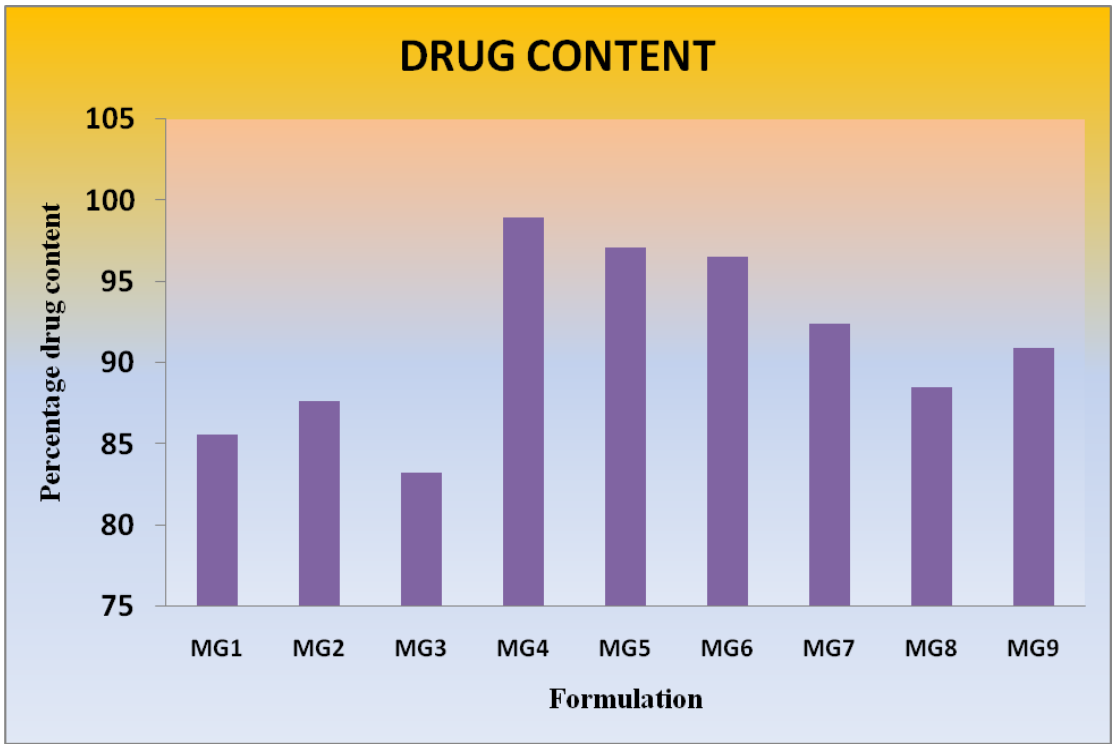


Fig. 9.15: Comparison of Drug Content

The percentage drug content of all the prepared *in situ gels* formulations swere checked and found to be in the range of 83.23-98.97%.

9.3.5. Measurement of Mucoadhesive Strength:

The measurement of mucoadhesive strength of nine formulations of Rizatriptan Benzoate mucoadhesive nasal *in situ gels* were recorded in Table 9.14.

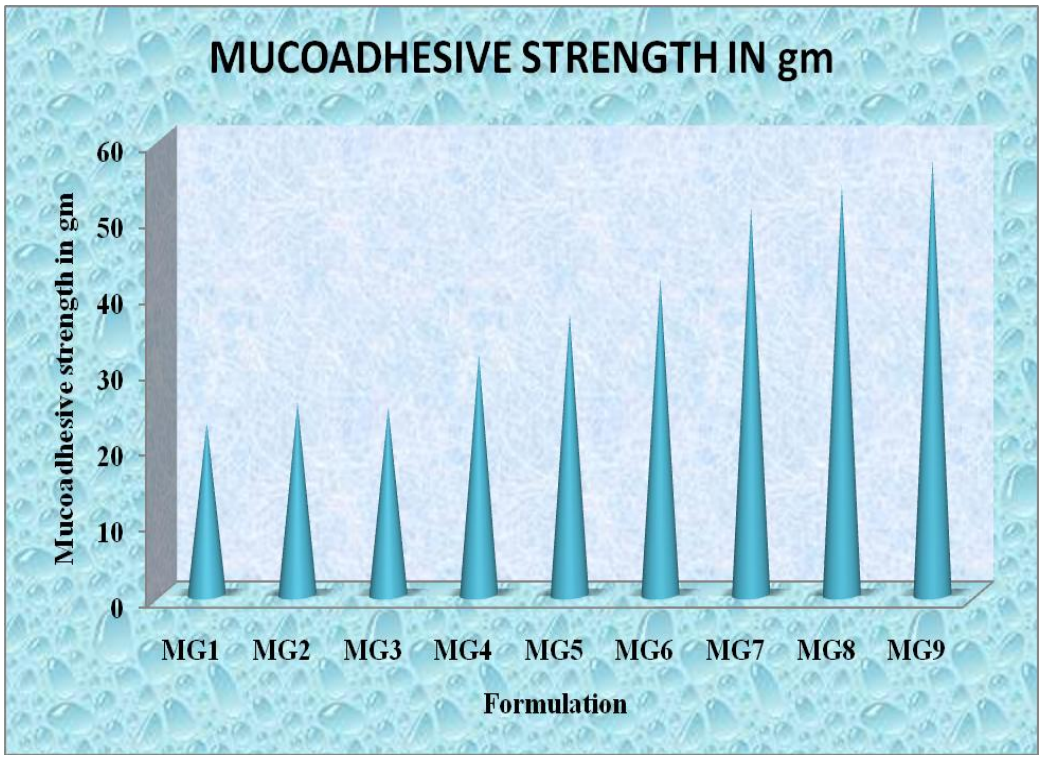
Mucoadhesive strength increases with increasing the concentration of mucoadhesive polymer.

Results of mucoadhesive strength indicates that the variable carbopol 974P and PEG 400 both are having the effect on mucoadhesive strength.

Table 9.14: Mucoadhesive Strength of Mucoadhesive Nasal *In situ Gels*

S.NO	FORMULATION CODE	MUCOADHESIVE STRENGHT* (g)±S.D.	MUCOADHESIVE STRENGHT* (d/cm^2)±S.D.
1	MG1	22.66±1.15	11056.90±0.23
2	MG2	25.33±1.52	11869.91±0.25
3	MG3	24.66±0.57	12032.52±0.31
4	MG4	31.66±1.52	15447.15±1.50
5	MG5	37.00±1.00	18687.80±0.15
6	MG6	41.66±1.52	20325.21±0.72
7	MG7	51.00±1.00	25040.65±0.73
8	MG8	54.00±1.73	27674.37±0.66
9	MG9	57.33±0.57	27967.47±0.99

All the values were expressed as mean S.D., n*=3



9.16: Comparison of Mucoadhesive Strength in gm

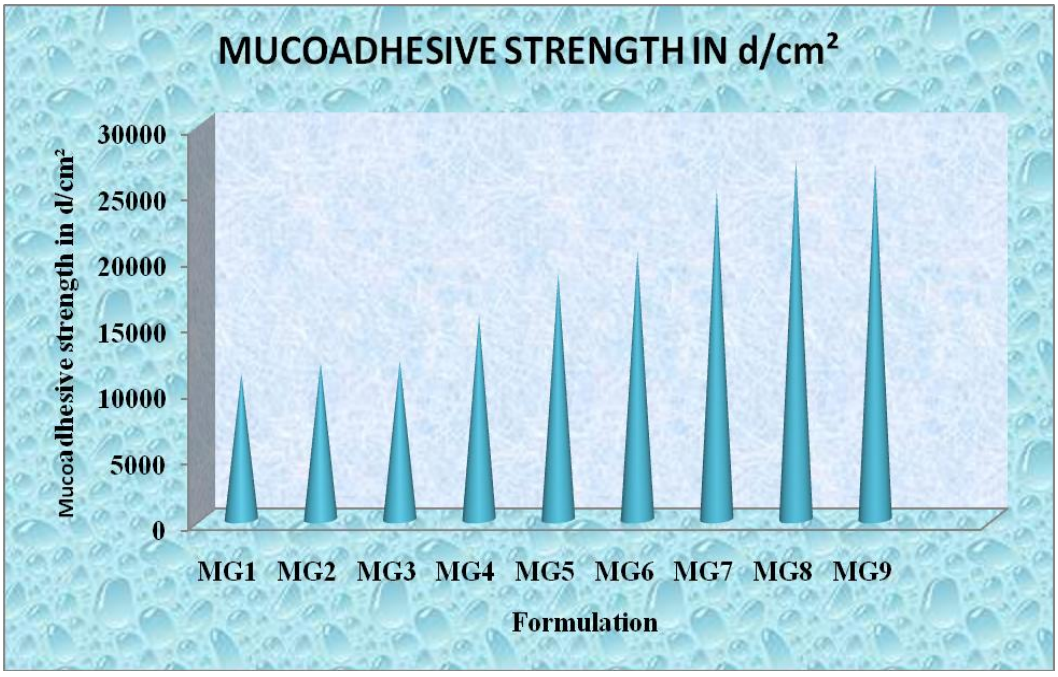


Fig. 9.17: Comparison of Mucoadhesive Strength in d/cm²

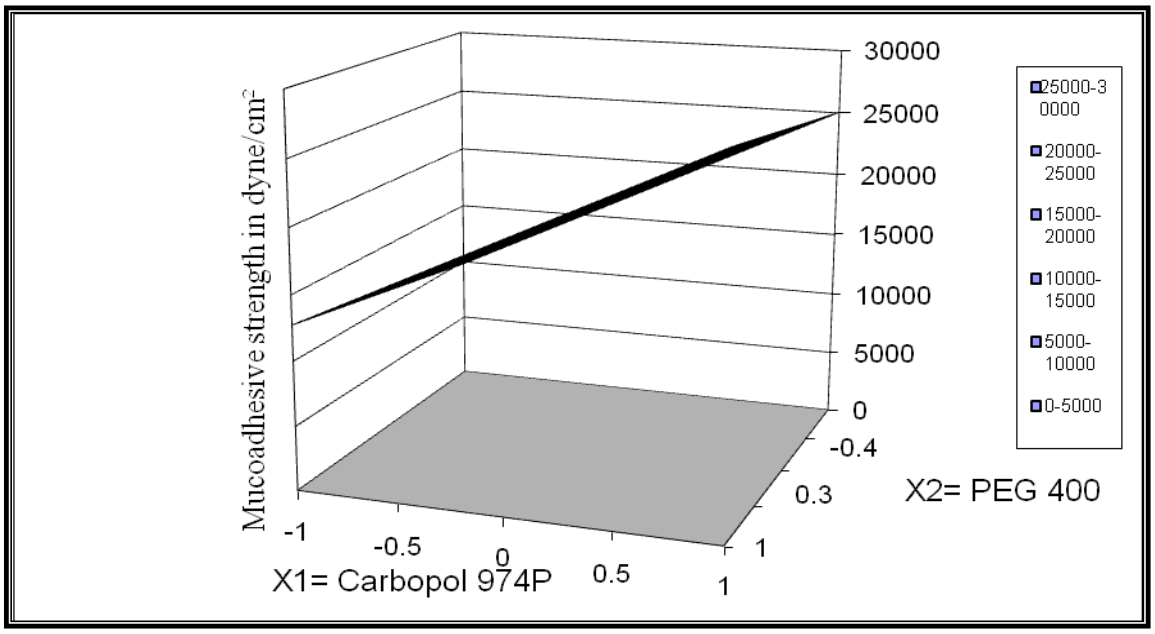


Fig.9.18: Quadric 3D surface plot showing the effect of Carbopol 974P and PEG 400 on Mucoadhesive strength

Mucoadhesive strength increases with increasing concentration of mucoadhesive polymer.

Determination of mucoadhesive strength in terms of detachment stress showed that adhesive property increases with addition of Carbopol 974P. The stronger the mucoadhesive strength is, the more it can prevent the gelled solution coming out of the nose. But if the bioadhesive strength is too excessive, the gel can damage the nasal mucosal membrane.

9.3.6. Measurement of Vicostiy: The measurement of of nine formulations of Rizatriptan Benzoate mucoadhesive nasal *in situ gels* were recorded in Table 9.15.

Table 9.15: Viscosity of Mucoadhesive Nasal *In Situ Gels*

TEMPERATURE °C	VISCOSITY MEASUREMENTS (cPs)±S.D.								
	Formulation code								
	MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8	MG9
20 °C	830.00 ±1.00	727.33 ±1.52	841.33 ±1.55	965.00 ±1.00	992.00 ±1.00	1022.66 ±1.52	1093.33 ±0.57	1247.00 ±1.00	1205.66 ±1.15
22 °C	840.00 ±1.52	780.00 ±1.00	871.33 ±1.52	981.00 ±1.00	1018.0 0±1.00	1114.33 ±0.57	1012.66 ±1.52	1372.33 ±1.52	1571.66 ±0.57
24 °C	861.66 ±1.52	793.00 ±0.57	893.00 ±1.00	1021.6 6±0.57	1126.3 3±1.52	1374.00 ±1.73	1231.00 ±1.73	1755.00 ±1.00	1922.33 ±1.52
26 °C	921.66 ±1.52	824.00 ±0.57	930.66 ±1.52	1072.6 6±0.57	1573.0 0±1.00	1731.00 ±1.00	1599.33 ±1.52	2002.33 ±1.52	2210.00 ±1.00
28 °C	1080.6 6±1.15	851.33 ±1.52	970.66 ±1.52	1160.6 6±1.52	1961.6 6±1.52	2142.00 ±1.73	1975.00 ±1.00	2242.33 ±1.52	2692.00 ±1.00
30 °C	1120.6 6±1.15	942.00 ±1.00	1160.6 6±1.15	1861.0 0±1.00	2262.0 0±1.73	2670.33 ±1.52	2273.00 ±1.00	2791.66 ±1.52	3097.66 ±1.15
32 °C	1185.0 0±1.00	1121.0 0±0.57	1251.6 6±1.50	2226.6 6±0.57	2591.0 0±1.00	2996.00 ±1.00	3001.33 ±1.52	3143.33 ±1.52	3211.33 ±1.15
34 °C	1200.6 6±1.52	1260.3 3±1.00	1337.0 0±1.73	2699.0 0±1.00	2780.6 6±1.52	3030.66 ±1.52	3247.00 ±1.00	3248.00 ±1.00	3481.00 ±1.00

All the values are expressed as mean± S.D., n=3.

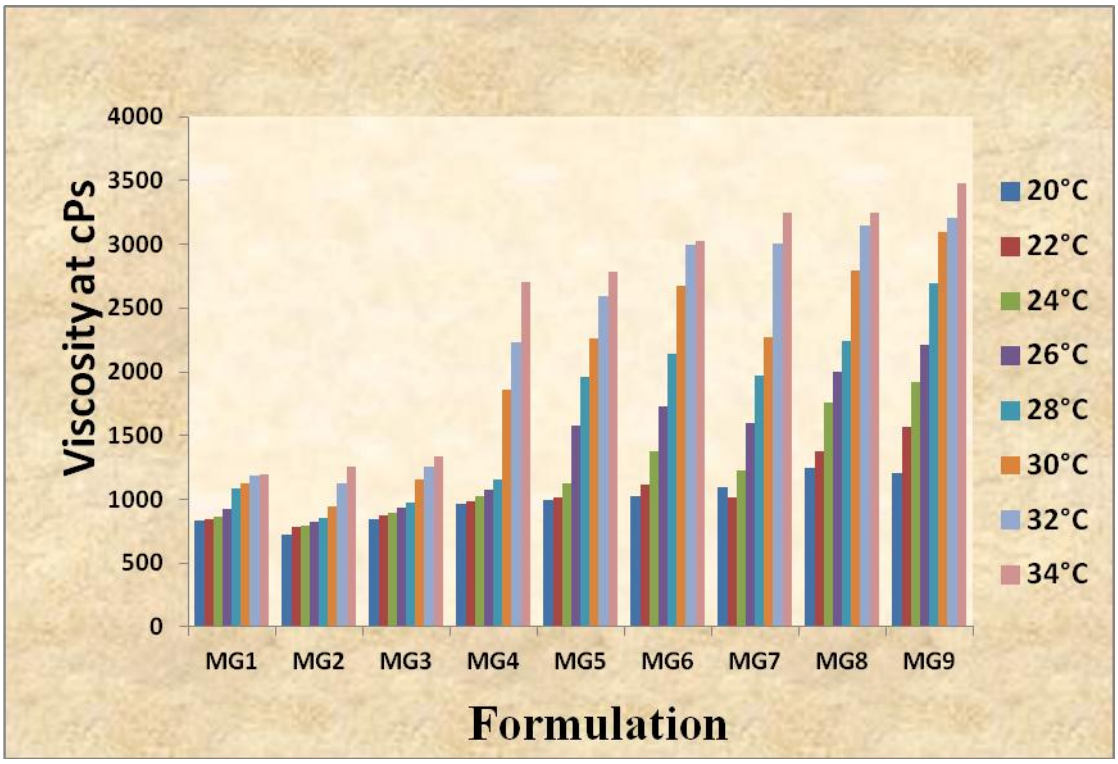


Fig. 9.19: Comparison of Viscosity of MG1-MG9 formulation.

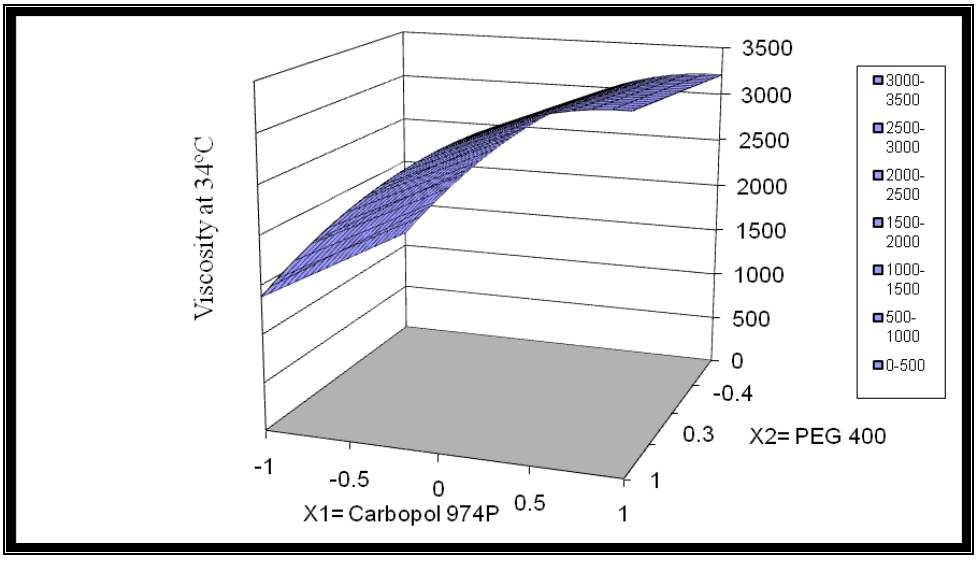


Fig.9.20: Quadric 3D surface plot showing the effect of Carbopol 974P and PEG 400 on viscosity

The figure (9.20) shows the viscosity profile of formulations at 34ºC. It shows that mucoadhesive polymer carbopol 974P had a viscosity enhancing effect. On the other hand, PEG polymer counteracted the viscosity enhancing effect caused by carbopol 974P.

Viscosity measurement of the nine formulations at various temperatures shows that there was increase in viscosity with increase in temperature. This indicates the temperature induced gel structure formation of pluronic F127.

9.3.7. *In-vitro* drug permeation studies:

Table 9.16: *In-vitro* drug permeation profile of formulation MG1, MG2, MG3.

S.No.	Time in hours	Medium	MG1 (%)	MG2 (%)	MG3 (%)
1	1	pH 6.4 PHOSPHATE BUFFER	0.00	0.00	0.00
2	2		5.29 ± 0.15	8.00 ± 0.13	6.40 ± 0.13
3	3		9.32 ± 0.20	12.05± 0.13	07.66 ± 0.31
4	4		13.15 ± 0.16	19.32± 0.13	12.87 ± 0.65
5	5		21.03± 0.09	27.26± 0.13	16.79 ± 0.39
6	6		37.03± 0.15	36.95± 0.13	22.94 ± 0.39
7	7		52.66± 0.50	47.26 ± 68.05	25.81 ± 1.41
8	8		70.40± 0.13	68.05± 0.14	28.48 ± 0.87
9	9		85.27± 0.16	87.34± 0.14	82.06± 0.16

All the values are expressed as mean± S.D., n=3.

Table 9.17: *In-vitro* drug permeation profile of formulation MG4, MG5, MG6.

S.No.	Time in hours	Medium	MG4 (%)	MG5 (%)	MG6 (%)
1	1	pH 6.4 PHOSPHATE BUFFER	0.00	0.00	0.00
2	2		17.86 ± 0.13	18.57 ± 0.15	17.86± 0.13
3	3		23.38± 0.14	20.56± 0.21	21.11 ± 0.13
4	4		31.93± 0.20	26.86± 0.15	25.79 ± 0.13
5	5		36.43± 0.13	33.08± 0.15	32.41± 0.15
6	6		45.28± 0.20	49.40 ± 0.23	48.18 ± 0.20
7	7		68.83± 0.16	71.36± 0.17	67.59± 0.14
8	8		75.97± 0.16	82.65± 0.22	81.94± 0.14
9	9		98.04± 0.15	96.56± 0.16	96.06 ± 0.19

All the values are expressed as mean± S.D., n=3.

Table 9.18: *In-vitro* drug permeation profile of formulation MG7, MG8, MG9.

S.No.	Time in hours	Medium	MG7 (%)	MG8 (%)	MG9 (%)
1	1	pH 6.4 PHOSPHATE BUFFER	0.00	0.00	0.00
2	2		17.95 ± 0.20	18.80 ± 0.40	18.00± 0.27
3	3		20.98± 0.27	20.93± 0.26	21.96 ± 0.27
4	4		25.88± 0.19	22.93± 0.34	25.54± 0.41
5	5		35.74± 0.22	30.54± 0.55	33.15 ± 0.25
6	6		52.31± 0.23	46.60± 0.62	51.44± 0.26
7	7		67.17± 0.43	62.31± 0.15	65.25± 0.15
8	8		80.20± 0.10	74.33± 0.50	78.26 ± 0.27
9	9		52.95 ± 0.86	88.24± 0.02	90.49± 0.20

All the values are expressed as mean± S.D., n=3.

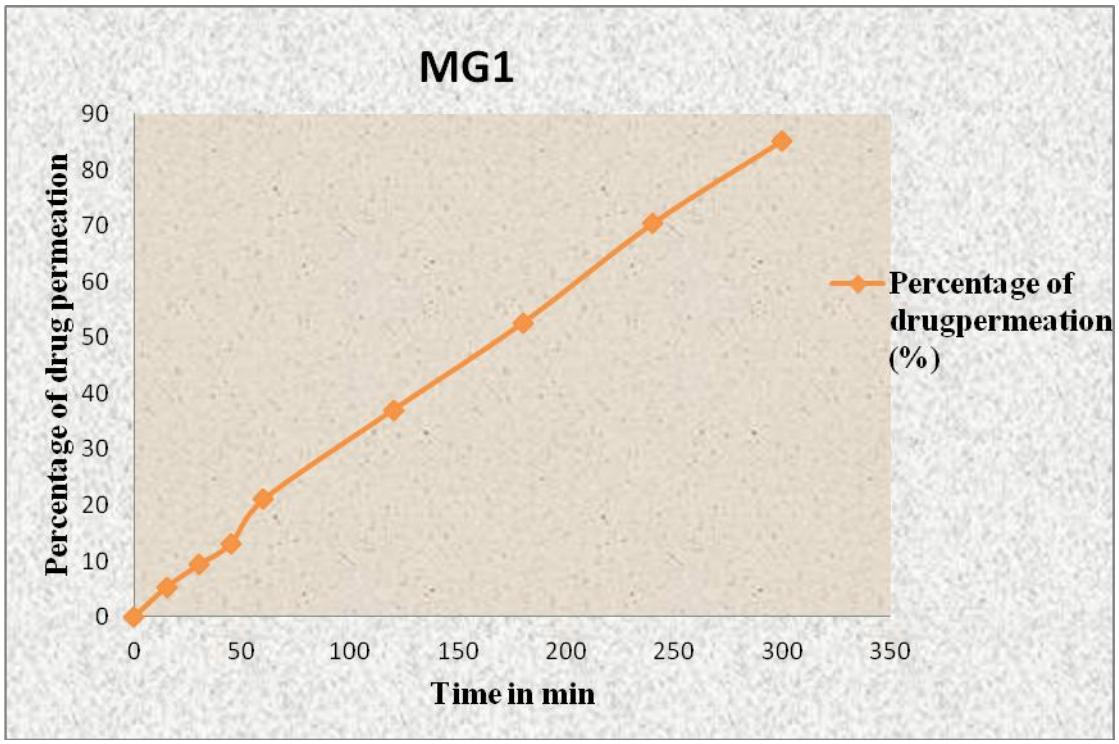


Fig. 9.21: *In-vitro* drug permeation profile of formulation MG1

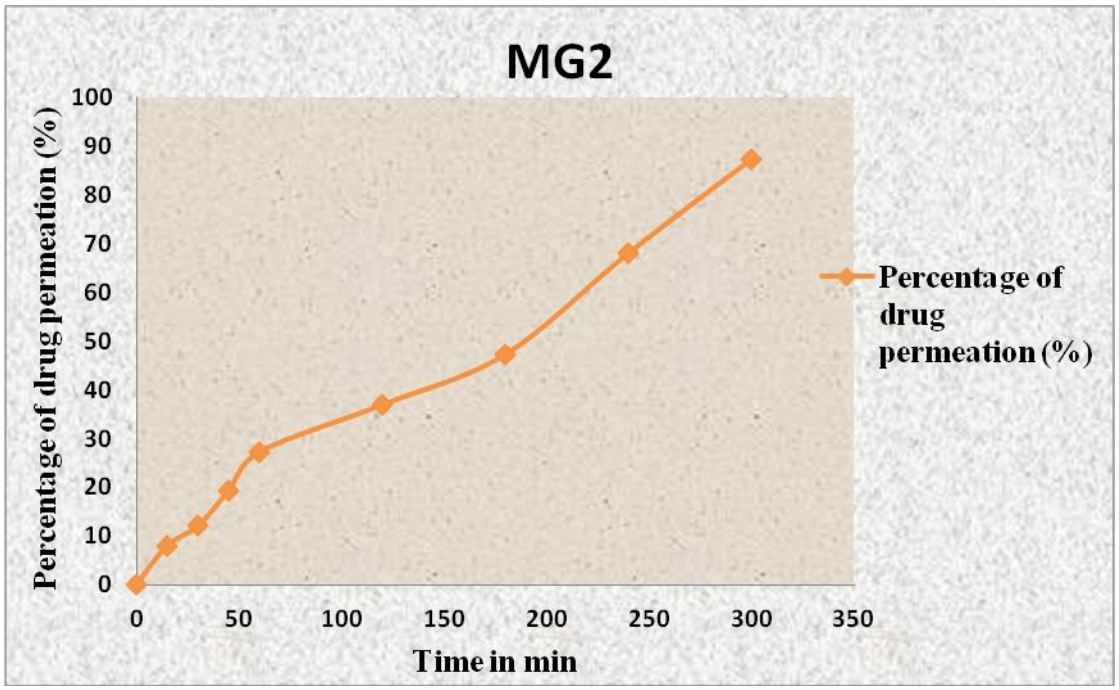


Fig. 9.22: *In-vitro* drug permeation profile of formulation MG2

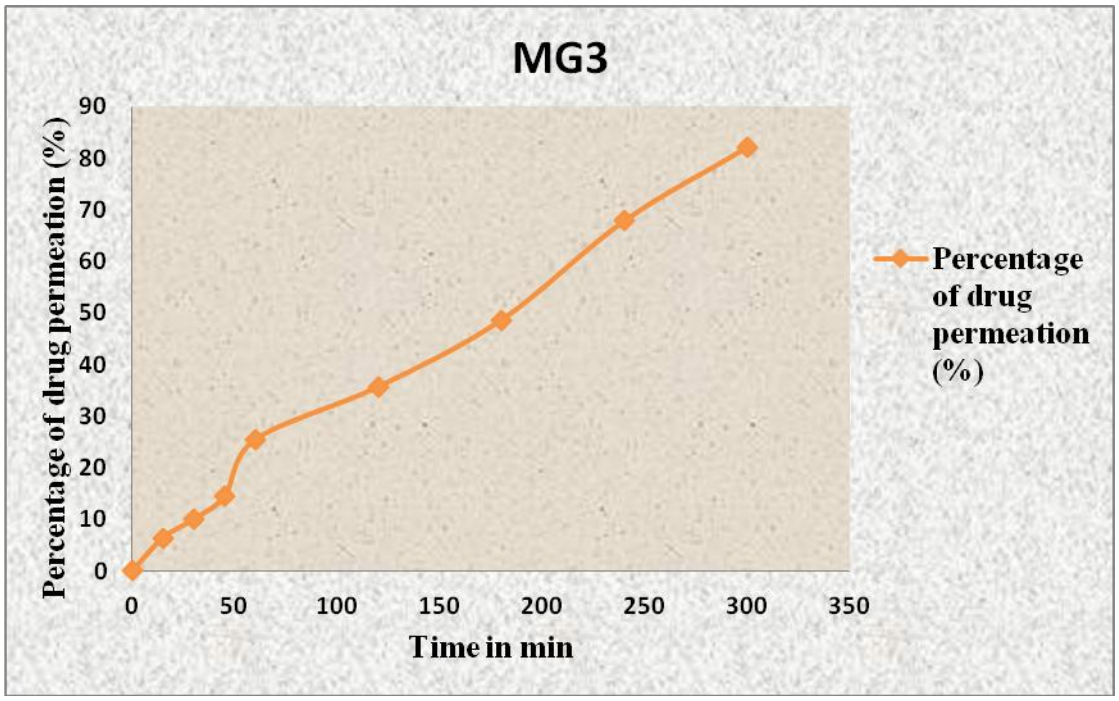


Fig. 9.23: *In-vitro* drug permeation profile of formulation MG3

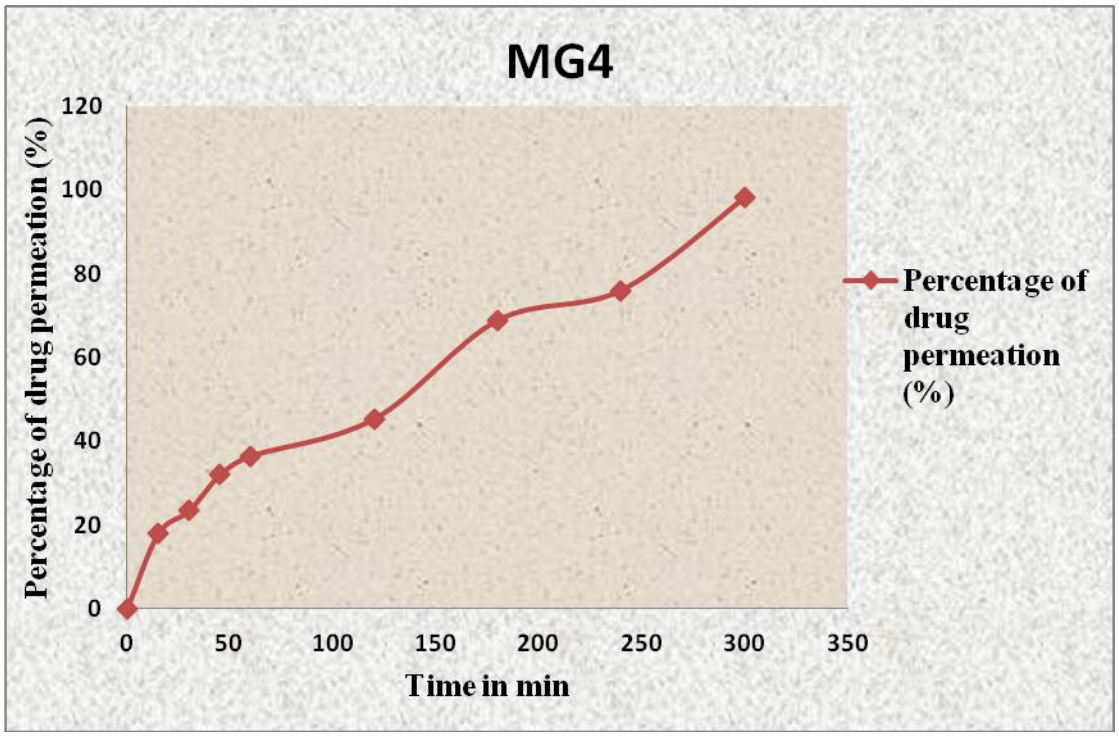


Fig. 9.24: *In-vitro* drug permeation profile of formulation MG4

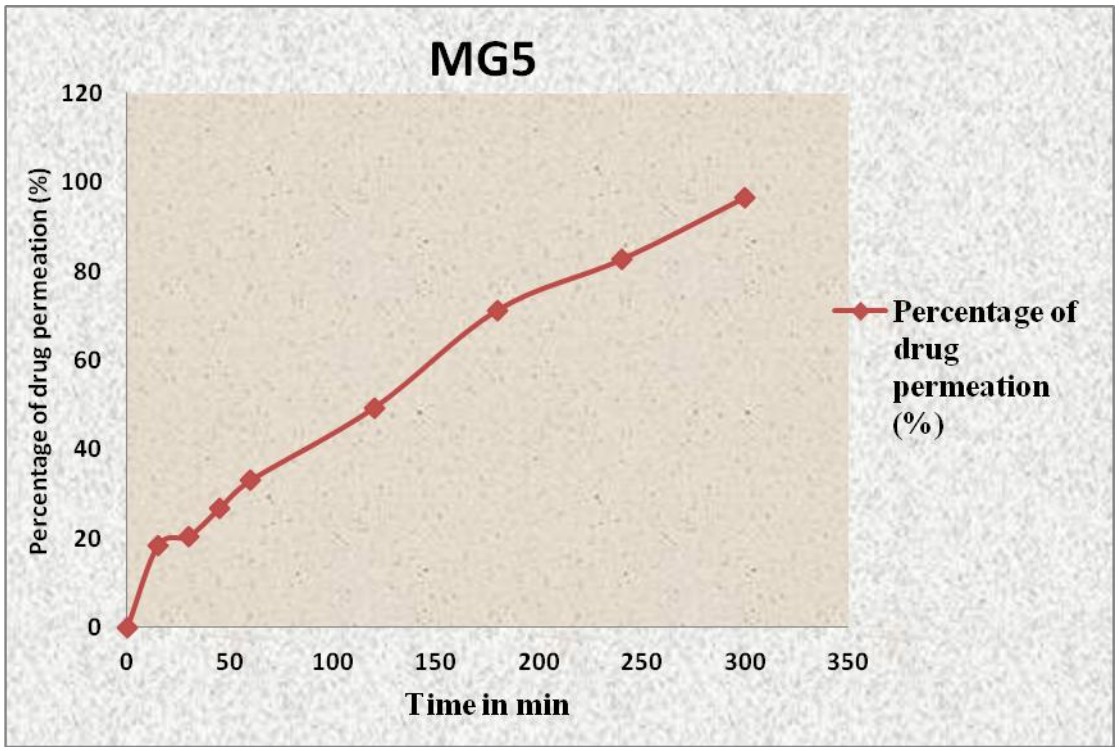


Fig. 9.25: *In-vitro* drug permeation profile of formulation MG5

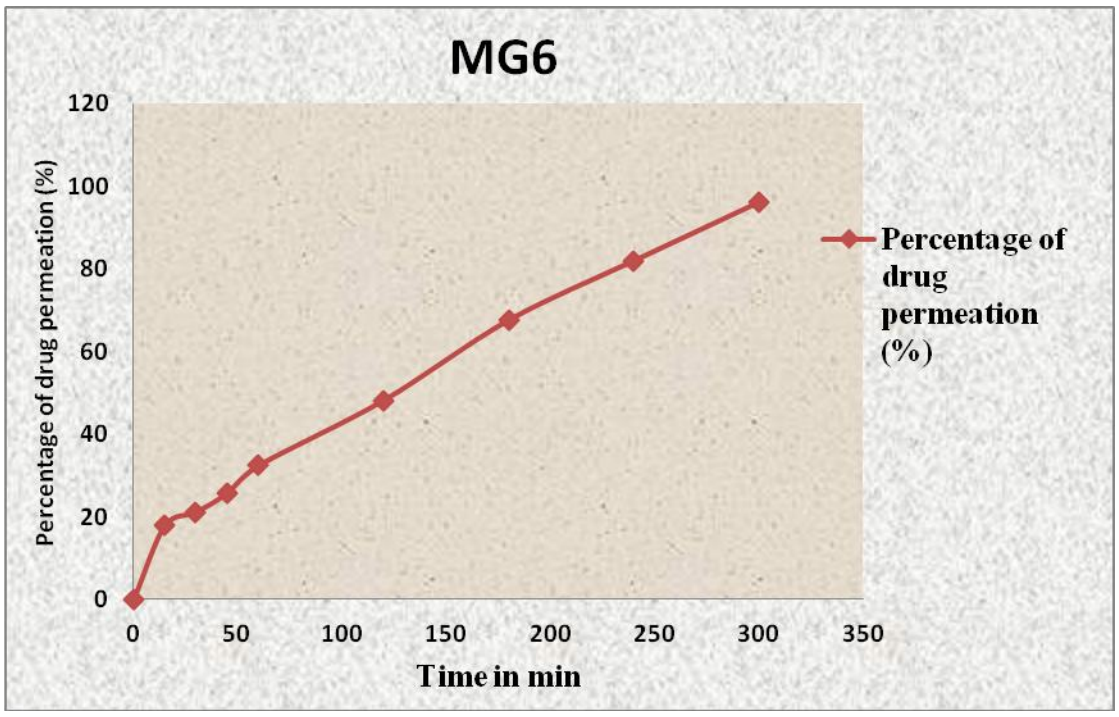


Fig. 9.26: *In-vitro* drug permeation profile of formulation MG6

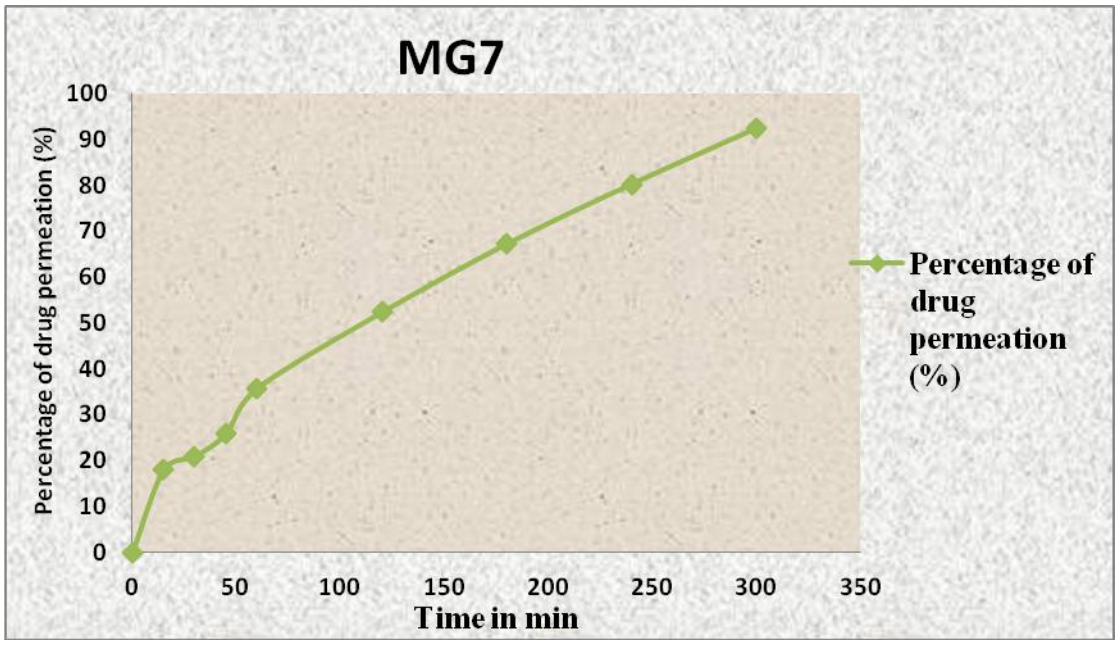


Fig. 9.27: *In-vitro* drug permeation profile of formulation MG7

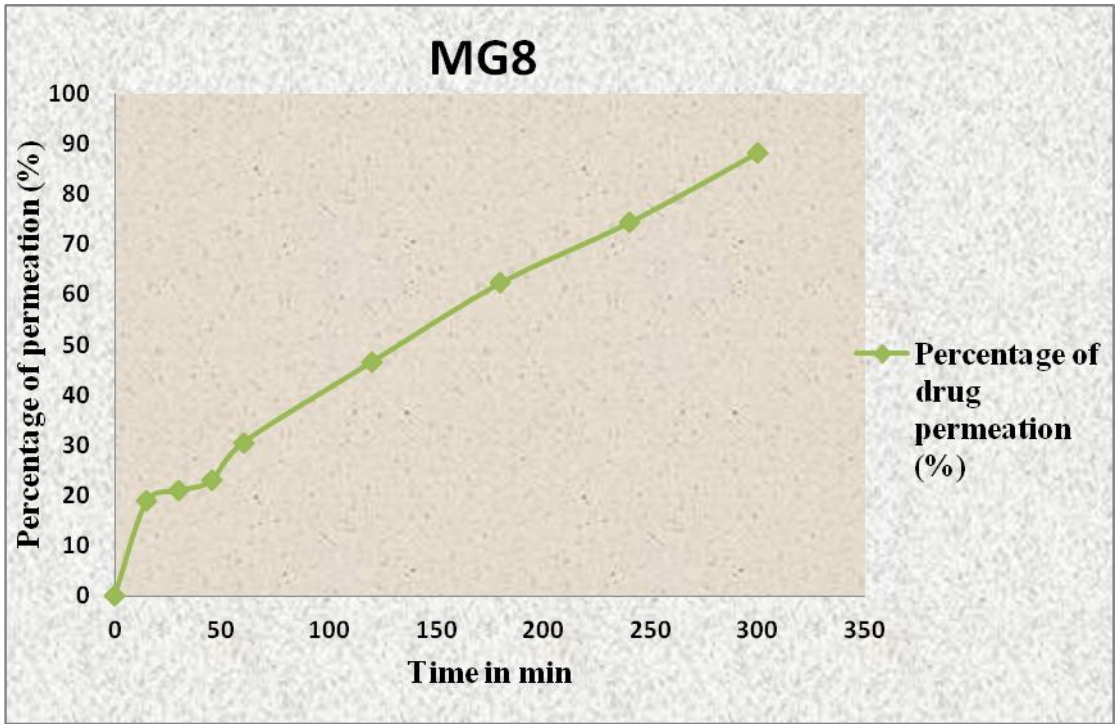


Fig. 9.28: *In-vitro* drug release permeation of formulation MG8

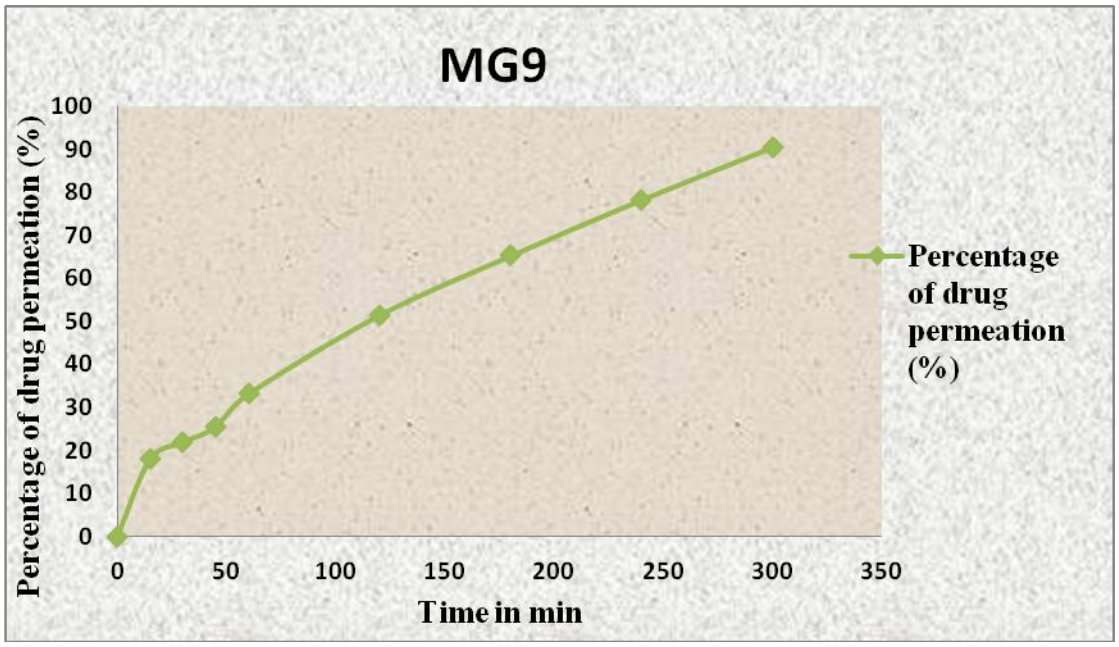


Fig. 9.29: *In-vitro* drug permeation profile of formulation MG9

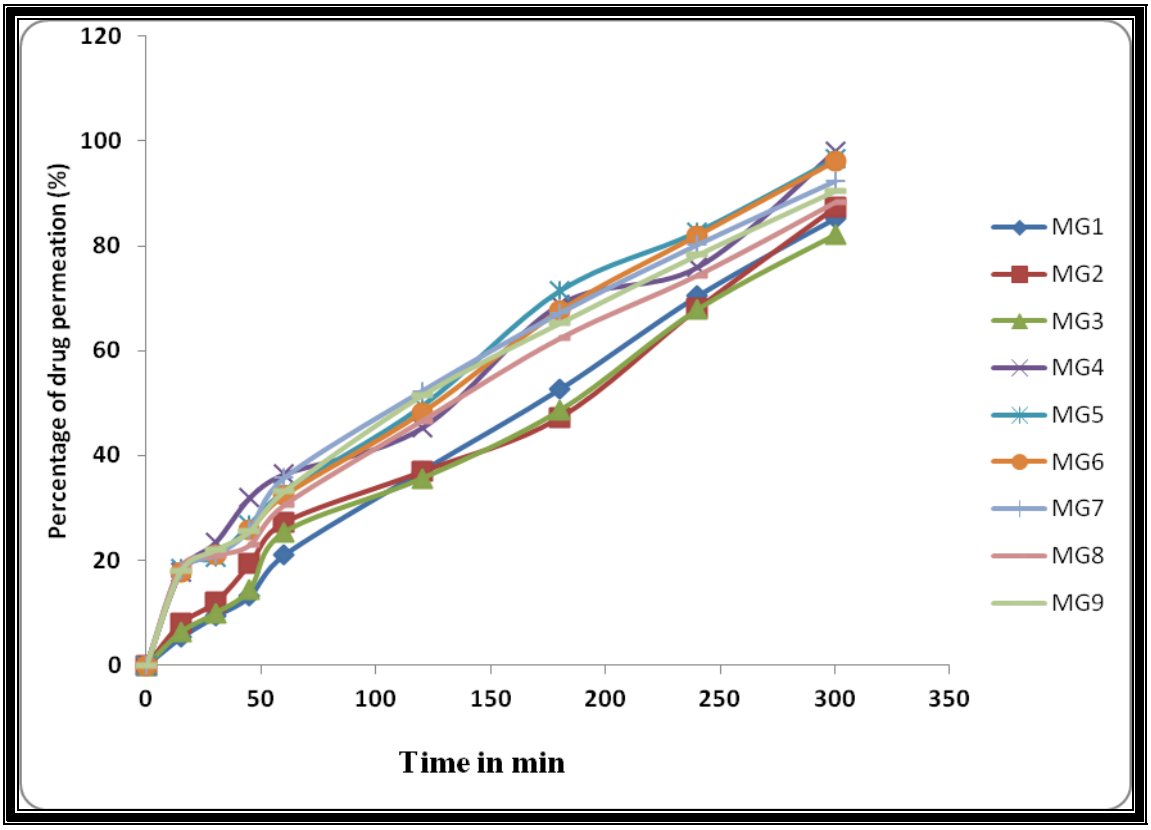


Fig. 9.30: Comparative *in-vitro* drug permeation of all formulations

In-vitro diffusion studies revealed that the release of Riztriptan Benzoate from different formulations varies with the characteristics and composition of polymers as shown in Figures 9.21 to 9.30.

The formulated mucoadhesive *in situ gels* showed a most favorable release within 5 hours. In the 5^{th} hour, the drug permeation was 85.27%, 87.34%, 82.06%, 98.09, 96.56, 92.32, 88.24, 90.49 and 97.17% for MG1, MG2, MG3, MG4, MG5, MG6, MG7, MG8 and MG9 respectively. This was followed by a steady drug release pattern.

From these above data, it showed formulation MG4 permeated drug mostly at the end of 5 hours. The in-vitro drug permeation rate of Rizatriptan Benzoate shows that with increasing carbopol 974P concentration influences the diffusion of drug particle while addition of PEG 400 enhances the drug permeation.

Among all the nine formulations, formulation MG4 (composed of Rizatriptan Benzoate 1%w/v, 18% w/v PF 127, 0.5% w/v carbopol 974P, 6% w/v PEG 400) exhibited the highest *in-vitro* drug permeation of 98.09 at 5 hours, while the lowest drug release of 82.06 was recorded for formulation containing (composed of Rizatriptan Benzoate 1%w/v, 18% w/v PF 127, 0.25% w/v carbopol 974P, 10% w/v PEG 400).

From the above evaluation parameters it was concluded that the **Formulation MG4** having a maximum percentage of drug release in a

controlled manner, so the formulation MG4 was selected as the optimized formulation.

9.3.8. Histopathological study of optimized formulation:

The histopathological study were determined by using sheep nasal mucosa. The histological report of both control and optimized formulation were showed in Figure 9.31 and 9.32

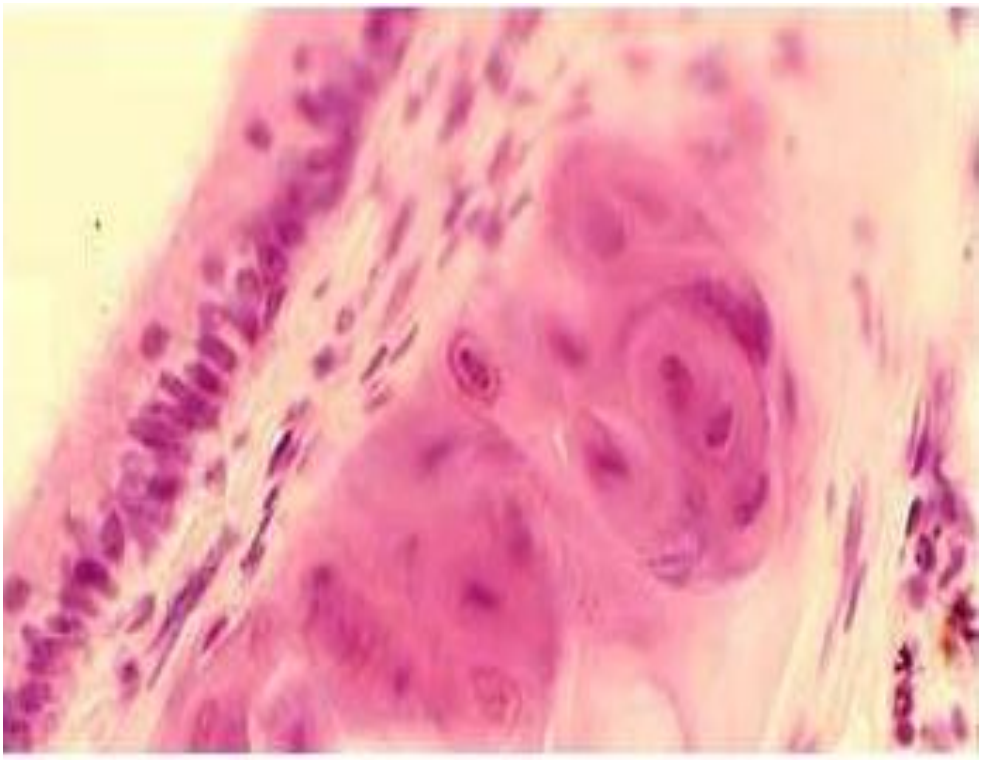


Fig. 9.31: Histopathology of nasal mucosa Control

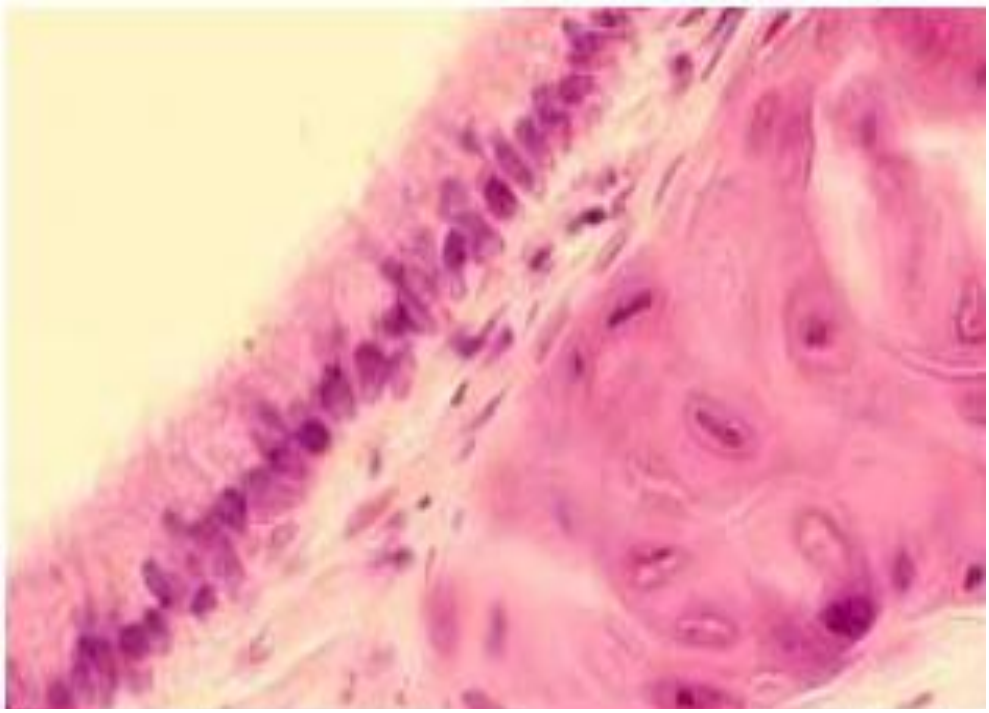


Fig. 9.32: Histopathology of nasal mucosa Optimized Nasal *In Situ Gel* formulation.

Photomicrographs of nasal mucosa after the permeation studies were observed for histopathological study with the phosphate buffer 6.4 treated mucosa.

The section of mucosa treated with optimized formulation MG4 showed no degeneration of nasal epithelium along with no signs of erotion.

9.3.9. Kinetics of *In-vitro* drug permeation:

The kinetics of *in-vitro* drug permeation was determined by applying the drug release data to various kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas. The result obtained was shown in Table 9.19.

Table 9.19: Different kinetic models for Rizatriptan Benzoate Nasal *In Situ Gels* (MG1 to MG9)

S. NO	F. Code	Zero order	First order	Higuchi	Korsemeyer-Peppas		Best fit model
		R^2	R^2	R^2	R^2	n	
1	**MG1**	0.9602	0.9608	0.9825	0.9915	0.5992	Peppas
2	**MG2**	0.9249	0.9258	0.9834	0.9878	0.5248	Peppas
3	**MG3**	0.9394	0.9401	0.9860	0.9892	0.5579	Peppas
4	**MG4**	0.8382	0.8400	0.9897	0.9865	0.5996	Higuchi
5	**MG5**	0.8743	0.8758	0.9956	0.9865	0.4596	Higuchi
6	**MG6**	0.8788	0.8758	0.9956	0.9837	0.4596	Higuchi
7	**MG7**	0.8432	0.8448	0.9973	0.9890	0.4505	Higuchi
8	**MG8**	0.8493	0.8509	0.9938	0.9769	0.4287	Higuchi
9	**MG9**	0.8459	0.8476	0.9966	0.9864	0.4417	Higuchi

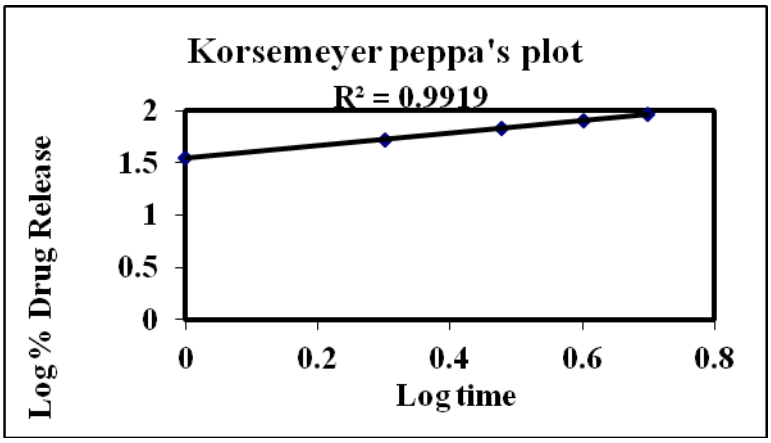


Fig. 9.33: Best fit kinetic release of formulation MG1

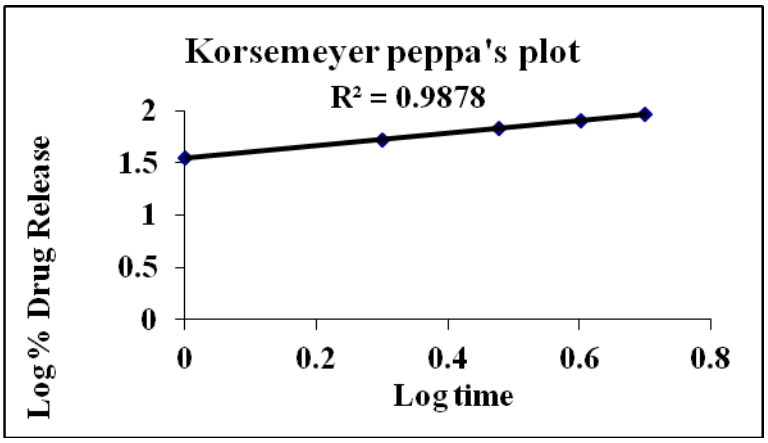


Fig. 9.34: Best fit kinetic release of formulation MG2

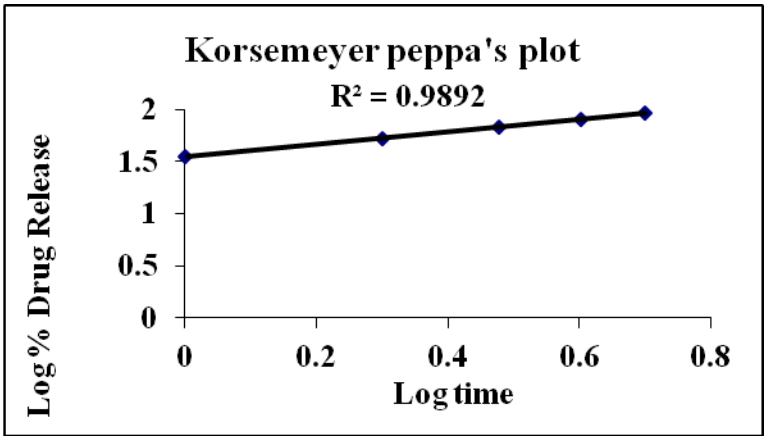


Fig. 9.35: Best fit kinetic release of formulation MG3

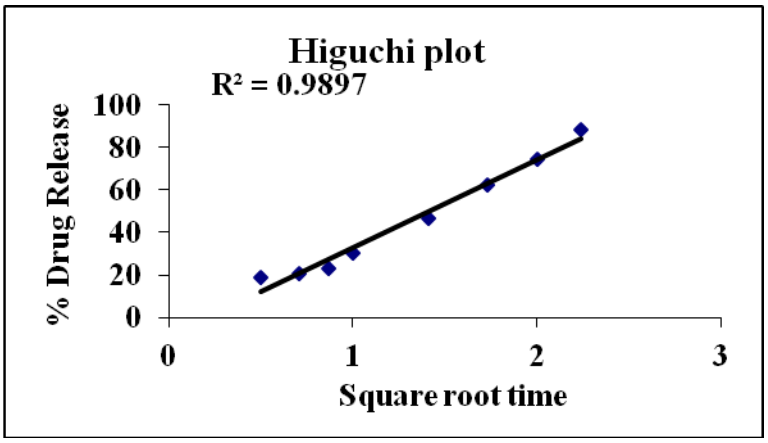


Fig. 9.36: Best fit kinetic release of formulation MG4

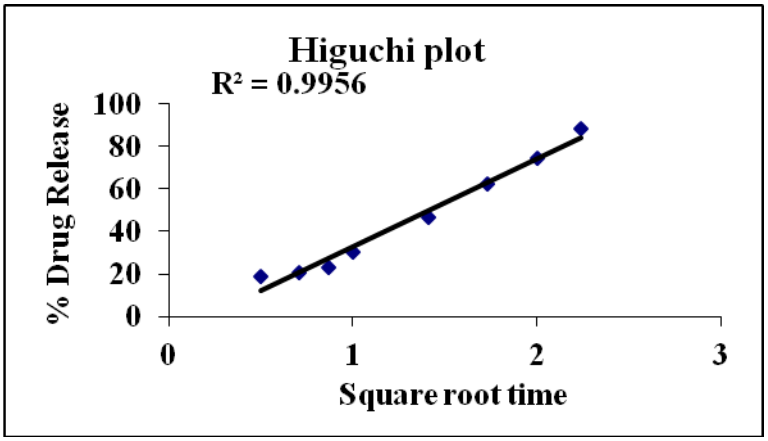


Fig. 9.37: Best fit kinetic release of formulation MG5

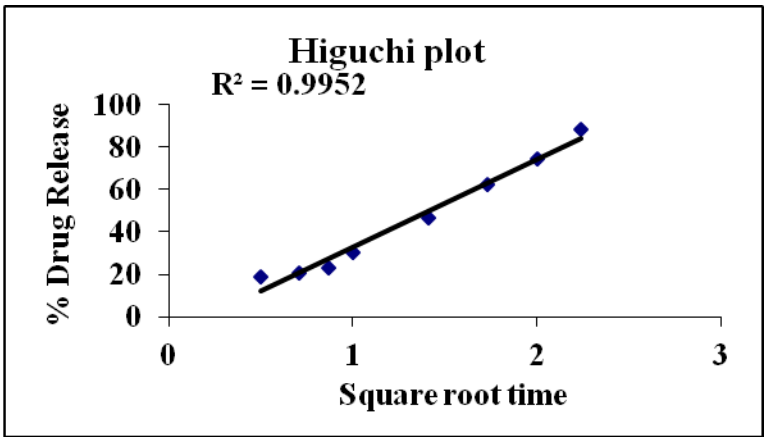


Fig. 9.38: Best fit kinetic release of formulation MG6

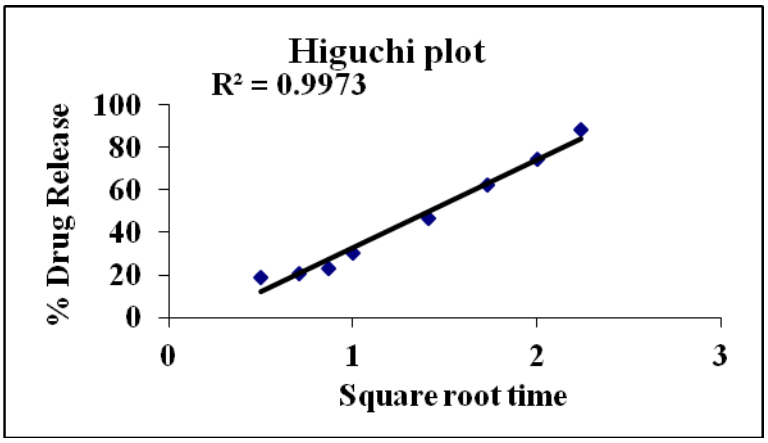


Fig. 9.39: Best fit kinetic release of formulation MG7

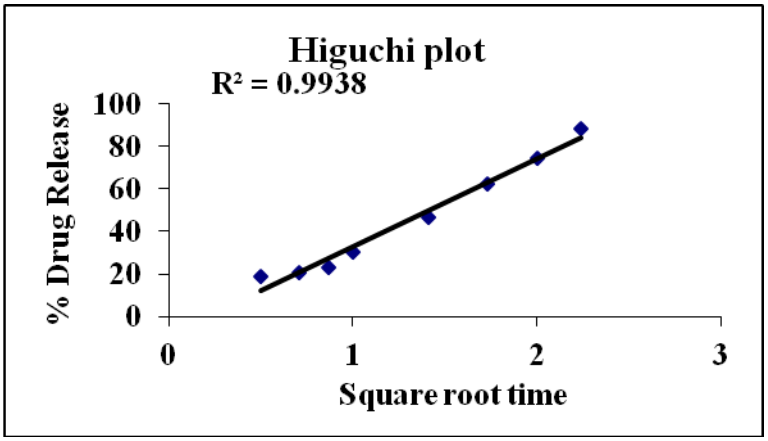


Fig. 9.40: Best fit kinetic release of formulation MG8

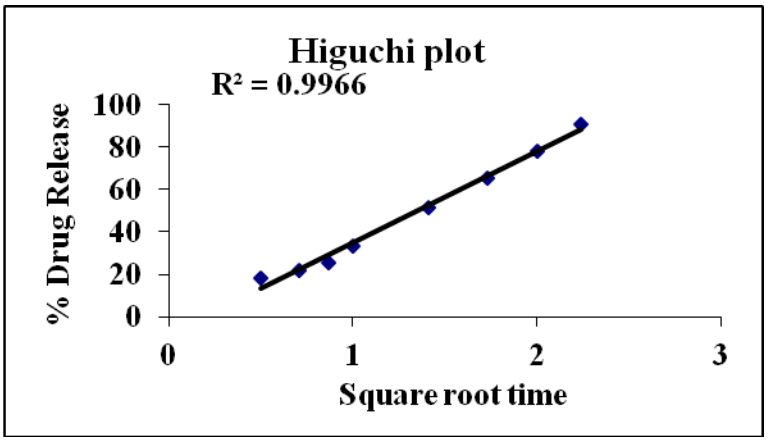


Fig. 9.41: Best fit kinetic release of formulation MG9

9.3.10: Stability studies:

After exposure to stability conditions (40ºC ±2ºC at 75% RH ±5% RH) the formulation was analyzed for various evaluation parameters; results are shown in Table 9.20-9.21.

Table 9.20: Stability studies of optimized formulation MG4

Characteristic	Initials	1st Month	2nd Month	3rd Month
Clarity	+++	+++	+++	+++
Gelation Temperature (T1)	33.86± 0.30	33.66± 0.30	33.50± 0.36	33.33± 0.41
Gel Melting Temperature (T2)	44.000.52	42.76±1.42	41.86±1.61	40.96±0.25
pH	6.40±0.02	6.38 ±0.05	6.53 ±0.02	6.21± 0.02
Drug Content	98.97±0.16	98.47±0.11	98.25±0.14	97.92±0.17
Invitro Drug Permeation at 5 h (%)	98.09± 0.15	97.61±0.15	97.44±0.14	97.03±0.08

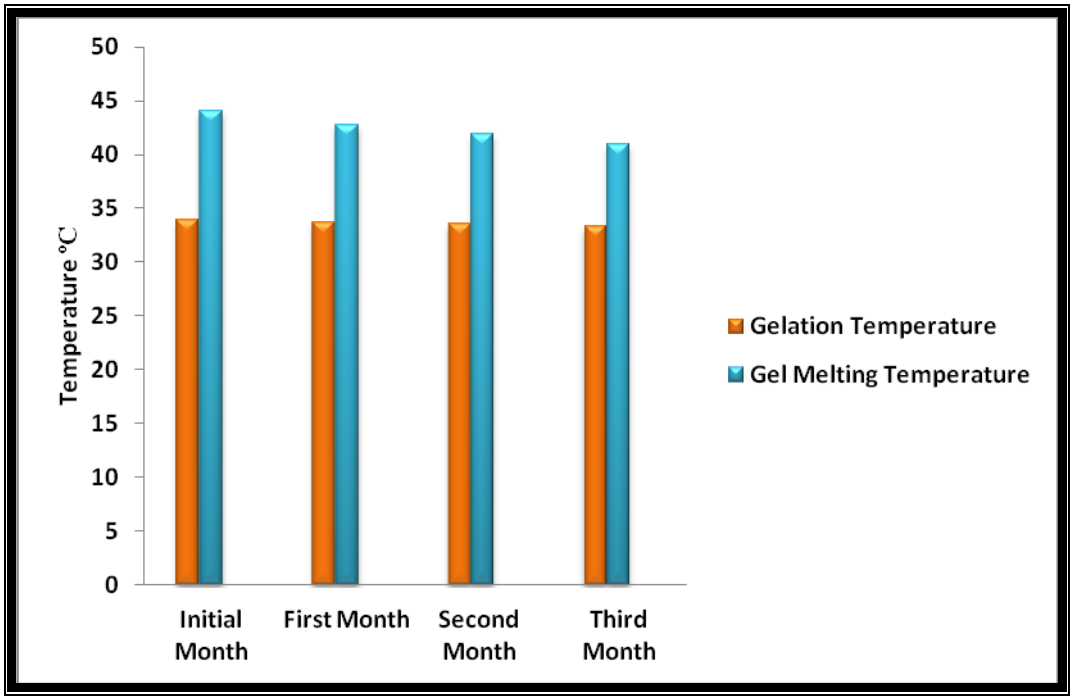


Fig. 9.42: Comparison of gelation ans ge lmelting temperature before and after stability studies at 40°C ±2°C at 75% RH ± 5% for optimized formulation MG4

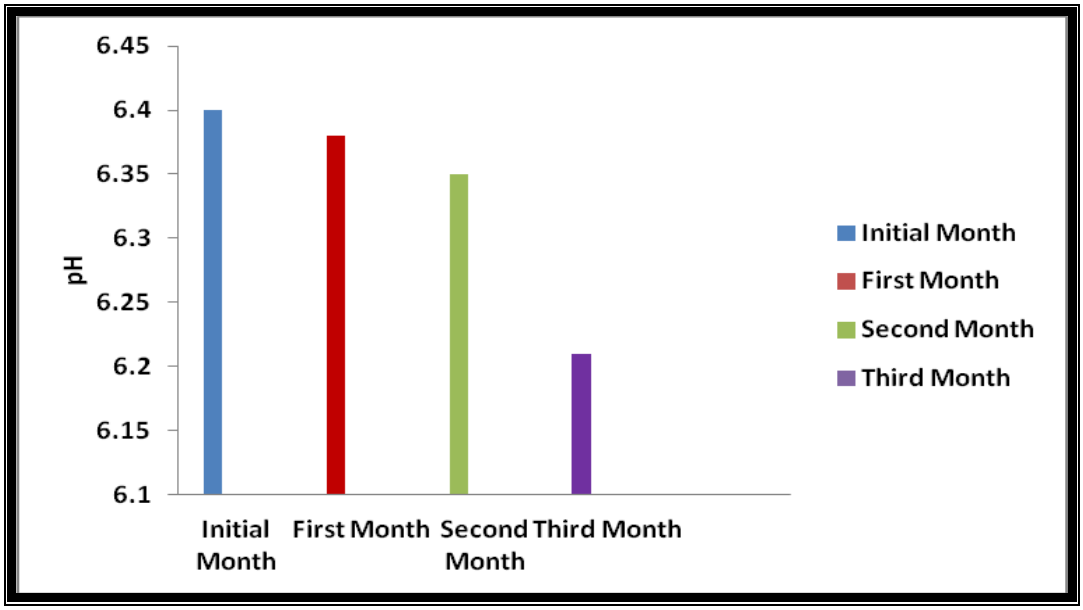


Fig. 9.43: Comparison of pH before and after stability studies at 40°C ±2°C at 75% RH ± 5% for optimized formulation MG4

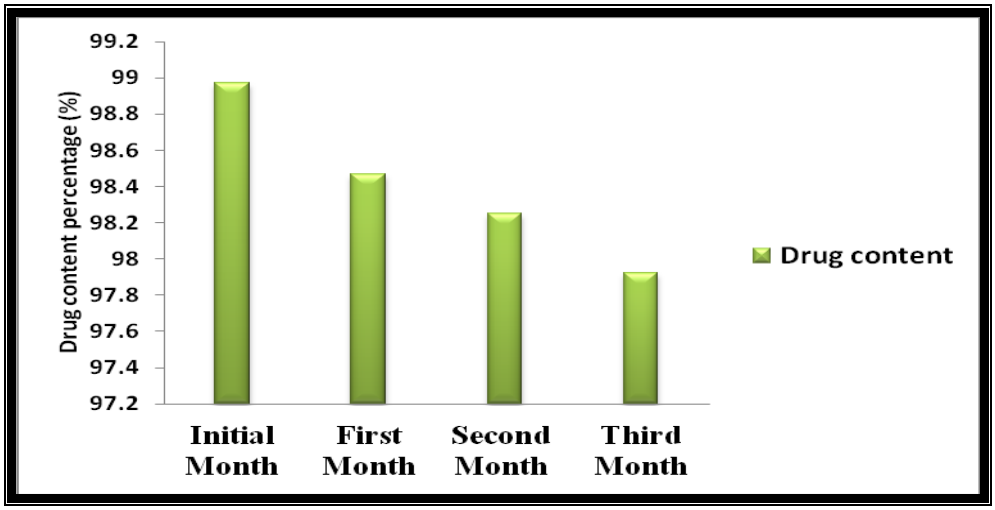


Fig. 9.44: Comparison of drug content of selected formulation MG4 after stability studies at 40°C ±2°C at 75% RH ± 5%

Table 9.21: Percentage *in-vitro* drug permeation of selected formulation MG4 after stability studies at 40°C ±2°C at 75% RH ± 5%.

S. No	Time in mins	40°C ±2°C at 75% RH ± 5%			
		Initial*	1st month*	2nd month*	3rd month*
1	1	17.86±0.13	17.20±0.13	16.93±0.13	16.80±0.13
2	2	23.38±0.14	22.82±0.14	22.63±0.08	22.31±0.07
3	3	31.93±0.20	31.83±0.14	31.73±0.08	31.44±0.08
4	4	36.43±0.13	36.07±0.08	35.96±0.14	35.66±0.13
5	5	45.28±0.20	45.08±0.19	45.01±0.24	44.84±0.13
6	6	68.83±0.16	68.41±0.27	68.29±0.14	68.02±0.08
7	7	75.97±0.16	75.80±0.20	75.68±0.12	75.49±0.13
8	8	98.09±0.15	97.61±0.15	97.44±0.14	97.03±0.08

All the values were expressed as mean S.D., n*=3

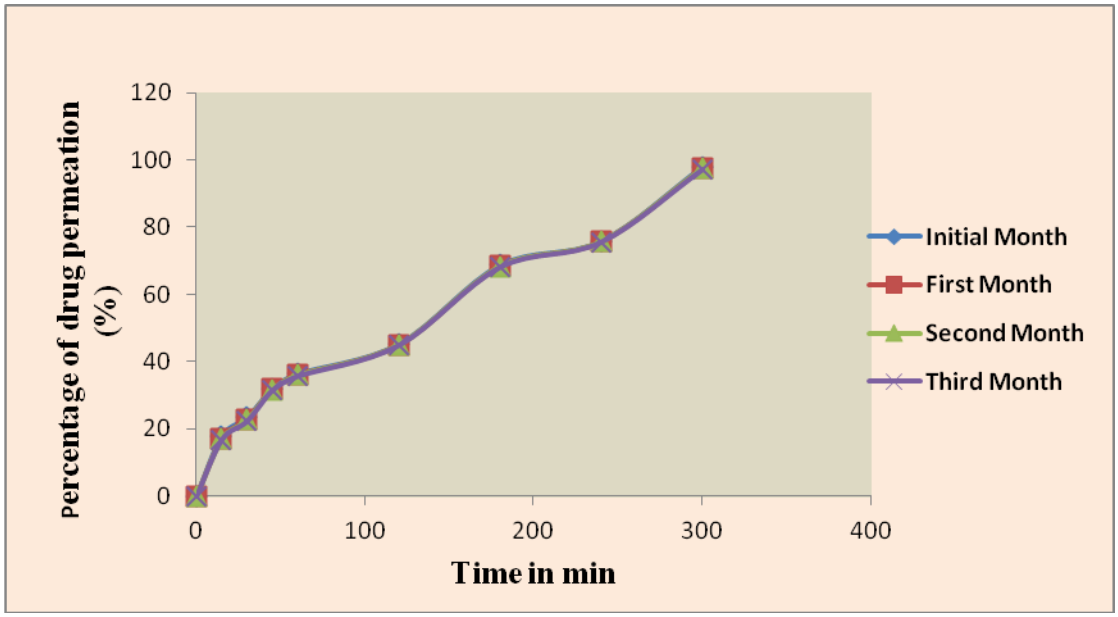


Fig. 9.45: Percentage *in-vitro* drug permeation of selected formulation MG4 after stability studies at 40°C ±2°C at 75% RH ± 5%

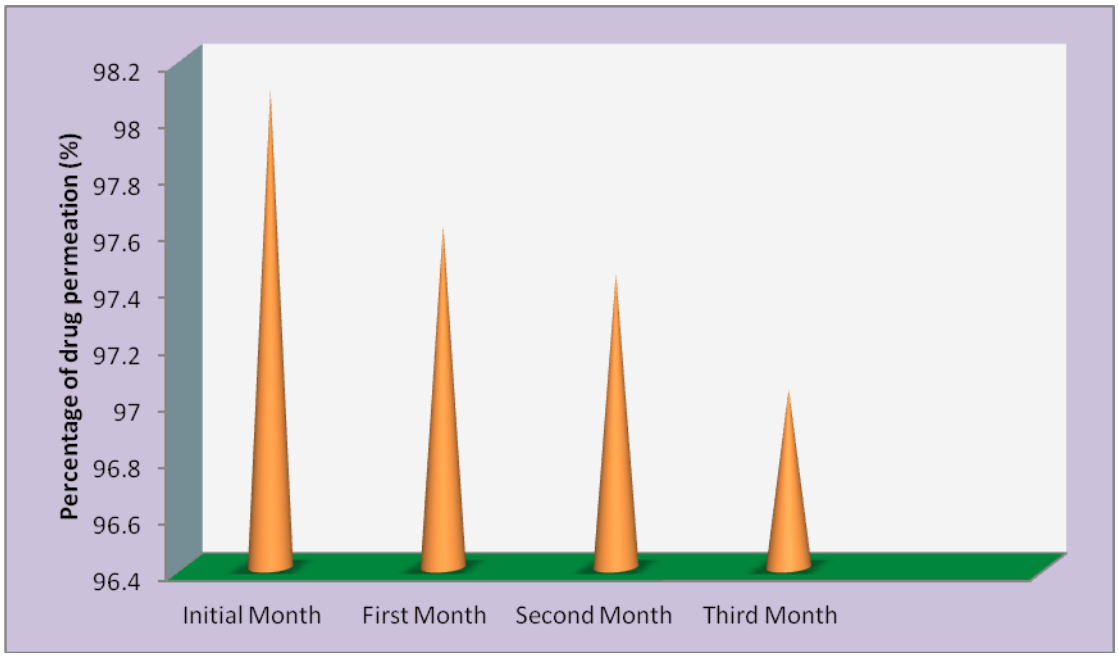


Fig. 9.46: Comparison of *in- vitro* drug permeation before and after stability studies at 40°C ±2°C at 75% RH ± 5% for optimized formulation MG4

The studies revealed that, there were no much significant changes in was intimate between the evaluated data from initial after stability studies of Clarity, pH, Gelation temperature (T1) and gel melting temperature (T2), drug content and *in-vitro* drug permeation studies and all the values were found in worth accepting limits after the stability studies at 40°C ±2°C at 75% RH ± 5% for optimized formulation MG4.

Summary And Conclusion

10. SUMMARY AND CONCLUSION

The present study was aimed to develop a mucoadhesive nasal *in situ gel* delivery system for the treatment of antimigraine activity. Nine batches of mucoadhesive nasal *in situ gels* (MG1, MG2, MG3, MG4, MG5, MG6, MG7, MG8, and MG9) were prepared by using carbopol 974P, PEG 400 and drug with 3^2 factorial design by Cold method.

Preformulation study was carried out for crude drug. The initial part of work was started from the identification of drug. Identification of drug was determined by melting point and solubility.

The compatibility studies by FTIR and DSC analysis of *in situ gels* suggest that the drug Rizatriptan Benzoate with polymers like carbopol 974P, PEG 400 and thermoreversible polymer Pluronic F 127 do not interact to form any additional chemical entity but remain as a mixture. Therefore, it could indicate that there was no incompatibility between drug and polymers. The similarity in peaks indicates there is no incompatibility between drug and the polymers.

The measurement of gelation temperature (T1) and gel melting temperature (T2) were found to be in the considerable range and it showed that addition of mucoadhesive polymer carbopol 974P has decreased T1 whereas addition of PEG 400 increased T1.

pH of the all formulations were found to be within 5.7-6.78 that is between physiological range of pH of nasal mucosa.

The percentage drug content of all the prepared *in situ gel* formulations were checked and found to be in the range of 83.23-98.97%.

Mucoadhesive strength increases with increasing concentration of mucoadhesive polymer. The results of prepared mucoadhesive nasal *in situ gels* indicates that the independent variables carbopol 974P (X1) and PEG 400 (X2) both are having the effect of mucoadhesive strength.

Viscosity measurement of the formulations at various temperatures shows that there was increase in viscosity with increase in temperature. This indicates that temperature induced gel structure formation of pluronic F 127.

From *in-vitro* permeation studies it was concluded that formulation MG4 **(composed of Rizatriptan Benzoate 1%w/v, 18% w/v PF 127, 0.5% w/v carbopol 974P, 6% w/v PEG 400)** found to be best formulation among other formulations, which showing the most desired drug permeation. It will be considered as optimized formulation.

The optimized formulation MG4 was subjected to histopathological and stability studies. The photographs of nasal mucosa after the permeation studies were observed for histopathological study. The section of mucosa treated with optimized formulation showed no degeneration of nasal epithelium along with no signs of erosion.

The studies revealed that, there were no much significant changes in percentage clarity, gelation temperature and gel melting temperature, pH, drug content and *in-vitro* drug permeation studies for three months at 40ºC.

Out of the nine formulations, it appears that **Formulation MG4** has the maximum potential in providing *In situ gel* nasal delivery system. This formulation was considered as best formulation for temperature induced nasal *in situ gelling* system for the treatment of anti-migraine activity with respect to its evaluation parameters like clarity, gelation temperature and gel melting temperature, pH, drug content and *in-vitro* drug release and this formulation may give patient friendly and needle free dosage form.

FUTURE PROSPECTS

11. FUTURE PROSPECTS

In the present work the Temperature induced mucoadhesive nasal in situ gel of Rizatriptan Benzoate were prepared by using carbopol 974P and PEG 400 and with 3^2 factorial design by cold method. In this investigation, the important parameters like physico chemical characterization, *in-vitro* evaluation, histopahhological studiest of mucoadhesive nasal in situ gelling system of Rizatriptan benzoate were done. Further detailed investigation and elaborate in-vivo studies using human volunteers need to be carried out to establish efficacy of this formulation.

In order to have a comparative evaluation of *in-vivo* performance such as pharmacokinetic analysis will be performed on plasma concentration time profiles of this formulation after nasal route of administration, since plasma profile is an important aspect in understanding biological action of a drug. *In-vitro/In-vivo* correlation studies to be performed in future.

In future the long term stability studies will be required to know the shelflife of the prepared muoadhesive nasal *in situ gels*.

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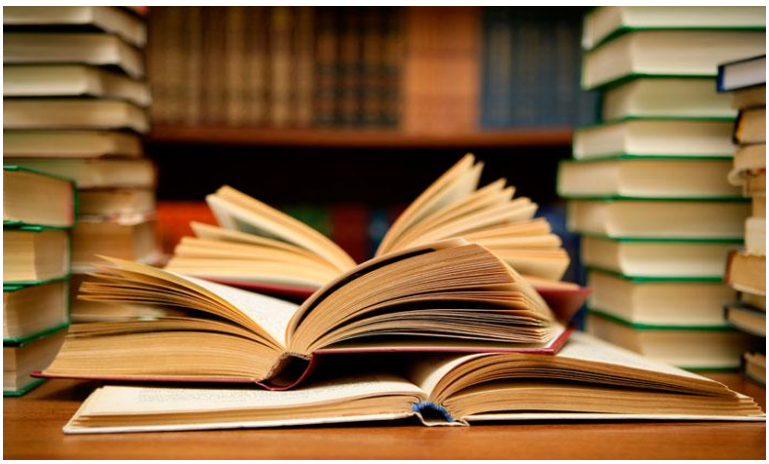

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