# FORMULATION DEVELOPMENT AND EVALUATION OF pH BASED INSITU VAGINAL GEL OF SECNIDAZOLE

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## ABSTRACT

*The aim of the study was to formulate and develop the insitu gel of secnidazole for vaginal route. Secnidazole is a second generation antibacterial drug with fewer side effects than other antibacterial drug. Present formulation is prepared to provide local action. With the help of pH triggered system, the present formulation convert from solution to gel when change occur from normal pH (3.5-4.5) .The insitu gel is prepared by using Carbopol 934 as pH sensitive agent and HPMC K-100M as Mucoadhesive agent. The preliminary trials were performed by using polymer of various concentrations of guar gum, Xanthan gum and Carbopol 934.The preliminary batches were studied for gelation property and selection of polymer is done. The prepared formulations were then evaluated for visual appearance, pH measurement, rheological studies, clarity, gelling capacity and drug content.*

**Keyword :** - *secnidazole, antibacterial, insitu gel, Carbopol 934, local action, Invitro-diffusion*

## 1. INTRODUCTION

### 1.1 Introduction To Vaginal Delivery

Vaginal delivery is a novel type of drug delivery which can act as local as well as systemic action, based on the disease condition and drug characteristic it can be used for long term treatment with the possibility of few side effects. Vaginal drug delivery include certain type of dosage form as Vaginal Rings Vaginal tablet, Vaginal Powder, Vaginal Capsule, Vaginal Ointment, Vaginal Gel, Vaginal Cream, Suppositories[1]. Due to its variety, the large number of formulation has been approached in order to treat the local infection, while most of them sometime cause irritation or discomfort during application. The majorly selected formulation for vaginal drug delivery is Insitu gels. It act by changing its state into gel after application, while remain liquid in idle state. It acts by several mechanisms as change in pH, temperature, stimuli response, ionic enzyme etc. Its thixotropic properties occurs because of presence of such polymer which can form conversion of liquid state into semisolid form, due to its interchangeability the bonding cause formation of weak charge which uphold the formulation and after application the changes occur in formulation due to interaction with body and result in release of api from polymer and provide particular therapeutic activity for local area or certain area of the vagina.

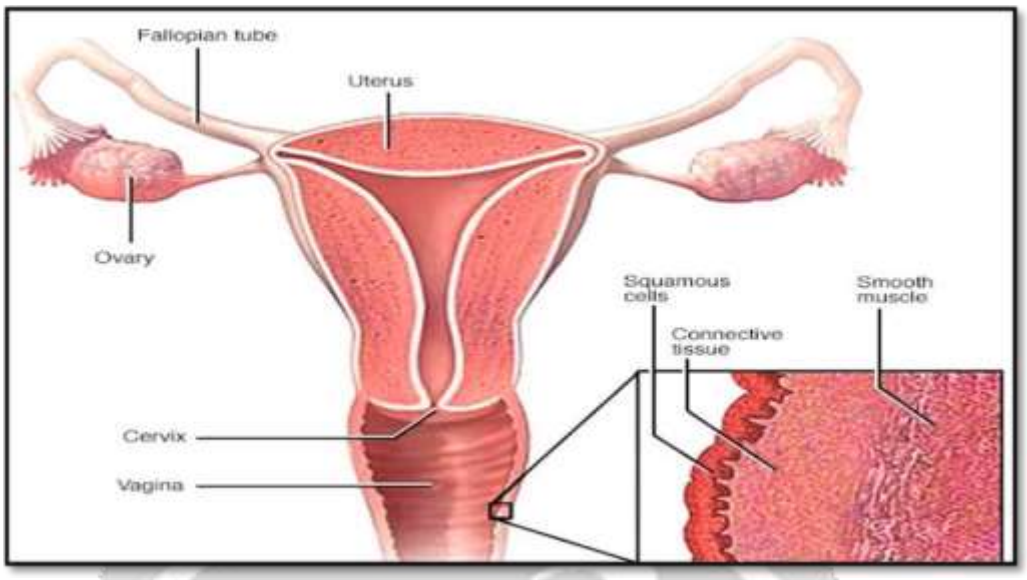


**Fig-1: Anatomy of female reproductive system**

### 1.2 Advantages Of Vaginal Delivery

- Convenient route for prolonged dosing.
- Avoidance of first pass effect.
- Avoidance of enzymatic deactivation in GIT.
- Non –invasive delivery
- Large permeation area, rich vascularization and comparatively low enzymatic activity.
- Self insertion and removal of the dosage form like vaginal films, gels, pessaries, etc.
- Avoid side effects like nausea and vomiting which is Observe after oral administration.

Rapid drug absorption and Quick onset of action

### 1.3 Disadvantages of Vaginal Delivery

- Cultural sensitivity
- Personal hygiene
- Gender specificity
- Local irritation
- Menstrual cycle-associated vaginal changes
- Sometimes leakage of medicine from vagina and wetting of under garments.
- Once the drug administered cannot be removed.

### 1.4 Factor affecting absorption

- Cyclic changes affect in thickness in vaginal epithelium
- Fluid volume
- Viscosity and composition
- pH
- Pathological condition
- Molecular weight
- Lipophilicity
- Ionization
- Surface charge
- Chemical nature (Aliphatic or Aromatic)

### 1.5 Vaginal Insitu Gel

In order to reduce the problem for the absorption and concentration of drug, the various methods were studied and apply. It was found out that insitu gel was one of the novel drug delivery system [2]. It is a kind of drug delivery system which is in solution form before administration in body, but undergoes change in gel form after application. In situ gel formulation, the conversion of liquid state occurs in to semisolid form which acts as Mucoadhesive reservoir.

### 1.6 Advantages of vaginal In situ Gel

- Increased residence time of drug in vaginal cavity.
- Decreased frequency of drug administration.
- Results in quick absorption and high onset.
- Avoids GIT drug degradation due to acidic or enzymatic degradation.
- Reduced dose concentration of drug.
- Improved patient compliance.

Secnidazole is a broad spectrum antibacterial drug which acts against gram-positive and -negative bacteria including Trichomonas Vaginalis, Giardia Duodenalis, Entamoeba Histolytica, Bacteriods Fragillis, Bacteria, and Protozoa.

## 2.0 METHODOLOGY:

**Material:** Secnidazole was gifted from Aarti drugs Limited. Boisar unit-II. Carbopol 934 was obtained from Avansecure life science, haryana. HPMC K100M was obtained from Rettenmaier, Mumbai. All the chemicals used are of analytical grade.

### 2.1 Method:

**Formulation of *in-situ* Gels**: Weigh the required amount of Polymers HPMC K100-M and dissolve in distilled water and allow it to soak for 24hr and then add drug secnidazole. Prepare another solution by dissolving preservatives as propyl Paraben and Gelling agent Carbopol 934 in Separate Beaker. Heat and Agitate above solution on Magnetic stirrer for 15 to 20 min at room temperature. Mix both the solution on magnetic Stirrer. Cool the above formulation on ice bath for 20 to 25 mins to form Gel. Nine batches were selected with varying concentration of Carbopol 934 and HPMC K100M as described in **Table 1**. [3]

**Table-1: Formulation of *In-situ* vaginal gels of secnidazole**

| Formulation | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| Secnidazole | 1% | 1% | 1% | 1% | 1% | 1% | 1% | 1% | 1% |
| Carbopol 934 | 0.2 | 0.4 | 0.6 | 0.2 | 0.4 | 0.6 | 0.2 | 0.4 | 0.6 |
| HPMC K100-M | 0.2 | 0.2 | 0.2 | 0.4 | 0.4 | 0.4 | 0.6 | 0.6 | 0.6 |
| Propyl Paraben | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Distilled water | Q.S | Q.S | Q.S | Q.S | Q.S | Q.S | Q.S | Q.S | Q.S |

### 2.2 Determination of λmax

#### 2.2.1 Preparation of Standard Stock Solution

In order to confirm λmax of Secnidazole, 100 mg of Secnidazole was weighed accurately and transferred to a 100 ml of volumetric flask. The volume was adjusted to 100 ml with suitable solvent to get a 1000 µg/ml stock solution. From stock solution (1000 µg/ml), 100 µg/ml working solution was prepared by diluting 10 ml of stock solution with 100 ml solvent in volumetric flask. The further stock solution was diluted appropriately, which was then analyzed by UV-visible double beam spectrophotometer between 200 to 400 nm against suitable solvent as blank solution.

### 2.2.2 Preparation of calibration curve of Secnidazole in Borate buffer pH 8.2

From stock solution (1000 µg/ml), 100 µg/ml solution was prepared by diluting 10 ml of stock solution with 100 ml Borate buffer pH 8.2 in volumetric flask. Accurately measure standard working sample solutions of Secnidazole (0.5,1.0,1.5,2.0,2.5 and 3.0ml) were transferred to a series of 10 ml of volumetric flasks and diluted to obtain the concentration of 05,10,15,20,25 and 30 µg/ml. The absorption of prepared Secnidazole solution was measured at 321 nm using UV-visible spectrophotometer against Water as blank. The experiment was performed in triplicate and based on average absorbance obtain the equation is generated. [4]

## 2.3 Evaluation of Insitu-Gel:

### 2.3.1 Appearance:

The prepared gel formulations were inspected for visual appearance (color, consistency, and homogeneity) under black and white background by using clarity test apparatus and it was graded as follows; Turbid +, Clear ++, Very clear (glassy) +++.

### 2.3.2 Viscosity measurement

The viscosity of gels was determined using Brookfield viscometer with spindle S96 at 37 ± 2 °C at 10rpm.Repeated procedure three times for each gel.

### 2.3.3 Determination of pH

Accurately weighed gel was dispersed in 10 ml of distilled water and pH value of the dispersion was measured by a digital pH meter at 37 ± 2 °C. Repeat procedure three times for each gel.

### 2.3.4 Gelation Study

- Gelation is the process in which the liquid phase makes a transition to gel. A 10 mL transparent vial containing a magnetic bar and 5 mL of each formulation was placed on a magnetic stirrer.
- The basic Naoh solution was added drop by drop slowly while stirring.
- The Gelation point was determined when the magnetic bar stopped moving due to Gelation. The consistency of formed gel was checked and graded as indicated in **Table 2.0.** [5]

**Table-2: Degree of Gelation**

| Indication | Grade of Gelation |
|---|---|
| - | **No Gelation** |
| + | **Weak Gelation** |
| ++ | **Rapid Gelation remains for few hrs** |
| +++ | **Rapid Gelation remains for extended period** |
| ++++ | **Very stiff gel** |

### 2.3.5 Gel Strength

- A sample of (20 g) was placed in a 100 ml graduated measuring cylinder.
- The weight of 15 g was then placed onto the disk whose diameter was 2.3 cm; clearance from side wall of cylinder 0.4 cm, thickness 0.5 cm and this disk was put onto the gel. The gel strength determined by (seconds) time for movement of piston 5 cm down through the gel.[6]

### 2.3.6 Mucoadhesive Strength

- Modified physical balance used for Mucoadhesive property. Buccal goat mucosa was used as biological membrane, which was fixed under one pan of the balance with the help of thread and was hydrated with Phosphate Borate buffer pH 8.2.
- To the inverted beaker (250 ml) weighed amount of 1 gram of gel was stuck using glue and a glass container below the pan where membrane was threaded, the height of balance was adjusted. A preload of 1gram was applied in order to allow the formulation of Mucoadhesive joints. Period of 3 minute was given, then the preload was abolished and the weight to other pan added up to the point where gel was removed from the mucosal surface.
- The total weight recorded for complete detachment of the gel. The Mucoadhesive force measured in dynes/$cm^2$ was judged from the lowest weight that required removing the mucosal tissue from surface of each formulation [7].

**Mucoadhesive Strength (dynes/$cm^2$) = mg/A**

Where, m: weight required for detachment in gram
g: acceleration due to gravity (980 $cm/s^2$)
A: surface area of mucosa exposed ($cm^2$)

### 2.3.7 Drug content study

The formulation equivalent to 10 mg of drug was weighed and then transferred to 10 ml volumetric flask containing distilled water. The flask was shaken to dissolve the drug and volume was adjusted with distilled water. Absorbance of resulted solution was measured at λmax 321nm in UV-visible spectrophotometer and concentration of drug was calculated. Repeat procedure thrice for each gel.[8]

### 2.3.8 *In-vitro* drug diffusion study

- *In-vitro* drug diffusion study was studied by Franz diffusion cell. Diffusion membrane (goat Buccal membrane) was immersed in receptor compartment having Borate buffer (pH 8.2) as diffusion medium, maintained at 37 ± $2^0$C for overnight for equilibrium.
- Diffusion cell was assembled on magnetic stirrer along with diffusion membrane, which separated donor and receptor compartments. Weighed amount of gel (1 gm) was placed on membrane in donor compartment. The contents were stirred using magnetic stirrer at 50 rpm and aliquots each of 1 ml were withdrawn from the release medium at time intervals 15,30 min and 1hr. Withdrawn samples was replaced by equal volumes of same fresh medium. Absorbance of these samples was measured spectrophotometrically by UV-Visible double beam spectrophotometer at 321 nm.[9][10]

## 3. RESULT:

### 3.1 Calibration curve of Secnidazole:

Secnidazole shows maximum absorbance at 321nm in Borate Buffer 8.2 respectively and shows linearity in range of 5-25µg/ml for Borate buffer 8.2.

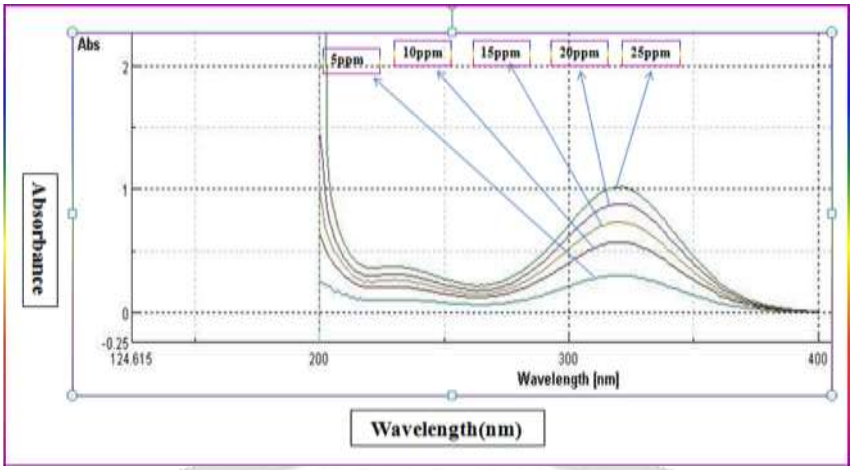


**Fig-2: UV Visible spectra of Secnidazole at 321nm**

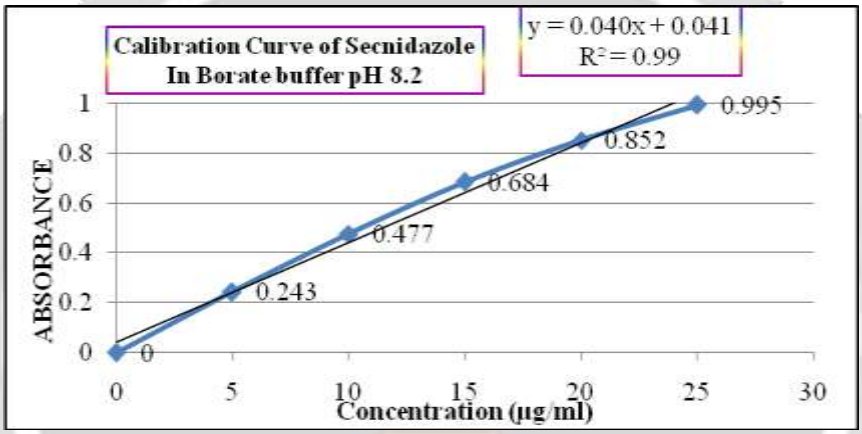


**Fig-3: Calibration curve of Secnidazole at 321nm**

**Table-3: Absorbance at 321nm**

| SR.NO | Concentration(µg/ml) | Absorbance (mean) (n=3) |
|---|---|---|
| **1.** | 0 | 0 |
| **2.** | 5 | 0.243 |
| **3.** | 10 | 0.477 |
| **4.** | 15 | 0.684 |
| **5.** | 20 | 0.852 |
| **6.** | 25 | 0.995 |

### 3.2 FT-IR studies:

IR spectra of drug were shown as the peaks obtained in the spectra of drug correlates with functional groups of Secnidazole which confirms purity of drug. All the characteristic peaks respective to their functional groups of drug are shown and comparison of graph done which reveal no interaction with polymer and drug mixture. Figure 4, 5, 6 and 7 are shown.[7][8].

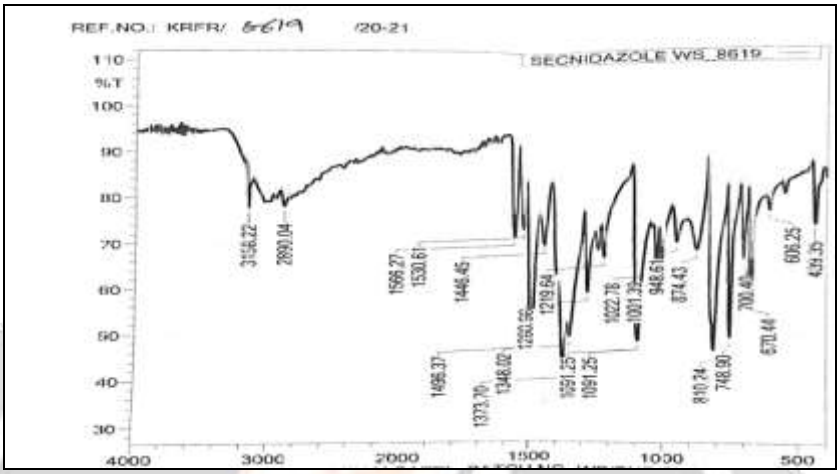


**Fig-4:FT-IR Spectra of Secnidazole**

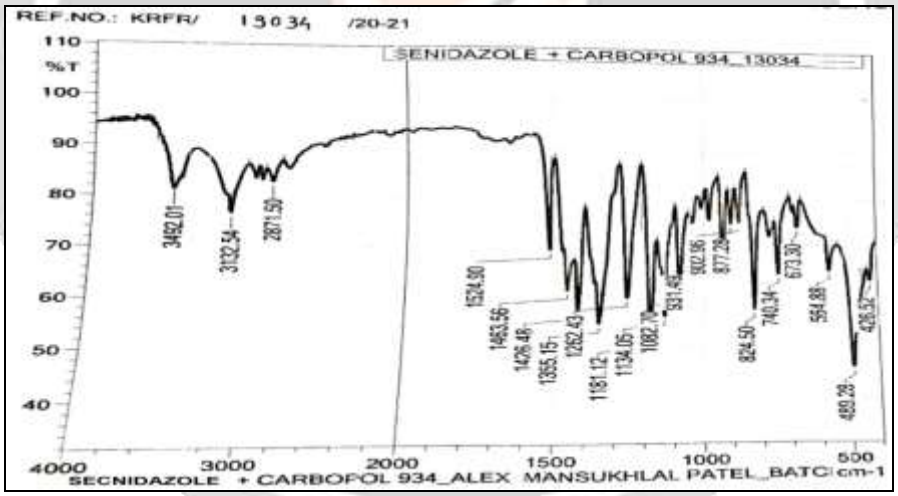


**Fig-5: FT-IR Spectra of Secnidazole with Carbopol 934**

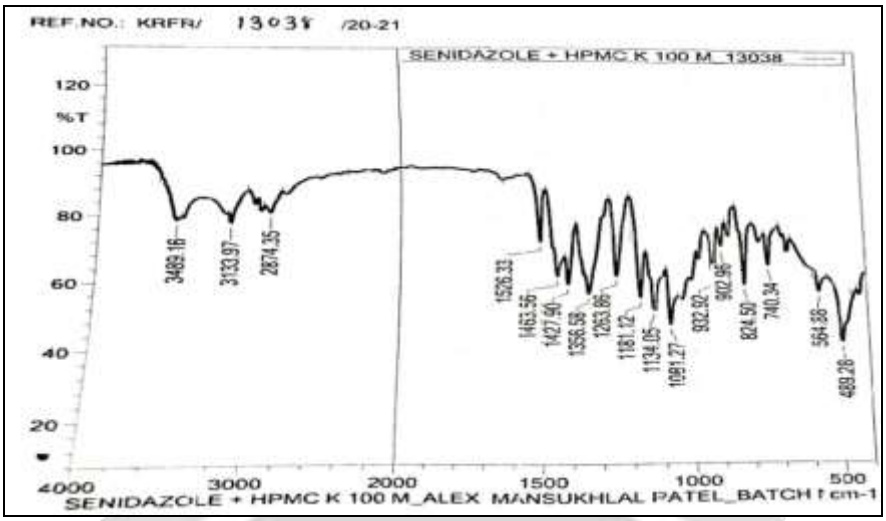


**Fig-6: FT-IR Spectra of Secnidazole with HPMC K100-M**

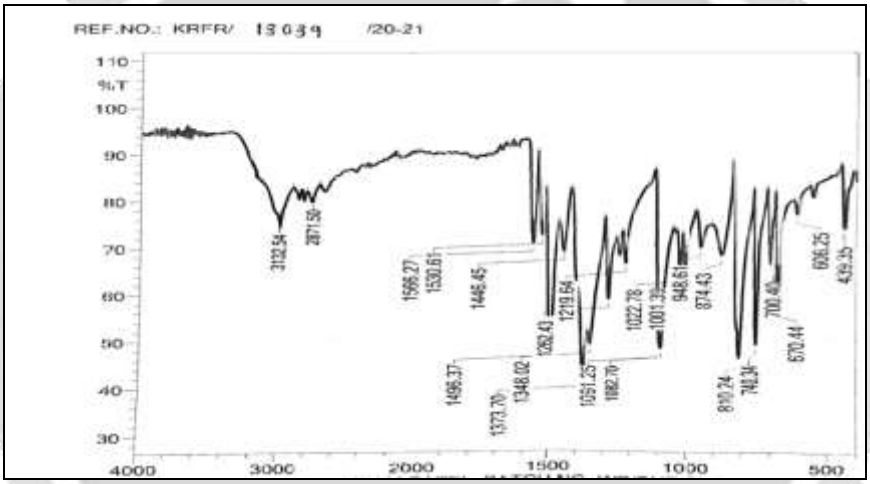


**Fig-7: FT-IR Spectra of Secnidazole with all excipients**

### 3.3 Physical Examination:

All the formulations were translucent in color and were found to be clear without impurities. The pH of the formulations was found to be in the range of 5.1 to 5.5. All these observations are shown in **Table 4**.

### 3.4 Gelling Capacity:

Gelling capacity is coded as described in **Table 4**.

Formulation F1 and F2 shows no gelation, and while formulation F9 shows immediate gelation and remained for a longer time.

### 3.5 Drug Content Estimation:

The % drug content was found to be in the range of 91.57% to 97.30%.

### 3.6 Gelling strength:

the gelling strength observes in F8 shows optimum result as compare to other formulation F1 and F4.

### 3.7 Viscosity:

The formulation F3 shows low viscosity in solution and gel while formulation F9 shows high viscosity.

### 3.8 Mucoadhesive strength:

The formulation F1 shows highest Mucoadhesive strength while, formulation F9 show lowest value. The Formulation F8 shows optimum result.

### 3.9 %Drug release:

The formulation F2 shows low drug release up to 80% while formulation F9 shows greater drug release of 92%.

**Table-4: evaluation data of batches**

| | | At pH 8 | | | | At pH 9 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Formulation No | | F1 | F2 | F3 | F4 | F5 | F6 | F7 | **F8** | F9 |
| Clarity | | clear | clear | Clear | clear | clear | clear | Clear | **clear** | Clear |
| Gelling capacity | | - | - | + | + | + | ++ | ++ | **++** | +++ |
| Gel Strength (Sec) | | 15±1.3 | 18±1.1 | 24±1.6 | 29±1.2 | 36±1.4 | 45±1.7 | 52±1.1 | **57±1.4** | 65±1.5 |
| Viscosity (cps) | **Solution** | 215±4. | 234±2.4 | 256±2.6 | 272±1.9 | 285±3.2 | 294±2.7 | 327±4.2 | **358±4.4** | 430±2.5 |
| | **Gel** | 980±1.5 | 1026±1.2 | 1151±1.8 | 1285±2.1 | 1346±1.9 | 1584±6.4 | 1725±1.7 | **1942±2.8** | 2016-±2.2 |
| Mucoadhesive Strength (Dynes/cm$^2$) | | 3018 | 2754 | 2415 | 2341 | 2214 | 1927 | 1527 | **1340** | 1033 |
| %Drug Content | | 91.57 | 92.61 | 91.67 | 93.41 | 94.59 | 93.28 | 96.29 | **97.30** | 97.68 |

**Table-5: Diffusivity study of factorial Batches**

| | %Drug release | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **At pH 8** | | | | **At pH 9** | | | | |
| Time (min.) | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0** | 0 |
| 15 | 10.89 | 11.53 | 10.21 | 9.65 | 10.54 | 9.87 | 8.35 | **9.39** | 11.38 |
| 30 | 14.2 | 14.38 | 12.42 | 12.42 | 13.27 | 11.53 | 11.28 | **12.02** | 14.27 |
| 60 | 19.67 | 21.06 | 19.63 | 18.25 | 19.83 | 18.59 | 17.46 | **18.76** | 20.45 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 120 | 31.88 | 33.2 | 32.15 | 30.59 | 32.09 | 31.28 | 30.51 | **33.24** | 35.69 |
| 180 | 42.35 | 44.54 | 44.77 | 43.56 | 44.36 | 43.05 | 44.29 | **48.06** | 47.52 |
| 240 | 55.19 | 56.36 | 58.9 | 57.05 | 58.48 | 57.2 | 58.69 | **62.32** | 61.28 |
| 300 | 66.58 | 68.75 | 71.64 | 68.42 | 70.51 | 72.09 | 73.77 | **76.28** | 78.89 |
| 360 | 79.64 | 80.33 | 84.66 | 80.55 | 83.48 | 85.19 | 87.37 | **90.17** | 92.74 |

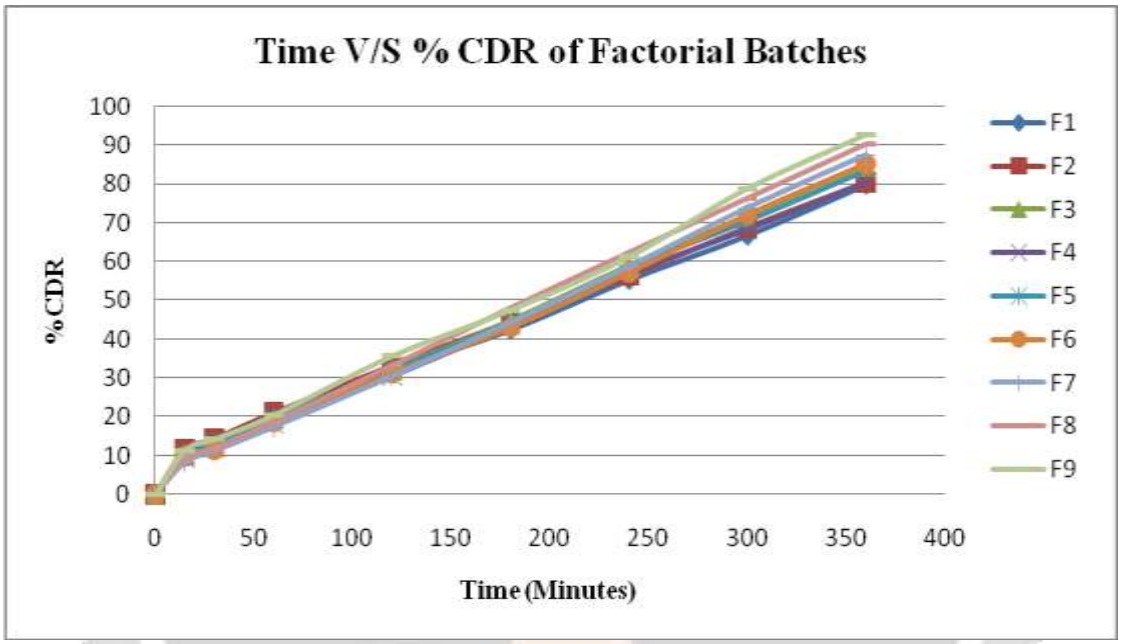


**Figure-8:%CDR of Factorial Batches (diffusion)**

## 4.0 CONCLUSION:

*In-situ* ocular gel of Secnidazole was successfully formulated by using pH-triggered gelation method and was developed to a satisfactory level, in terms of gelling capacity, viscosity, physical appearance, Gelling strength, Mucoadhesive strength and drug content. All formulations are clear and translucent in appearance. Formulation F8 with 0.4% Carbopol 934 and 0.6% HPMC K100-M shows immediate gelation and remained for extended period. Formulation F8 also shows the highest % drug content of 97.30% along with the drug release of 90.17%.

## 5.0 REFERENCE:

**[1].** Vermani, K. & Garg S., "The scope of potential of vaginal drug delivery." Research Focus. **2000**, *3*,359-64.

**[2].** Kalia et al., "Insitu gelling system: A review." *Journal of Drug Delivery & Therapeutics*; **2014**, *4*, 93-103**.**

**[3].** R. Arun Karthick, D. Ramya Devi, B.N. Vedha Hari., "Investigation of sustained release Mucoadhesive in-situ gel system of Secnidazole for the persistent treatment of vaginal infections." *Journal of Drug Delivery Science and Technology.* **2017**, 2-23.

**[4].** Mandal T.K., "Swelling controlled release system for the vaginal delivery of miconazole."*Eur. J.Pharm. Biopharm*, **2000**, *50*,337-43.

**[5].** Das Neves, J.; Amaral, M.H.; Bahia, M.F., "Vaginal Drug Delivery. In Pharmaceutical Manufacturing Handbook: Production and Processes." *Wiley: New York*, NY, USA, **2007**, pp. 809–878**.**

**[6].** Esra Baloglu et al., "Mucoadhesive *in situ gel formulation for vaginal* delivery of clotrimazole: formulation, preparation, and in vitro/in vivo evaluation" Pharmaceutical development and Technology. **2017**,*22*,*4*, 551–561.

**[7].** Himanshu gupta et al., "Sustain ocular drug delivery from temperature and pH triggered novel in situ gel system" *Informa healthcare* **2008**, *14*, *8*,*507*-515.

**[8].** Haiyan Hu., "Preparation and characterization of bear-bile loaded pH sensitive in-situ gel eye drops for ocular drug delivery." *Iranian journal of basic medical sciences.* **2020**, *23*, 922–929.

**[9].** Neha Bisht et al, "Preparation and evaluation of insitu topical gel of levofloxacin." *Indian Journal of Drugs* .**2014**, *2, 4*, 142-151.

**[10].**Varsha Gaikwad et al, "Formulation and evaluation of *In –Situ gel of metoprolol tartrate* for nasal delivery." *Journal of Pharmacy Research*.**2010**, *3, 4*, 788-793.