International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

Formulation Design and Evaluation of Clindamycin Microspheres in a Bioadhesive Gel for Local Therapy of Vaginal Candidiasis.

Dr. Shajan Abraham[1], Aswathy Sasi[2], Feba Elsa Mathew[*3], Dr. Elessy Abraham[4]

[1]*Head of the Department, Nazareth College of Pharmacy, Othera P. O, Thiruvalla, Kerala, 689546, India*
[2]*Student, Shree Devi College of Pharmacy, Kenjar Airport Road, Dakshina Kannada, Mangalore, Karnataka, 574142, India*
[3]*Student, Nazareth College of Pharmacy, Othera P. O, Thiruvalla, Kerala, 689546, India*
[4]*Principal, Nazareth College of Pharmacy, Othera P. O, Thiruvalla, Kerala, 689546, India*
**Corresponding Author: Feba Elsa Mathew*

Date of Submission: 15-09-2021 Date of Acceptance: 28-09-2021

ABSTRACT
The purpose of the study was to design a novel vaginal drug delivery system of Clindamycin microspheres incorporated in a bioadhesive gel. Microspheres were prepared by a solvent evaporation method using Eudragit RS100 and Eudragit RL100 polymers with different drug-polymer ratios. The microspheres were found to be discrete, spherical with free-flowing properties and evaluated for particle size analysis shape (Scanning Electron Microscopy), drug entrapment efficiency, percentage drug loading, and in vitro drug release studies. The selected microsphere formulation (F7 and F8 containing drug-polymer ratio 1:1:1 and 1:0.25:1.75 respectively) was incorporated in bioadhesive Carbopol 93Pgel. Both the gel formulation was evaluated for in vitro release studies. The formulation (F8 CD-MG) which showed a maximum of 91.03% release at 8h was then subjected to stability studies and anti-microbial activity. The antimicrobial activity of F8 CD-MG and placebo gel was evaluated against Candida albicans J1012 by using the cup plate method. The result showed that CD-MG was capable to control the growth of Candida albicans for more than 14h. Placebo gel did not show any zone of inhibition. Stability studies were done as per ICH guidelines for a period of 6 months. Initial and third-month studies were done and evaluated for parameters such as pH, drug content, drug content uniformity, extrudability, spreadability, viscosity and in vitro drug release. The result showed that there were no significant changes in drug content and in vitro drug release. It may conclude from the present study that CD-MG can be used as a novel delivery system for local therapy of vaginal candidiasis.

KEYWORDS: Bioadhesive gel, Clindamycin, Microsphere, Vaginal Candidiasis.

I . INTRODUCTION

A well-designed controlled drug delivery system can overcome some of problems of conventional therapy and enhance therapeutic efficacy of the given drug. The main goal of drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and then maintain the desired drug concentration [1]. The drug must be delivered for a prolonged period and many mediums must be taken simultaneously in case of chronic patients. Drugs have short half-lives, and this leads to decrease in patient compliance. To overcome these problems various types of controlled release dosage forms are formulated. So patient compliance increase through prolonged effect and adverse effect decreases by lowering peak plasma concentration.

Microspheres are defined by a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles having a size range of 1-1000µm[2,3]. The spherical shape of microspheres also increases the surface area which increases the bioavailability of dosage form [3]. It provides constant and prolonged therapeutic effect and reduced GI toxic effect and dosing frequency. Therefore, patient compliance is increased. They could be injected into the body due to spherical shape and smaller in size. The morphology of microsphere allows a controllable

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

variability in degradation and drug release [4]. Microspheres have many applications in medicine with the main use being for the encapsulation of drugs and proteins. By active means or passive means the drug loaded microspheres are delivered to the target area and the encapsulated drug release slowly over desired time. Ideal microspheres have (a) controlled particle size and dispersibility in aqueous vehicle injection, (b) biocompatibility with a controllable biodegradability, (c) susceptibility to chemical modification, (d) ability to incorporate reasonably high concentration drug.

Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules. The diameter of microcapsules between a few millimetres. The material place inside the microcapsule is referred to as the core and the wall is sometimes called a shell, coating, or membrane. Microencapsulation is also defined by which solids, liquids and gases may be enclosed in microscopic particles which results in the formation of thin coating of wall material around the substance [5].

In solvent evaporation method, a core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. The process is carried out in a liquid manufacturing vehicle. Therefore, appropriate size microcapsule is formed by dispersing the solvent for the polymer solution, polymer shrinks around the core. The core material may be either water soluble or water insoluble materials. The capacity of continuous phase is insufficient to dissolve the entire volume of dispersion to obtain hardened microsphere.

The term bioadhesion refers to the phenomenon where natural and synthetic adhere to biological surfaces. It can be epithelial tissue or mucus coat on the surface of a tissue. Bioadhesive drug delivery system prolongs the residence time at the site of drug absorption [6,7].

Vaginal drug delivery system refers to the delivery of drugs within or through the vaginal mucosa for local or systemic pharmacological action. Vaginal cavity is an important area of the reproductive tract. The vagina has a great potential for systemic delivery of wide range of compounds including proteins and peptides. Creams, tablets, gels, suppositories, ointments, are commonly used as vaginal drug delivery systems.

Bioadhesive vaginal delivery systems have several advantages when compared to conventional dosage forms. The formulation is readily localized in the region of application thus improving the bioavalability of drugs. Then the delivery systems provide intimate contact by the formulation with the underlying absorption surface. It permits continuous and prolonged residence of the dosage form at the site of application. At last, it reduces side effects due to avoidance of repeat administration of the drug [8]. A bioadhesive formulation not necessarily contains a therapeutic agent and can be used as a moisturizer for the treatment of dry vagina. Several bioadhesive polymers have been reported for different mucosal sites such as the buccal cavity, stomach, and intestine. In most of vaginal preparations, either Carbopol or polycarbophil has been used as the bioadhesive polymer [9].

Vaginal Candidiasis is a most fungal disease and one of the most common vaginal infections in women, in fertile period. Many psychological and emotional stress related problems are associated with vaginitis such as reduced immunity, prolonged antibiotic therapy, use of contraceptive, malnutrition, pregnancy, obesity. The infectious vaginitis is of three types: Candidiasis, trichomoniasis, and bacterial vaginosis [10]. Vaginitis is defined as a spectrum of conditions that cause vaginal spectrum of conditions that cause vaginal and sometimes vulvar symptoms, such as itching burning, irritation, odour, and vaginal discharge [11].

Candida albicans is most important cause of vaginal candidiasis [12,13]. During pregnancy vaginal secretions fall from a pH of greater than 7(an alkaline pH) to 4 or 5 (an acid pH). It is believed that higher oestrogen level and higher glycogen content in vaginal secretions during pregnancy increases in women's risk of developing vulvar vaginal candidiasis.

A gel is a solid jelly like material that can have properties ranging from soft and weak to hard and tough. Gels are defined as a substantially dilute cross-linked system which exhibits no flow when in steady state. There are hydrophilic and hydrophobic gels. Ideal gel should not be tacky. Higher molecular weight gel is difficult to dispense and applied. It should exhibit little viscosity, changes under the temperature variation of normal use and storage. Medicated gels used by various routes of administration including skin, nose, eye, vagina, and rectum [14].

Polymers are essential for preparation of gels forming polymers are classified as: natural polymers (gelatin, agar), semi-synthetic polymer (methyl cellulose, hydroxyethyl cellulose), synthetic (carbomer, poloxamer), inorganic substance (bentonite, aluminium hydroxide) and

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

surfactants (Brij- 96, Cetostearyl alcohol) [15]. Thermal changes, flocculation, and chemical reaction of polymer cause formation gels [16].

The present work is to formulate and evaluate Clindamycin microspheres in a bioadhesive gel for local therapy of vaginal candidiasis. Vaginal infection can be diagnosed by clinical examinations, pH measurement and microscopic examination of vaginal discharge. The conventional formulations of vaginal drug delivery systems (VDDS) are associated with poor retention due to self-cleansing action of vaginal tract. Leading to poor compliance. To overcome these problem bioadhesive polymer are added to VDDS. Topical Clindamycin tented to cause a lower rate of adverse effects (metallic taste in the mouth, nausea, vomiting) than oral Metronidazole. The antibiotic binds preferentially to the 50S ribosomal subunit at the level of the bacterial ribosome. Objective of the study include: preformulating studies, preparation, and evaluation of Clindamycin microspheres, formulating Clindamycin microsphere using bioadhesive gel and analysing data statistically [17].

Ⅱ. MATERIALS AND METHODS

Materials:

Clindamycin (Yarrow Chem Product, Mumbai), Eudrajit RS100 (EvonicDeggusa PVT LTD, Mumbai), Eudrajit RL 100 (EvonicDeggusa, PVT LTD, Mumbai), Magnesium Stearate (Loha chemic PVT LTD, Mumbai), Liquid Paraffin (Nice Chemicals PVT, LTD, Kerala), n-hexane (Loba Chemic PVT, LTD, Mumbai), Acetone (Loba Chemic PVT, LTD, Mumbai).

Preformulation study

Solubility:

Preformulation solubility analysis was done to select a suitable solvent system to dissolve the drug and to test its solubility in the dissolution medium which was to be used. The solubility of clindamycin in 10µg/ml of different solvent was carried out.

Determination of λmax:

A solution of clindamycin containing concentration of 10µg/ml was prepared in methanol and UV spectrum was taken using Shimadzu (UV1800) double beam spectrometer and scanned between 200-400 µm. The maxima obtained in the graph were considered as λmax for drug Clindamycin.

Sample preparation and analysis by FTIR:

IR spectra of drug and excipients were taken using FTIR spectrophotometer (Jasco 4100 type A). The drug and polymer were mixed physically in 1:1 ratio and mixtures were stored in an oven at 40°C and 75% relative humidity in closed containers for one month. FTIR spectrum of samples was taken by using KBr pellet method [18].

Sample preparation and analysis by DSC:

The samples were prepared by physical mixture of drug and excipients (1:1) using a clean dried glass mortar and pestle. Sample 5 – 10 mg were accurately weighed and hermetically sealed in aluminium pans. Thermograms were obtained using Shimadzu (DSC 60) instrument, heating at a constant rate of 10°C/ min, over a temperature range of 40 – 600°C. To maintain an inert atmosphere nitrogen gas was purged at a rate of 10ml/min [19].

Development of Analytical Method

Preparation of stimulated vaginal fluid:

Composition of stimulated vaginal fluid shown in **(Table 1)**. The pH of mixture was adjusted to 4,2 using 0.1m HCl [20].

Standard calibration curve for Clindamycin 4.2 SVF:

Accurately weighed quantity of 100mg Clindamycin was dissolved in 5ml methanol using 100ml volumetric flask and volume was made up to 100ml with 4.2 pH SVF to produce 1000µg/ml of stock solution. 5ml of stock solution pipette into 100ml volumetric flask and the volume was made up using 4.2 SVF. This gives concentration of 50µg/ml. 0.6,1.0, 1.4, 1.8, 2.2, 2,6, and 3.0ml stock solution were pipette out in 10ml volumetric flask and volume was made up using pH 4.2 SVF. This solution gives concentration of 3,5,7,9,11and 15 µ/ml of solution of Clindamycin. The absorbance was measures in UV-Visible spectrophotometer (Shimadzu 1800) at 207nm using 4.2 pH SVF as blank. **(Figure. 1)** show the calibration curve. Data are mentioned in the **(Table. 2)**.

Method:

Preparation of microspheres by solvent evaporation

Clindamycin microspheres were prepared by solvent evaporation method using Eudrajit RS100 and Eudrajit RL100 with different drug/polymer ratios. Weighed quantity of polymer was dissolved in 30ml of acetone with stirring 1g of drug and 100mg Magnesium Stearate dispersed in polymer solution. The resultant milky white dispersion was poured into a vessel containing a mixture of 270 ml liquid paraffin and 30 ml of n-hexane. Then stirred for 5h using a homogenizer fitted with a four blade “butterfly” propeller with a diameter of 50mm, stirring was continued for 3h at 1000rpm or until the acetone was completely

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

evaporated [21]. Following removal of acetone, the resultant microspheres were harvested by vacuum filtration after which they were washed four times with 25ml of n-hexane and dried at room temperature for 24 h, Formulation for Clindamycin microspheres are shown in **(Table 3)**.

Preparation of Gel:

Accurately weighed quantity of 1g Carbapol 934P was dispersed in 88g of distilled water by continuous stirring for 15 – 20 min with the help of glass rod in which 10g of glycerol was previously added. Mixture was stirred until thickening occurred and neutralized by dropwise addition of 50% w/w triethanolamine. Mixing was continued until a transparent gel appeared [21].

Incorporation of Microspheres in a Gel:

Microsphere containing Clindamycin incorporated into the 1%w/w Carbopol 934 gel. Mixing well by using an electrical mixer at 25 rpm for 2min to get Clindamycin microsphere incorporated gel [21].

Ⅲ. EVALUATION

Evaluation of Microspheres:

Appearance:

The general appearance of microsphere was identified visually. It includes size, shape, colour, presence or absence of an odour, taste, surface texture.

Particle Size:

Particle size analysis carried by optical microscopic method. A minute quantity of microspheres was dispersed in glycerine and then spread on clean glass slide and average size of 100 microspheres was determined in each batch.

Percentage yield:

To know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method or production. Practical yield was calculated as the weight of Clindamycin microspheres recovered from each batch in relation to the sum of starting material

The percentage yield of prepared Clindamycin microsphere was determined by using the formula.

Percentage yield = (Actual weight of product / Total weight of excipients and drug) × 100

Determination of percentage drug entrapment efficiency (PDE):

Each batch was calculated in terms of percentage drug entrapment as per the following formula.

PDE = (Practical drug content / Theoretical drug content)×100

Theoretical drug content was determined by calculation assuming that the entire Clindamycin (CD) present in the polymer solution used gets entrapped in CD microspheres. No loss occur at any stage of preparation of CD microspheres and the percent drug loading was calculated by using the formula.

% loading = (Weight of drug/ Weight of microspheres) ×100

Practical drug was determined by weighing CD microspheres equivalent to 100mg of CD, dissolved in 100ml pH 4.2 SVF. This solution was kept overnight for the complete dissolution of the CD in SFV. This solution was filtered and further diluted to make a concentration of 10 µg/ ml solution. The absorbance of the solution was measured at 207 nm using UV-Visible spectrophotometer against SVF as blank and calculated for the percentage of drug present in the sample [22,23].

Scanning Electron Microscopy (SEM):

It is used to determine particle size distribution, surface topography, texture and to examine the morphology of the fractured section surface. SEM studies were carried out by using the JEOL JSM 6380 LA (Japan). Dried Clindamycin microspheres were placed on an electron microscope brass stub and coated with an iron sputter. Picture of clindamycin microspheres were taken by random scanning of the stub [24].

Procedure for in-vitro dissolution studies of microspheres:

Clindamycin microsphere containing 100mg equivalent weight of CD were placed in the basket of the dissolution vessel containing 900ml of pH 4.2 SVF as dissolution medium at 50 rpm. The dissolution media were maintained at the temperature of 37±0.5℃. 2ml of the dissolution media was withdrawn at predetermined time intervals and same amount of fresh dissolution media was replaced. The withdrawn samples were filtered through Whatman filter paper, and the absorbance was measured using UV-Visible spectrophotometer at 207nm. Dissolution profile was analysed by plotting drug release versus time plot.

Evaluation of Gel:

Physical appearance:

Clindamycin incorporated gel were visually inspected for colour, clarity, homogeneity presence of particles and fibres.

Determination of pH:

The pH of microsphere incorporated gels were determined by using digital pH one gram of gel was dissolved in 25ml water, and the electrode was the dipped into gel formulation 30 min until constant reading obtained [25].

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

Drug content analysis:
Accurately weighed gel equivalent to 10 mg of drug was suspended in 25ml of SVF. The volume was made up to 100ml. After proper dilution absorbance was measured using UV 207 nm [26].
Drug content uniformity:
To ensure the homogeneity of drug content in the formulation of gel, five tubes were sampled from the different location in the mixer and assayed for the drug content [27].
Extrudability study:
The prepared gel was based upon the quantity of percentage gel extruded from the tube on application of certain load. The formulation under study was filles in a clean, lacquered aluminium collapsible one ounce tube with nasal tip 5mm opening. It was then placed in between two glass slides and was clamped and extrudability was determined by weighing the number of gels extruded through the tip when a constant load of 1 kg was placed on the slides and gels extruded was collected and weighed [28].
Spreadability:
Spreadability of Clindamycin incorporated gel were determined 48h after preparation by measuring two 20×20 glass plates after 1min. The mass of the upper plate was standardized at 125g. To calculate the spreadability by using formula S = ml/t, where S, is spreadability, m is the weight tied to the upper slide, l is the length of glass slide, and t is the time taken [29].

Viscosity Measurement:
A Brookfield (DV-11^{+}) viscometer were used to determine the viscosity of microsphere incorporated gel. The gel was placed in the sample holder and the suitable spindle selected was lowered perpendicularly into the sample. The spindle attached to the viscometer and then it was allowed to rotate at a constant optimum speed at room temperature and readings of the viscosity of the formulation were measures after 2 minutes [30].
In-vitro release studies of gel:
A modified open diffusion cell was used for drug release from the clindamycin microsphere gel. Egg membrane was soaked overnight in SVF before the study. 1g of gel kept carefully between the donor and receptor compartment. The donor compartment as empty and open to the atmosphere but the receptor compartment contained 25ml of pH 4,2 SVF as dissolution medium. The dissolution medium was maintained at 37±0.5℃ and stirred on a magnetic stirrer with a stirring speed of 25 rpm 1ml of dissolution media was withdrawn at predetermined time interval (every 1 hour) and replaced with equal volumes of fresh medium. The absorbance of the samples was analysed by UV-spectrophotometer 207nm [28].
In-vitro antimicrobial activity:
Antimicrobial activity of clindamycin microsphere gel and placebo gel was evaluated against Candida albicans j 10 by using Cup Plate Method. Culture media Sabourand Dextrose Agar (Dextrose 4%), peptone (1%), agar (1.5%), distilled water up to (100ml).
Method: the composition of sabourand's dextrose agar was taken in a 250ml of conical flask and was dissolved in 100ml of distilled water. The pH was adjusted to 5.6. the medium was sterilized in an autoclave at 15 lbs for 15 minutes. After completion of sterilization, the medium was kept aside at room temperature. 0.5ml diluted suspension NaCl 0.9% were added to 100ml of medium at 47±2℃ and used as inoculated layer. The medium (20ml) was poured into a sterilized petri dish to give a depth of 3-4 mm and was assured that the layer of medium of is uniform in thickness by placing petri dish on a level surface. Petri dish was divided into two sectors. After solidifying the medium at room temperature, with the help of a sterile cark borer, cup of 6mm diameter were punched and scooped out from the petri dish. Each bore in different sector was loaded with equal quantity of the placebo gel (gel without the drug) and clindamycin microsphere gel. The petri dish was then incubated for 24 hours at 37℃. After incubation zone of inhibition was measured [31].
Stability studies:
Formulated F8 Clindamycin microspheres incorporated gel was kept at a temperature of 40℃ ±2℃ and relative humidity (RH) 75% RH ± 5% RH for a period of six months. Initial, third month, six month the samples were evaluated for parameters such as colour, pH, extrudability, spreadability, viscosity, drug content and in-vitro drug release [32,33].

Ⅳ. RESULTS AND DISCUSSION

Preformulation study:
Solubility analysis:
The solubility of Clindamycin in 10mg/ml of solvent was carried out and it revealed that it is freely soluble in methanol and alcohol.
Determination of λmax:
A solution of CD containing concentration 10µg/ml was prepared in methanol and absorbance was taken using UV-visible spectrophotometer. The λmax for CD was found to be 207nm.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

FTIR studies:
FTIR studies were carried out to investigate the possible interactions between drug and polymers. IR spectrum for pure drug and physical mixture of drug polymer were obtained and analysed. As shown in **(Figure 2,3).**
DSC studies:
DSC curve obtained for pure CD, Eudragit RS100, Eudragit RL100, and their mixture are shown in **(Figure 4,5)** respectively. Pure CD powder showed a melting endotherm at 88.43℃. Physical mixture of CD and Eudragit RL100 showed melting and endotherm at 93.2℃. These endotherm peaks showed that there is no interaction between the drug and polymer.

Formulation studies:

The popular method of the encapsulation of drugs within water insoluble polymers is the emulsion solvent evaporation method. This technique offers several advantages and is preferred over other preparation method such as spraying, sonication and homogenization because it requires only mild condition such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed without compromising the activity of the drug. Both polymers are soluble in acetone, so acetone was used as solvent. Liquid paraffin was used as dispersion medium. Eight formulations were prepared to investigate the effect of the increasing amount of polymer and dispersing agent on the microsphere formation the drug – polymer and drug dispersing agent ratio was altered while the amount of solvent and stirring speed were kept constant.

Evaluation of microspheres:
Percentage yield:
The percentage yield for CD microspheres were given in the **(Table.4)**. Graphical representation for percentage yield and entrapment efficiency shown in **(Figure 6).**
Percentage drug entrapment efficiency:

Entrapment efficiency increases with increase in polymer concentration. Entrapment efficiency was found to be un the range of 65.12% to 82.10%. the results obtained are given in efficiency was obtained for the formulation F6 which contain 1:3 drug polymer ratio. Actual and theoretical drug loading shown in **(Table. 6)**.
Particle size:

Particle size was highly influenced by the stirring speed and the polymer concentration increased. As shown in **(Table. 5)** the formulation F6 and F3 was higher when compared to all other formulation. It was observed that when the speed of stirrer was below 1000 rpm, there was no formation of spherical microspheres. This could be due to in adequate agitation to disperse the inner phase in the total mass. Therefore, stirring speed was maintained at 1000rpm.
Scanning electron microscopy (SEM):

Scanning electron microscopy of the formulation F8 was carried out. It was observed that the microsphere was spherical in shape with smooth surface. Indicating that CD was well dispersed inside the carried **(Figure.7)**. It is also evident that the microspheres exhibited slightly porous surfaces probably due to high concentration of drug in the microspheres.
In-vitro drug release studies:

The drug release from batches F1-F8 **(Table.7)** indicates that as the polymer concentration is increased the release rate decreased. The formulation F8 containing 1:0.25:1.75 concentration of drug polymer shows maximum of 90.04% drug release at 12 hours compared to other formulations. In-vitro release profile shown in **(Figure. 9)**.

Evaluation of gel:
Drug content and uniformity:
The drug contents of the prepared gels were found to be 65.18% and 80.10% for F7 CD-MG and F8 CD-MG respectively indicating the applications of present method for the preparation of novel Clindamycin bioadhesive gel system with high drug content uniformity.
pH measurement:
The pH of the gels was found to be 6.6 and 6.5 which is within the limit of the semisolid specifications. The almost neutral pH reflected that the gel will be non-irritant to vagina.
Spreadability:
The spreadability plays an important role in patient compliance and helps in uniform application of gel to the skin. A good gel takes less time to spread and will have high spreadability.
Extrudability:
The extrusion of gel from tube is important during application and for the patient compliance. Good extrudability was observed for the prepared gel.
Viscosity:
Viscosity is an important parameter for characterising that gel as it affects the spreadability,

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

extrudability and release of drug. The data are given in **(Table. 8)**.

In-vitro drug release studies:

The in-vitro drug release profile was presented in the **(Table.9)** and **(Figure10)** indicate release from microsphere retarded by incorporating in gel network. The formulation F8 CD-MG containing drug polymer ratio 1:0.25:1.75 showed 90.12% drug release at 8 hours.

Antimicrobial activity:

The antimicrobial activity of CD-MG and placebo gel was evaluated by cup plate method. The placebo gel did not show any zone of inhibition. The zone of inhibition was observed with the CD-MG formulation containing loaded microspheres. Antimicrobial study with the Sabouraud culture showed that the CD-MG was capable of control the growth of Candida albicans for more than 14 hours. Zone of inhibition of CD-MG was found to be 19mm **(Figure. 8)** shows the zone of inhibition.

Stability studies:

Stability studies were done according to ICH guidelines. The prepared gel F8 CD-MG were packed in aluminium collapsible tube and kept at a temperature of 40 ± 2℃ and relative humidity 75% RH ± 5% RH for a period of 6 months. Initial and third month studies were done for the following parameter pH, drug content, drug content uniformity, extrudability, spreadability, viscosity, and in-vitro drug release and results were mentioned in **(Table. 10)**.

CONCLUSION

The preformulation studies like solubility, melting point and UV analysis of CD were compiled with standards. The FTIR spectra reveals that there was no interaction between polymers and CD. Surface smoothness of CD microspheres was increased by increasing the polymer concentration, which was confirmed by SEM. As the drug to polymer ratio was increased, the mean particle size of CD microspheres was also increased. Entrapment efficiencies increase with increase in the polymer concentration. The in-vitro release study revealed that the drug released decreases with an increase in the polymer concentration. The melting point of CD was estimated by DSC and found to comply with the IP standards. The in-vitro release study of the CD-MG revealed that drug release is increased with the optimum concentration of drug polymer ratios. Anti-microbial study with Sabouraud culture shows that the F8 CD-MG was capable to control the growth of Candida albicans for more than 14 hours. Stability studies of the formulation were carried out as per ICH guidelines. The best formulation F8 CD-MG was subjected to stability studies at 40 ± 2℃ / 75% RH ± 5% RH for a period of 6 months. The physical stability was assessed by the appearance and chemical stability by change in the drug content and drug release studies for initial and third month. The results showed that there were no significant changes in the drug content and in-vitro drug release studies. The stability studies will be continued further according to ICH guidelines. From the present study it may be concluded that the ability of the system to adhere to the vaginal mucosa for an extended period as well as to improve the drug availability has great appeal for the convenienttreatment of vaginal Candidiasis.

REFERENCE

[1]. Chowdary KPR, Yarraguntla SR. Mucoadhesive microsphere for controlled drug delivery. Biol Pharm Bull 2004; 1717-24.

[2]. Freiberg, Zhu S. Polymer microspheres for controlled drug release. Int Pharm 2004; 282:1-18.

[3]. Arshady R. Preparation of polymer nano- and microspheres by vinyl polymerization techniques. J Microencapsulate 1988; 5:101-14.

[4]. Chella N, Yada KK, Vempati R. Preparation, and evaluation of ethyl cellulose microspheres containing Diclofenac sodium by novel W/O/O emulsion method. J Pharm Sci and Res 2010; 2(12): 884-888.

[5]. Alagusundaram M, Chetty MSC, Umashankari K, Badarinath AV, Lavanya C, Ramkanth S. Microspheres as a Novel Drug Delivery System- A Review. Int J Chem Tech Res 2009; 1:526-34.

[6]. Bernkop-Schnurch A. Mucoadhesive polymers: strategies, achievements, and future challenges. Adv Drug Del Rev 2005; 57:1553-55

[7]. Khanna R, Agarwal SP, Ahuja A. Mucoadhesive Buccal Drug Delivery: A Potential Alternative to Conventional Therapy. Indian J Pharm Sci 1998; 60(1):1-11.

[8]. Dobaria N, Mashru R, Vadiya NH. Vaginal drug delivery systems. East Cent Afr J Pharm Sci 2007; 10:3-13.

[9]. Vermani K, Garg S. The scope and potential of vaginal drug delivery. Pharm Sci Technolo Today 2000;3(10):359-64.
[10]. Yellanki SK, Nerella NK, Goranti S, Sambit Kumar. Development of Metronidazole intra vaginal gel for treatment of bacterial vaginosis. Int J Pharm Tech Res 2010; 2(3):1746-50.
[11]. Hainr BL, Gibson MV. Vaginitis: diagonosis and treatment. Am Fam Physician 2011; 83(7):807-15.
[12]. Hani U, Shivakumar HG, Bhat SR. Formulation design and evaluation of Metronidazole microspheres in a bioadhesive gel for local therapy of vaginal candidiasis. Lat Am J Pharm2011; 3(1):161-67.
[13]. Patel A, Patel J. Design, development and invitro evaluation of Sertaconazole mucoadhesive vaginal tablet. Der Pharmacia Lettre 2012;4(2):418-27.
[14]. Muir GG. Multiple enzyme studies in the vaginal fluid in relation to benign and malignant gynaecological conditions. British J Cancer 1996; 20:448-62.
[15]. Goldberg DM, Hart DM, Watts C. Distribution of enzymes in human vaginal fluid: relevance to the diagnosis of cervical cancer. Cancer 1968; 21:964-73.
[16]. Esposito E, Menegatti E, Cortesi R. Hyaluronan-based microspheres as tools for drug delivery: a comparative study. Int J Pharm 2005; 288:35-49.
[17]. Bonucci E, Ballanti P, Ramires A, Richardson JL, Benedetti LM. Prevention of ovariectomy osteopenia in rats after vaginal administration of microspheres containing salmon calcitonin. Calcif Tissue Int 1995; 56:274-79.
[18]. Rajamanickam D, Rajappan M, Varadharajan M, Srinivasan B. Formulation, and evaluation of albumin microspheres containing aceclofenac; Int J. Pharm Sci Rev 2010;4(1):112-7.
[19]. Sahoo SK, Dalai SR, Pani NR, Barik BB. Formulation and in vitro evaluation of alginate beads of Aceclofenac by ionotropic gelation technique. Indian Drugs 2007;44(11):324-7.
[20]. Marques MRC, Loebenberg R, Almukainzi M. Simulated biological fluids with possible appilication in dissolution testing. Dis Tech 2011; 22-3.
[21]. Hani U, Surendra RB, Shivakumar HG. Formulation Design and Evaluation of Metronidazole Microspheres in a Bioadhesive Gel for Local Therapy of Vaginal Candidiasis. Lat Am J Pharm 2011;30(1):161-67.
[22]. Dandagi PM, Manvi FV, Gadad AP, Mastiholimath VS, Patil MB, Balamuralidhara V. Microencapsulation of Verapamil hydrochloride by ionotropic gelation technique. Indian J Pharm Sci 2004;66(5):631-5.
[23]. Deore BV, Mahajan HS, Deore UV. Development and characterization of sustained release microspheres by quassi emulsion solvent diffusion method. Int J Chem Tech Res 2009;1(3):634-42.
[24]. Magharla DD, Nandhakumar S, Vankayalu DS, Suresh C. Preparation of poly (epsilon-caprolactone) microspheres containing etoposide by solvent evaporation method. Asian J Pharm Sci 2010;5(3):114-22.
[25]. Sankar V, Chandrasekaran AK, Durga S, Prasanth KG, Nilani P, Geetha G, Ravichandran V, Raghuraman S. Formulation, and stability evaluation of diclofenac sodium ophthalmic gels. Indian J Pharm Sci 2005;67(4):473-76.
[26]. Panigrahi L, Ghosal SK, Pattnaik S, Mahasana L, Barik BB. Effect of permeation enhancer on the release and permeation kinetics of Lincomycin hydrocloride gel formulation through mouse skin. Indian J Pharm Sci 2006:68(2):205-11.
[27]. Hiuguchi T. Mechanism of rate of sustained-action medication. J Pharm Sci 1963;52(11):45-9.
[28]. Gupta GD, Goud RS. Release rate of tenoxicam from acrypol gels. The Indian Pharmacist 2005;69-75.
[29]. Panigrahi, John T, Sharif, Rani S, Hiremath R. Formulation, and evaluation of Lincomycin HCl gels. Indian J Pharm Sci 1992:330-32.
[30]. Padamwar MN and Pawar AP. Preparation and evaluation of sericin gels containing choline salicylate. Indian Drugs 2005;40(9):526-31.
[31]. Staub I, Elfrides ES, Schapoval, Ana M, Bergold. Microbiological assay of ketoconazole in shampoo. Int J Pharm 2005; 292:195-99.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

[32]. Carstensen JT. Drug Stability, Principles & Practices. New York: Marcel Dekker; 1989. p. 17-58.

[33]. Lachman L, Patric D. Kinetic principles, and stability. Theory and practices of Industrial pharmacy. 3rd ed. Philadelphia: Varghese publishing House;1987. p.767

Table1. Composition of stimulated vaginal fluid

Composition (in water)	**g/ml**
Sodium Chloride	3.51
Potassium Hydroxide	1.4
Calcium Hydroxide	0.222
Bovine Serum Albumin	0.018
Lactic Acid	2
Acetic Acid	1
Glycerol	0.16
Urea	0.4
Glucose	5

Table 2. Calibration curve of clindamycin microspheres

Concentration	**Absorbance**
0	0
3	0.13
5	0.184
7	0.265
9	0.334
11	0.416
13	0.478
15	0.558

Table 3. Formulation for clindamycin microspheres

Formulation Code	**Drug (mg)**	**Eudrajit Rs100(mg)**	**Eudrajit RL100 (mg)**	**Magnesium Stearate (mg)**	**Acetone (ml)**
F1 (1:1)	1000	1000		100	30
F2 (1:2)	1000	2000		100	30
F3 (1:3)	1000	3000		100	30
F4 (1:4)	1000		1000	100	30
F5 (1:2)	1000		2000	100	30
F6 (1:3)	1000		3000	100	30
F7 (1:1:1)	1000	1000	1000	100	30
F8 (1:0.25:1.75)	1000	250	1750	100	30

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

Table 4. Percentage yield and entrapment efficiency of formulation f1- f8

Formulation Code	Percentage Yield	Entrapment Efficiency
F1	80.2	70.1
F2	80.3	73.5
F3	77.31	78.12
F4	91.12	70.78
F5	81.92	76.12
F6	92.91	80.1
F7	82.06	65.12
F8	92.03	82.1

Table 5. Mean particle size of formulation f1 – f8

Formulation Code	Mean Particle Size
F1	176 ± 1.4
F2	287 ± 1.1
F3	420 ± 1.66
F4	180 ± 0 .56
F5	315 ± 0.59
F6	458 ± 0.35
F7	293 ± 0.8
F8	291 ± 1.1

Table 6. Actual drug loading and theoretical drug loading of formulation f1 – f8

Formulation code	Actual Drug Loading %	Theoretical drug loading %
F1	32.13	42.2
F2	24.21	32.33
F3	18.52	24
F4	34.25	48.92
F5	37.8	34.3
F6	20.42	24
F7	22.18	32.33
F8	25.78	32.3

Time	Cumulative Drug Release							
	F1	F2	F3	F4	F5	F6	F7	F8
1	8.44	7.52	5.21	17.18	14.28	10.84	18.38	22.44
2	12.73	11.13	7.71	22.78	21.13	17.82	23.21	27.38

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

3	22.42	18.72	14.8	26.44	26.18	22.84	28.76	36.13
4	28.12	25.7	20.82	34.82	32.31	28.73	36.11	42.12
5	34.33	33.12	24.13	39.92	37.49	34.62	45.22	50.29
6	42.52	40.2	32.39	44.07	39.28	38.05	53.12	57.82
7	46.31	42.28	36.09	49.01	45.19	40.57	56.17	63.17
8	56.3	53.77	41.93	5213	52.32	45.58	63.72	71.73
9	58.2	56.39	47.42	61.63	58.25	53.5	73.16	79.13
10	60.35	58.23	48.52	71.1	67.09	61.23	74.12	82.19
11	62.12	60.5	50.3	74.82	71.34	65.13	81.12	87.61
12	64.18	62.52	54.28	79.16	72.38	67.07	84.18	90.06

Table 7. In-vitro drug release profile of formula f1- f8

Table 8. Evaluation of parameter for cd microsphere incorporated gel

Evaluation parameters	**F7 (CD- MG)**	**F8 (CD- MG)**
Physical Appearance	White translucent	White translucent
pH	6.7	6.8
Drug content (%)	65.18±0.42	80.10±1.1
Drug content uniformity	Good	Good
Extrudability	Good	Good
Spreadability	25.52±0.168	25.48±0.178
Viscosity	3.611	3.614

Table 9. In-vitro drug release profile of gel formulation f7 and f8

Time(h)	**% Cumulative Drug Release**	
	F7(CD-MG)	**F8(CD-MG)**
1	35.17	48.21
2	39.42	58.42
3	42.18	61.38
4	47.32	67.12
5	53.12	72.71
6	59.11	75.82
7	63.07	84.13
8	71.21	90.12

Table 10. Stability studies of selected formulation (f8 cd-mg) at temperature 40 ± 2℃.

Evaluation parameters	**F8(CD-MG)**	
	Initial	**3 Months**
Physical appearance	No change	No change
pH	6.6	6.5
Drug content (%)	80.10	80.03

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

Extrudability	Good	Good
Spreadability	27.60	27.69
Viscosity	3.626	3.620
In vitro release studies 8(h)	92.12%	91.04%

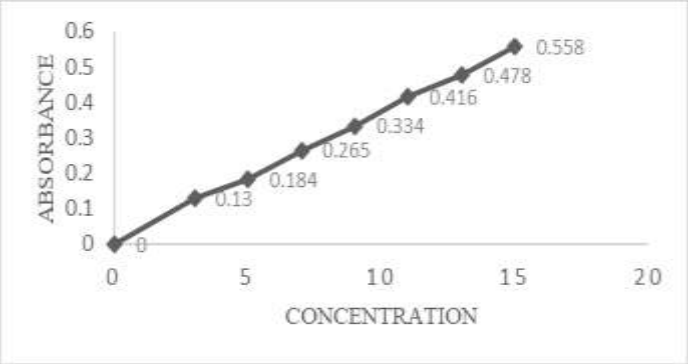

Figure. 1. Calibration curve of Clindamycin in SVF Concentration in µ/ml

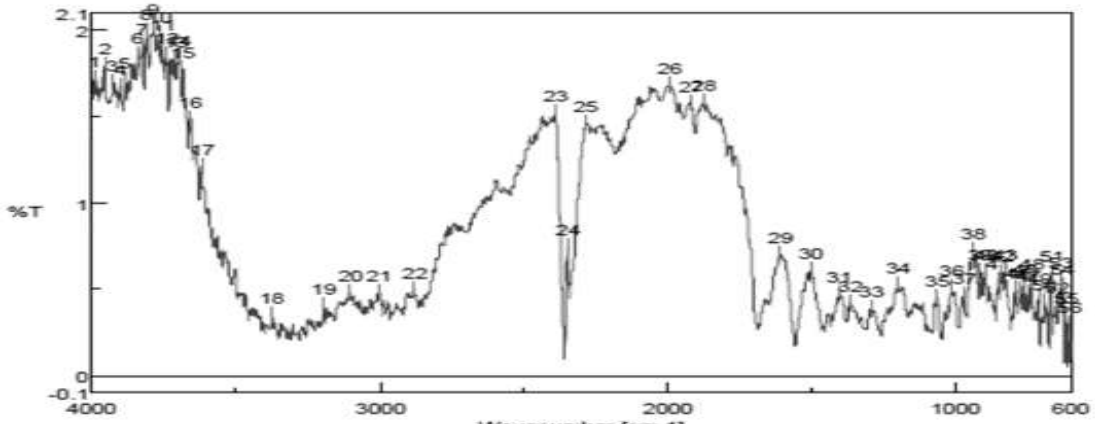

Figure. 2. FTIR spectra of pure Clindamycin

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

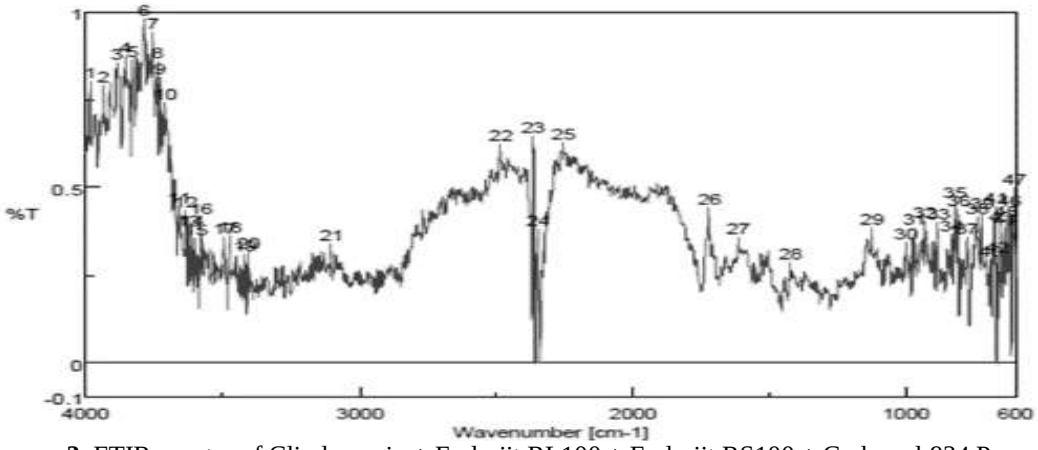

Figure. 3. FTIR spectra of Clindamycin + Eudrajit RL100 + Eudrajit RS100 + Carbopol 934 P

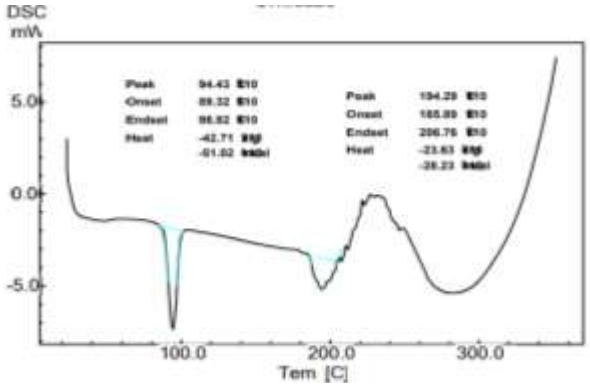

Figure. 4. DSC curve of pure Clindamycin

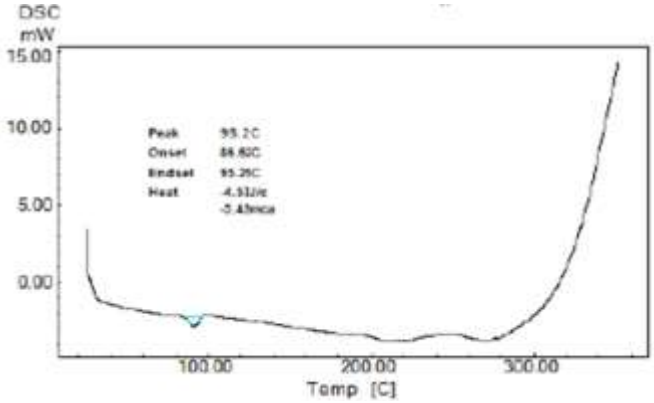

Figure. 5. DSC curve of clindamycin + Eudrajit RL 100

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

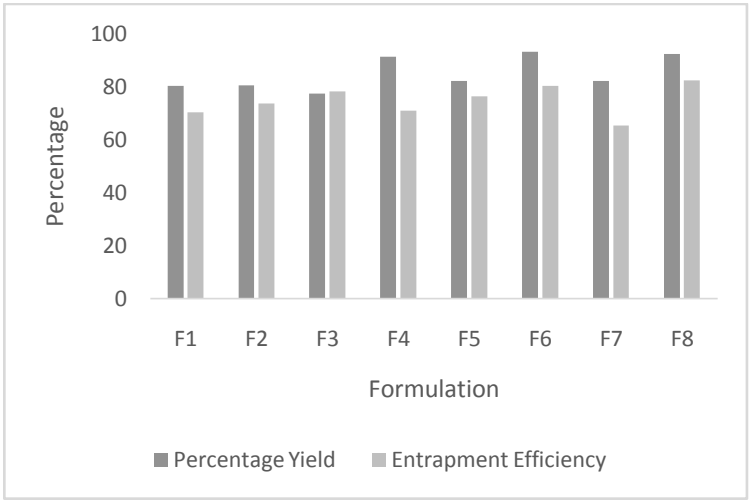

Figure. 6. Percentage yield and entrapment efficiency data of formulation F1 to F8

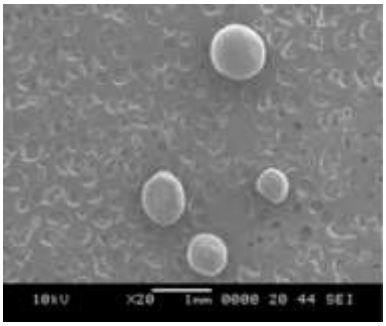

Figure. 7. Scanning Electron Microscopy F8

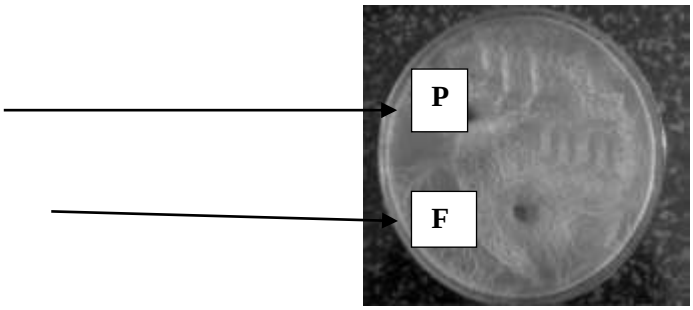

Figure. 8. Antimicrobial activity study for formulation
Comparative antifungal activity study of selected CD-MG with Placebo gel F= Formulation P= Placebo

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

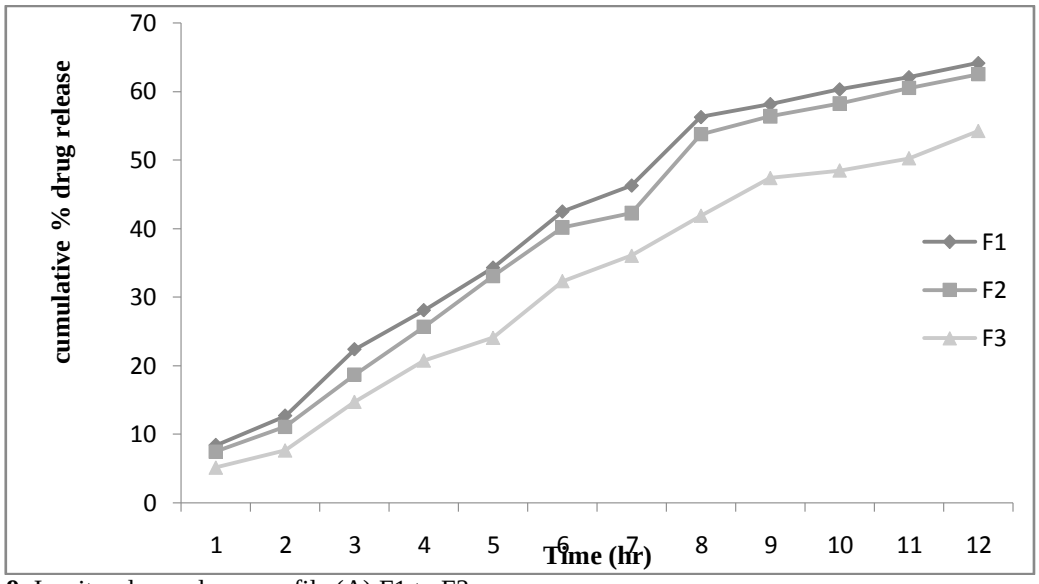

Figure. 9. In-vitro drug release profile (A) F1 to F3

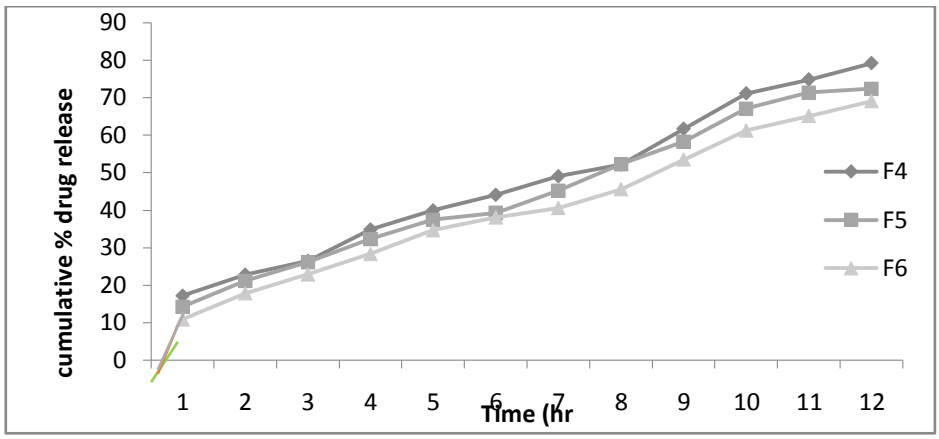

Figure. 9. In-vitro drug release profile (B) F4 to F6

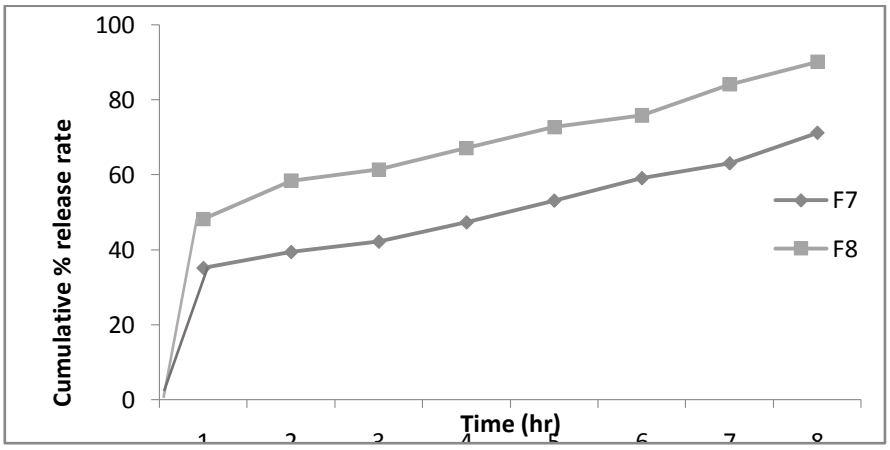

Figure. 10. In-vitro profiles of gel formulation F7 and F8

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 612-627 www.ijprajournal.com ISSN: 2249-7781

Table and figure titles