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Review Article

FORMULATION AND IN-VITRO EVALUATION OF NYSTATIN MICROSPHERES LOADED TRANSDERMAL GEL

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ABSTRACT
Nystatin is an antifungal medication. It is used to treat Candida infections of the skin including diaper rash, thrush, esophageal candidiasis, and vaginal yeast infections. It may also be used to prevent candidiasis in those who are at high risk. Nystatin may be used by mouth, in the vagina, or applied to the skin. The aim of the present study is to formulate and evaluate microspheres loaded transdermal gel containing Nystatin as a model drug microsphere were prepared by using aqueous ionotropic gelation method. Different polymers, different drug to polymer(s) ratio(s) and other parameters were screened to study their effects on properties of microspheres and to optimize each parameter. The microspheres obtained were subjected to preformulation studies like bulk density, tapped density, angle of repose, carr's index, hausner's ratio the results obtained were within the limit. The microspheres were characterized by Percentage yield, Drug entrapment efficiency, Particle size analysis, then the optimized microspheres formulation F3 were incorporated into the gel prepared with various grades of carbopol polymer(s) ratio(s) and was evaluated by parameters like Visual inspection , pH measurement , Spreadability studies , Viscosity and invitro drug release by using franz diffusion cell for results from the diffusion results F5 showed maximum percentage drug release of 96.72 hence it was considered as the optimized formulation.

Keywords: Nystatin, microspheres loaded transdermal gel.

INTRODUCTION

Microspheres

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm). Microspheres are sometimes referred to as microparticles [1]. Microspheres can be manufactured from various natural and synthetic materials [2]. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available [3]. Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material. Solid microspheres have numerous applications depending on what material they are constructed of and what size they are. Polyethylene and polystyrene microspheres are two most common types of polymer microspheres [4].

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Advantages

- Microspheres provide constant and prolonged therapeutic effect.
- Reduces the dosing frequency and thereby improve the patient compliance.
- They could be injected into the body due to the spherical shape and smaller size.
- Microsphere morphology allows a controllable variability in degradation and drug release [5].

Limitation

- The modified release from the formulations.
- Differences in the release rate from one dose to another.

Dosage forms of this kind should not be crushed or chewed [6].

Drug Transport Through Skin

The penetration of drug into viable epidermis and dermis is viable to achieve. But once trans epidermal penetration has been achieved, the continued diffusion of drug into epidermis is likely to result in drug transfer into microcirculation of the dermis and then into general circulation [7]. Topical products may unintentionally reach systemic circulation, it is usually in sub-therapeutic concentrations, and does not produce effects of any major concern except possibly in special situations, such as pregnant or nursing patient. On the other hand, transdermal drug delivery systems use the percutaneous route for systemic drug delivery, but the skin is not the primary target organ [8].

Permeation pathways of transdermal gel through stratum corneum

For some lipophilic drugs the principle barrier to permeation may reside in the essentially aqueous viable epidermis membrane, for most molecules the stratum corneum is the rate limiting barrier to delivery [9].

- Transappendageal Transport
- Transcellular Route
- Intercellular Pathway
- Hair follicles and sweat ducts

Factors Affecting Drug Permeation

I. Properties of the permanent:

1. Molecular Size
2. Solubility/ Melting Point
3. Ionization

II. Physiochemical properties of drug delivery systems:

- Release Characteristics
- Enhancement of Transdermal Permeation

III. Physiochemical and Pathological Conditions of Skin

- Reservoir Effect of Horny Layer
- Lipid Film
- Skin Hydration
- Regional Variation
- Pathological Injuries to The Skin
- Skin Temperature
- Cutaneous Self-Metabolism

Penetration Enhancement

I. To increase the penetration of drug across stratum corneum, penetration enhancers are used. Some of the desirable properties of penetration enhancers are:
II. They should be nontoxic, non irritating and non-allergic.
III. The activity and duration of action of penetration enhancers should be predictable and reproducible.
IV. They should not have any pharmacological action in the body. The penetration enhancers should work unidirectional i.e should allow therapeutic agents into the body whilst preventing the loss of endogeneous material from the body.
V. The penetration enhancers should be compatible with drugs and excipients [10,11].

Types of penetration enhancers

- Chemical penetration enhancers
- Physical penetration enhancers

Mechanism of action of penetration enhancers which directly act on skin are

- They directly act on stratum corneum intercellular keratin, denature it or modify their confirmation by causing swelling and by increasing hydration.
- They act on desmosomes which maintain the cohesion between corneocytes.
- They cause the disruption of the lipid bilayer and the enhancer gets heterogeneously concentrated within the domains of bilayer lipids.
- They alter the solvent nature of the stratum corneum to modify partitioning of the drug or of a cosolvent into the tissue [12].

The above mechanisms of action are of peneteration enhancers which effect the stratum corneum and increases the penetration of drug by causing pilid disruption, protein modification and by promoting partitioning of drug. In addition, the indirect mechanisms of actions of enhancers are:

By modifying the thermodynamic activity of the vehicle. Rapid permeation of a good solvent from the donor solution, such as ethanol, leave the permeant in a more thermodynamically active state than when the solvent was present—even to the point of super saturation.

By solubilising the permeant in the donor (e.g. with surfactants), especially where solubility is very low as with steroids in aqueous donor solutions, can reduce depletion effects and prolong drug permeation [13,14].

MATERIALS

Nystatin, Xanthan gum, Methocel K4M, Methocel K15M, Calcium chloride, Methanol, Sodium hydroxide pellet, Potassium di hydrogen phosphate, Carbopol 934, Carbopol 940, Triethanolamine, PEG.

METHODOLOGY

Standard Graph of Nystatin

Preparation of Standard Calibration Curve of Nystatin

100 mg of **Nystatin** was accurately weighed and dissolved in100 ml of 6.8pH phosphate buffer to prepare stock solution. The 10 ml of stock solution was further diluted with 6.8pH phosphate buffer in 100ml to get

100µg/ml (working standard). Then 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml were prepared. Then the absorbance was measured in a UV spectrophotometer at 297 nm against 6.8pH phosphate buffer as blank. The absorbance so obtained was tabulated as in Table 1 Calibration curve was constructed and shown in Fig 1.

Compatibility Studies

A proper design and formulation of a dosag e form requires considerations of the physical, chemical and biological characteristics of both drug and excipients used in fabrication of the product. Compatibility must be established between the active ingre-dient and other excipients to produce a stable,
efficacious, attractive and safe product. If the excipient(s) are new and if no previous literature regarding the use of that particular excipient with an active ingredient is available, then compatibility studies are of paramount importance. Hence, before producing the actual formulation, compa-tibility of Nystatin with different polymers and other excipients was tested using the Fourier Transform Infrared Spectroscopy (FT-IR) technique.

Fourier Transform Infrared Spectroscopy (FT-IR):

In order to check the integrity (Compatibility) of drug in the formulation, FT-IR spectra of the formulations along with the drug and other excipients were obtained and compared using Shimadzu FT-IR 8400 spectrophotometer. In the present study, Potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the spectrophotometer and the spectrum was recorded. The FT-IR spectra of the formulations were compared with the FT-IR spectra of the pure drug and the polymers.

Scanning electron microscopy (SEM)

Morphological characterization of the microcapsules was done by using Scanning electron microscope (JEOL JSM -5200). The samples were coated to 200A° thickness with gold-palladium prior to microscopy. Microcapsules before dissolution study were only subjected to SEM study.

Method of preparation

Ionotropic Gelation Method

Batches of microspheres were prepared which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. The API, Nystatin (100 mg) were added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a 22G needle into calcium chloride (4% w/v) solution. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air-dried.

Table 1: formulation of Biooadhesive Microspheres

S. no	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Drug	300	300	300	300	300	300	300	300	300
2	Methocel K4M	100	200	300	-	-	--	-	-	-
3	Methocel K15M	-	-	-	100	200	300	-	-	-
4	Xanthan gum	-	-	-	-	-	-	100	200	300
5	Methanol	5	5	5	5	5	5	5	5	5
6	Water	20	20	20	20	20	20	20	20	20
7	Calcium chloride (5%)	QS	QS	QS	QS	QS	QS	QS	QS	QS

Characterization of microspheres

- Percentage yield
- Drug entrapment efficiency
- Particle size analysis
- Micromeritic properties
- Bulk density
- Tapped density
- Hausner's ratio
- Compressibility index (carr's)
- Production yield (%)

Evaluation of mucoadhesive property:

The mucoadhesive property of microspheres was evaluated by an in vitro adhesion testing method known as wash-off method. Freshly excised pieces of goat stomach mucous were mounted on to glass slides with cotton thread. About 20 microspheres were spread on to each prepared glass slide and immediately thereafter the slides were hung to USP II tablet

disintegration test, when the test apparatus was operated, the sample is subjected to slow up and down movement in simulated gastric fluid pH 6.8 at 37^0C contained in a 1-litre vessel of the apparatus. At an interval of 1 hour up to 8 hours the machine is stopped and number of microspheres still adhering to mucosal surface was counted.

Table 2: Preparation of gel Formulations

S. no	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Carbopol 974 (mg)	100	200	300	-	-	--	-	-	-
2	Carbopol 934 (mg)	-	-	-	100	200	300	-	-	-
3	Carbopol 940 (mg)	-	-	-	-	-	-	100	200	300
4	Triethanolamine(v/v)	2%	2%	2%	2%	2%	2%	2%	2%	2%
5	PEG(%V/W)	30%	30%	30%	30%	30%	30%	30%	30%	30%
6	Distilled water(ml)	QS	QS	QS	QS	QS	QS	QS	QS	QS

Evaluation of Nystatin microsphere gel

Visual inspection

The organoleptic properties, such as color, texture, consistency, homogeneity, and physical appearance of gel containing microspheres were checked by visual observation.

pH measurement

Gel formulation pH was recorded using digital pH meter. 5 g gel was dispersed in 45 ml distilled water at 27°C and solution pH was measured

Spreadability studies

One of the requisite qualities for an ideal gel is to pursue excellent spreadability. Spreadability is used to express the extent of the area of skin or affected part to which gel readily spreads. A spreading value significantly affects therapeutic efficacy of the formulation. Expression of spreadability is given in terms of time (in seconds) taken by two slides to slip off from gel placed in between under application of specific load. Better spreadability is indicated by minimum time required for slides separation. Mathematical expression used for spreadability was calculated.

Viscosity

The viscosity of the gel formulation was measured with a Brookfield viscometer (Brookfield, USA; Capcalc Version 2.2) using 1x model and cone number 01, with an angular velocity of 5 rpm at 25 ºC. An average of five readings was used to calculate viscosity.

In Vitro Drug Release

In vitro drug release studies of the microspheres, emulgel formulations and commercial cream product were carried out using modified Franz diffusion cell during 12 h. Mixture of PBS pH 7.4: ethanol (70:30) was used as receptor medium and sink condition was determined. The receptor phase was kept at a constant temperature of 37°C and stirred by a magnetic stirrer. Spectra/Por 2® dialysis membrane, MWCO: 12– 14 kDa (Spectrum Lab.,USA) was used as the diffusion membrane. At appropriate time intervals, 0.5 ml of samples were collected and replaced by an equal volume of fresh receptor medium. Nystatincontent was analysed by UV at a wavelength of 297 nm.

Application of Release Rate Kinetics to Dissolution Data:

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

Zero order release rate kinetics:

To study the zero–order release kinetics the release rate data ar e fitted to the following equation.

$$F = K_o t$$

Where, 'F' is the drug release at time't', and 'K_o' is the zero order release rate constant. The plot of % drug release versus time is linear.

First order release rate kinetics: The release rate data are fitted to the following equation

$$\text{Log } (100\text{-}F) = kt$$

A plot of log cumulative percent of drug remaining to be released vs. time is plotted then it gives first order release.

Higuchi release model: To study the Higuchi release kinetics, the release rate data were fitted to the following equation.

$$F = k\ t1/2$$

Where, 'k' is the Higuchi constant.

In higuchi model, a plot of % drug release versus square root of time is linear.

Korsmeyer and Peppas release model:

The mechanism of drug release was evaluated by plotting the log percentage of drug released versus log

time according to Korsmeyer- Peppas equation. The exponent 'n' indicates the mechanism of drug release calculated through the slope of the straight Line.

$$M_t/ M_\infty = K t^n$$

Where, M_t/ M_∞ is fraction of drug released at time 't', k represents a constant, and 'n' is the diffusional exponent, which characterizes the type of release mechanism during the dissolution process. For non-Fickian release, the value of n falls between 0.5 and 1.0; while in case of Fickian diffusion, n = 0.5; for zero-order release (case I I transport), n=1; and for supercase II transport, n > 1. In this model, a plot of log (M_t/ M_∞) versus log (time) is linear.

Hixson-Crowell release model:

$$(100-Q_t)^{1/3} = 100^{1/3} – K_{HC}.t$$

Where, k is the Hixson-Crowell rate constant.

Hixson-Crowell model describes the release of drugs from an insoluble matrix through mainly erosion [15, 16, 17].

RESULTS AND DISCUSSION

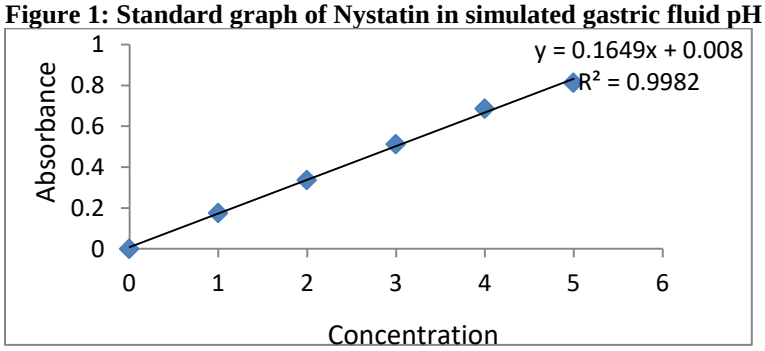
Calibration curve

Suitable analytical method was developed for Nystatin using UV spectroscopy and analytical wavelength of λmax at 297 nm was identified in pH6.8 buffer solution.calibration curve was constructed in this media.the method have shown good reproducibility.Beer Lambert's law was obeyed in the range of 1 to 5ug\ml and pH 6.8 buffer solution.

Table 1: Calibration curve data for Nystatin in simulated fluid pH 6.8 buffer at 297 nm

Conc [µg/l]	Abs
1	0.175
2	0.336
3	0.512
4	0.685
5	0.813

Figure 1: Standard graph of Nystatin in simulated gastric fluid pH 6.8



DRUG AND EXCIPIENT COMPATABILITY STUDIES

Figure 2: FTIR spectrum of pure drug

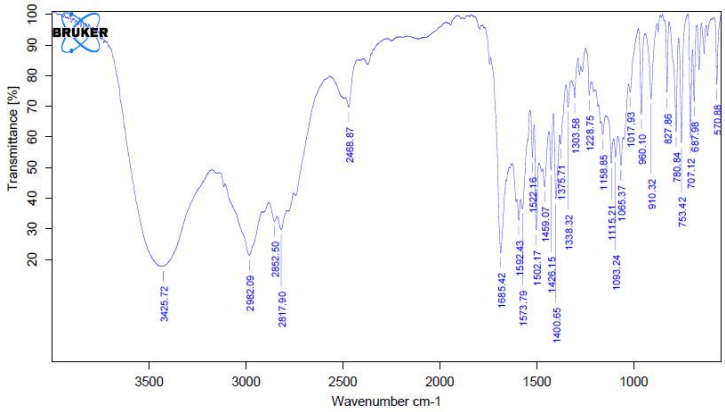
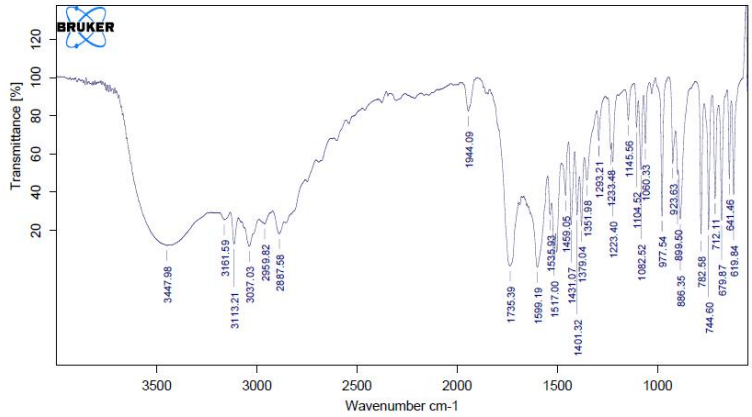


Figure 3: FTIR spectrum of optimized formulation



Evaluation and Characterization

Table 2: pre-formulation parameters

Formulation Code	% yield	Mean particle size (µm)	Bulk density Gm/cm^3	Tapped density	Hausner's Ratio	Carr's index	Angle of repose	Percentage Entrapment efficiency
F1	79.12	651	0.46	0.53	16.52	1.23	22.41	69.18
F2	70.03	730	0.51	0.56	17.53	0.86	25.08	76.93
F3	66.24	639	0.53	0.51	16.87	0.99	23.31	71.12
F4	81.50	680	0.45	0.57	17.62	1.14	24.09	74.37
F5	75.32	727	0.59	0.52	16.48	1.24	25.27	72.18
F6	63.41	769	0.52	0.59	17.42	1.20	21.83	77.61
F7	69.27	692	0.46	0.52	16.13	0.96	26.41	68.92
F8	71.52	663	0.59	0.58	17.48	1.23	25.92	71.47
F9	76.87	795	0.58	0.56	16.42	0.98	23.18	74.32

Figure 4: Dissolution profile of F1, F2, F3 formulations

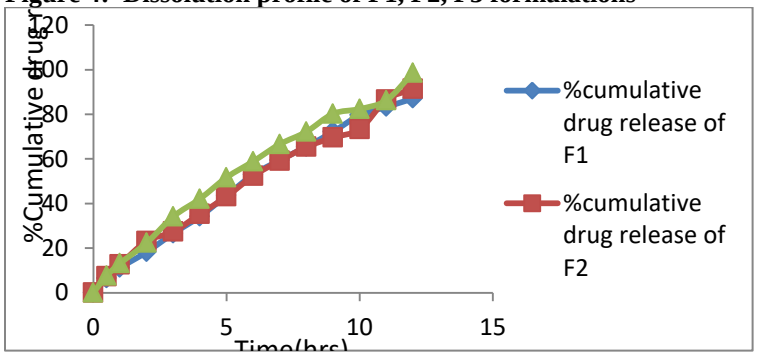


Figure 5: Dissolution profile of F4, F5, F6 formulations

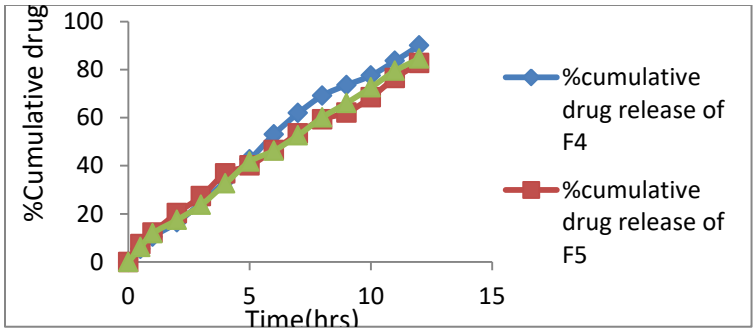
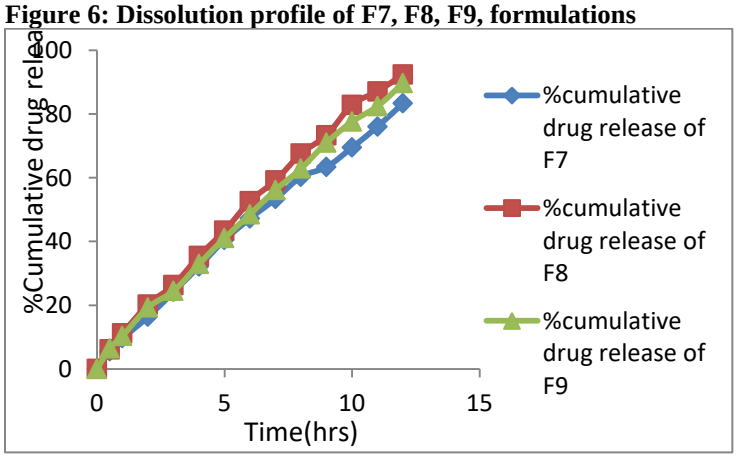


Figure 6: Dissolution profile of F7, F8, F9, formulations



Evaluation of Microspheres Loaded Topical Gel

Visual inspection

The organoleptic properties, such as color, texture, consistency, homogeneity, and physical appearance of gel containing microspheres were checked by visual observation.

Table 3: Physical parameters of microspheres loaded topical gels of Nystatin

S.NO	Formulation code	% Drug content (mg)	Spreadability(gm.cm\sec)	Viscosity(cps)	PH	Extrudability study
1	F1	97.31	15.73	221.12	6.63	96.21
2	F2	96.27	13.62	228.46	6.49	97.93
3	F3	95.38	15.09	209.47	6.58	96.27
4	F4	97.62	14.71	219.13	6.91	98.51
5	F5	98.51	16.34	216.51	6.07	96.58
6	F6	94.41	13.23	224.62	6.28	99.37
7	F7	95.72	16.18	219.52	6.51	98.71
8	F8	97.13	14.69	228.69	6.16	96.07
9	F9	98.39	15.76	229.58	6.63	99.51

In-vitro release studies

Figure 7: Dissolution profile of F1, F2, F3 formulations.

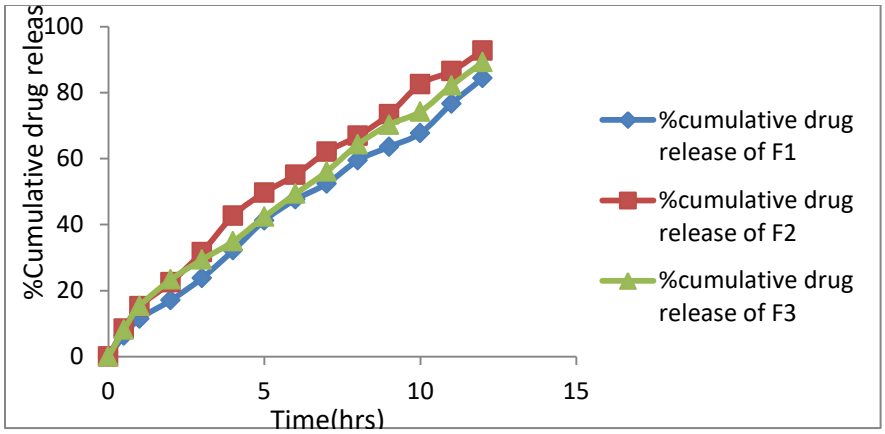


Figure 8: Dissolution profile of F4, F5, F6 formulations

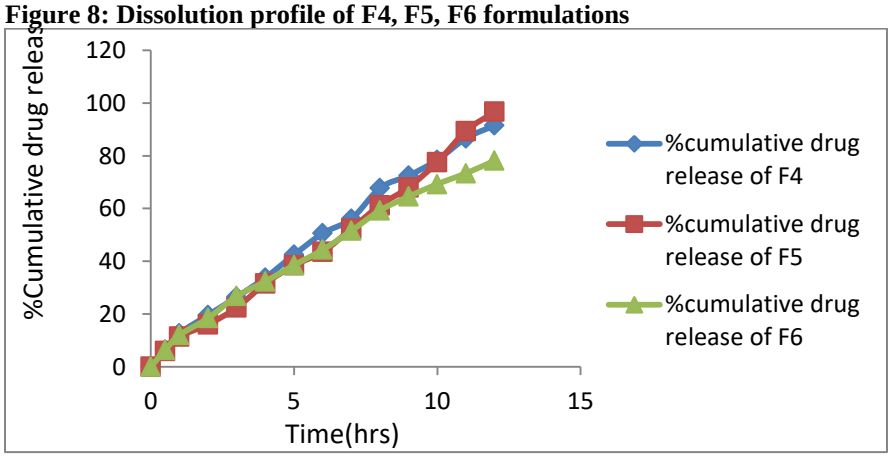
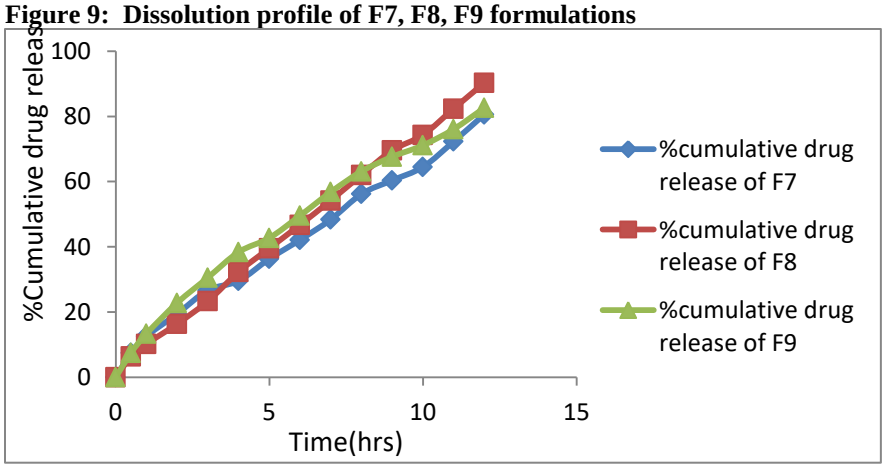


Figure 9: Dissolution profile of F7, F8, F9 formulations



Application of Release Rate Kinetics to Dissolution Data
Figure 10: Zero order release kinetics graph

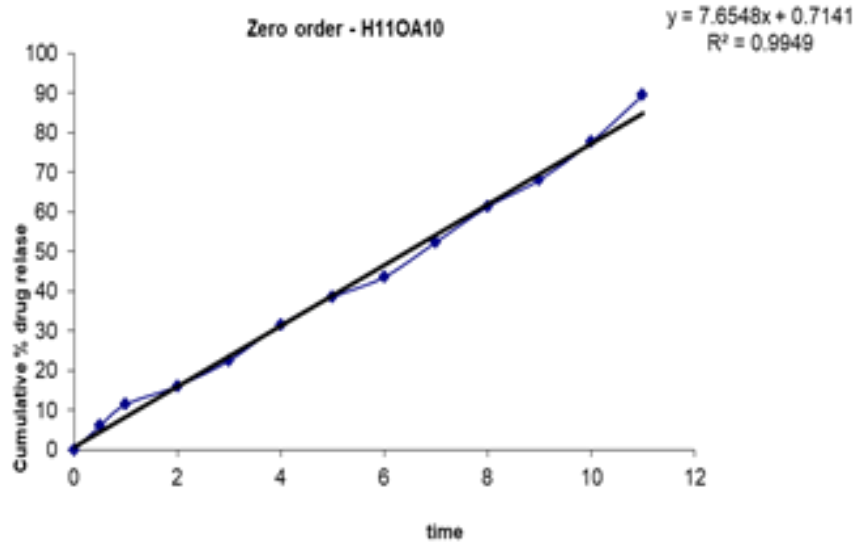


Figure 11: Higuchi release kinetics graph

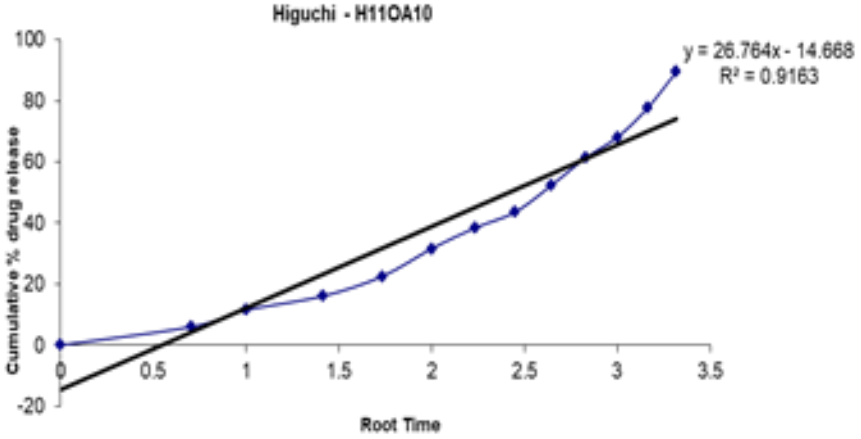


Figure 12: Kars mayer peppas graph

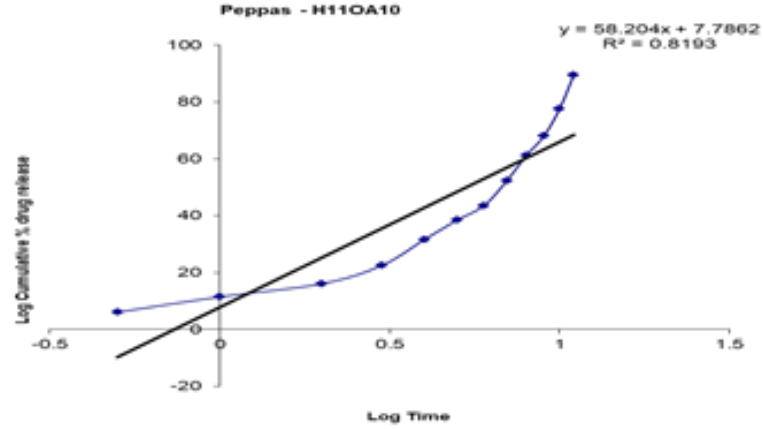
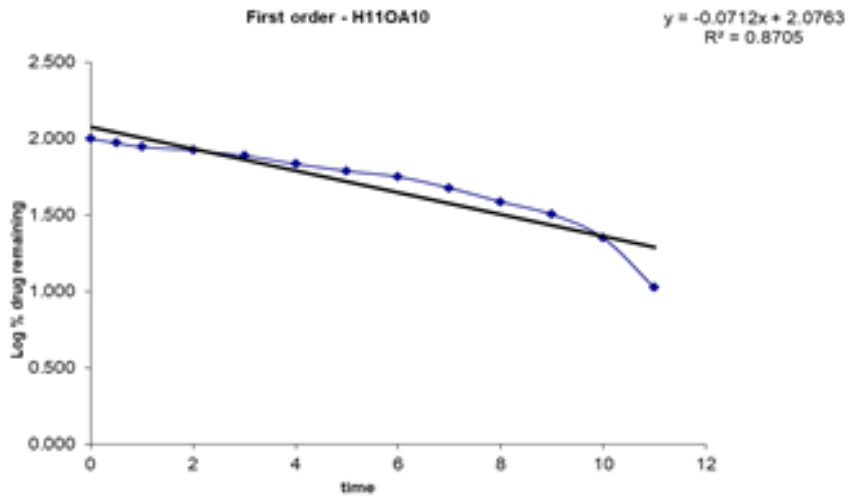


Figure 13: First order release kinetics graph



From the above graphs it was evident that the formulation F5 was followed zero order kinetics.

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