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"FORMULATION AND EVALUATION OF MICROSPHERE LOADED ANTIFUNGAL CREAM"

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ABSTRACT

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature and ideally having a particle size less than 200µm. This is the important approach in delivering therapeutic substance to the target site in sustained and controlled release fashion. Size of microspheres affects the rate of drug release. As size decreases, the surface area-to-volume ratio of the particle increases. Thus, for a given rate of drug diffusion through the microsphere, the rate of flux of drug out of the microsphere, per mass of formulation, will increase with decreasing particle size.Microspheres were prepared by optimizing various parameters and studying their effects on particle size as well as entrapment efficiency. It was then incorporated into a cream base to form a suitable topical drug delivery system. From the experiments, it was concluded that; as polymer amount increases, particle size and drug entrapment increase; as surfactant concentration increases, particle size and drug entrapment decreases; as stirring speed increases, particle size and entrapment decrease. Microspheres was prepared with aim to deliver the drug which passes through transdermal route as it provides quick onset of action when compared to oral route.

INTRODUCTION

Sustained release dosage forms (CRDFs) have been created during the last 3 decades because of their significant therapeutic benefits including as ease of administration, dosing frequency, and composition flexibility. Micro-sponges transportation systems built from living organisms biodegradable materials have received substantial attention in the field of maintained medication orders for some years. Capsules that also can effective completion release rates and target medicines to a specific anatomical location have recently had a significant influence on the formation and execution of nano medicine. Nanoparticles are an essential component of such new medication delivery vehicles. Micelles are regarded as a dependable way of delivering the medication to the target site with sensitivity, and of maintaining the desired concentration at the location of interest without adverse effects, if adjusted. 1 Because of their tiny size and effective carrier properties, microspheres play a key role in these drug delivery systems. Through the selection and formulation of diverse drug–polymer combinations, micro sphere-based therapy allows drug release to be carefully customised to the individual treatment location. The elements that may be adjusted to obtain the desired outcome are the overall dosage of medicine and indeed the timing of absorption. Micro-spheres may be created into an optimum drug delivery system with the required release profile using novel encapsulation capabilities and by changing the polymer ratios, monomer of the polymer, and so on. They haves a wides ranges of uses and are made from a variety of polymers. To entrap and distribute medicines, several synthetic and natural polymers like as viscose, bentonite, jelly, and polyamide have been utilised to produce controlled drug delivery.[2,3]

- **DRUG PROFILE**
- **MICONAZOLE NITRATE**
- **Synonyms** MCZ
- **Proprietary names** Fungidal® BT, Loramyc, Tibozole.
- **Chemical name** 1-[2,-4-Dichloro-β-[(2,-4-dichlorobenzyl) oxy] phenethyl] imidazole mononitrate
- **Molecular formula** $C_{18}H_{14}Cl_4N_2O.HNO_3$

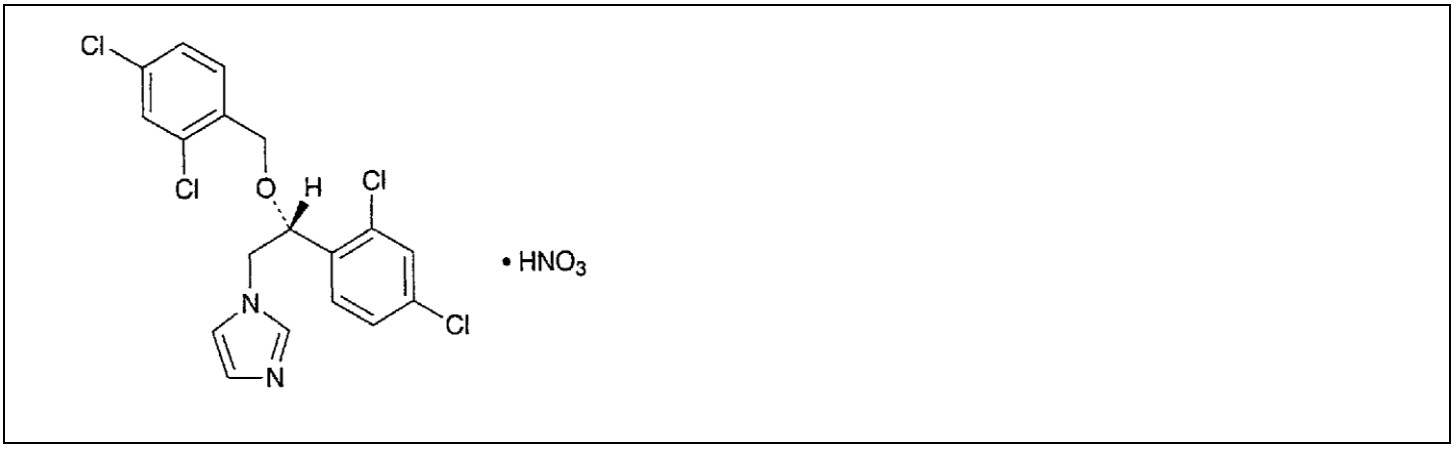


Figure : Structural presentation of mirconazole nitrat

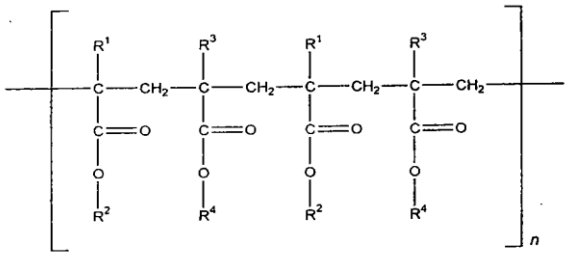
- **Molecular weight** 479.14 g/ml
- **Appearance** white to cream colored crystalline powder, odorless.
- **Solubility** freely soluble in N, N-dimethylforamide, sparingly soluble in methanol, slightly soluble in ethanol, in acetone and in acetic acid, and very slightly soluble in water and in diethyl ether.
- **Dissociation constant** 6.65
- **Partition coefficient** 5.86
- **Mechanism of action :**

MCN interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. MCN may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis.

EXCIPIENTs

1.Polymethacrylates [3,4]

Structural Formula



For Eudragit RL and Eudragit RS:

R1 = H, CH_3

R2 = CH_3, C_2H_5

R3 = CH_3

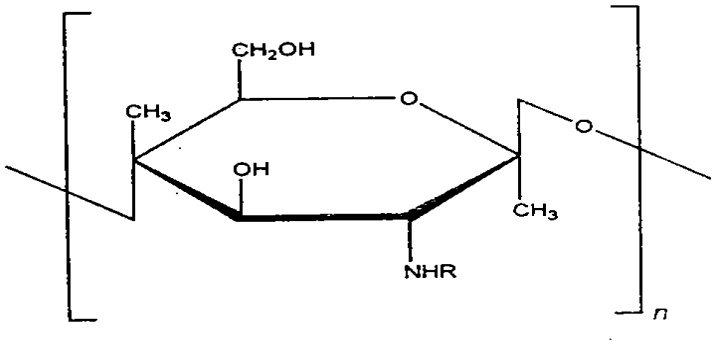
R4 = $CH_2CH_2N(CH_3)_3^+Cl^-$

Functional Category

Film former; tablet binder; tablet diluent.

2.Chitosan[4,5]

Structural Formula



R = H or $COCH_3$

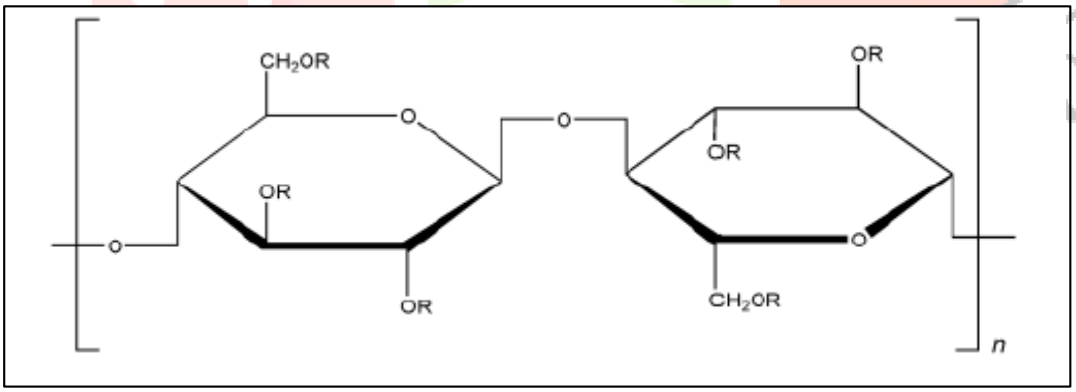
Functional Category

Coating agent; disintegrant; film-forming agent; mucoadhesive;

tablet binder; viscosity-increasing agent.

3.HYDROXY PROPYL METHYL CELLULOSE [3]

Structural Formula:



EXPERIMENTAL WORK

Procurement of Drug and Excipients:

The following drug, polymers, excipients and chemicals were used for the formulation and evaluation of mirconazole nitrate micro spheres.

Table : List of Drug and Excipients

Sr.No.	Material	Supplier
1	Micronazole nitrate	GSK India.
2	Eudragit RSPO	Alkem, India.
3	Eudragit RLPO	Alkem, India.
4	HPMC K4M	Alkem, India.
5	Chitosan	Alkem, India.
6	Paraffin wax	Loba chemie, Pvt Ltd, Mumbai.
7	Span 80	Loba chemie, Pvt Ltd, Mumbai.
8	Acetone	Loba chemie, Pvt Ltd, Mumbai.
9	Petroleum ether	S.D. Fine chemicals, Ltd.

Table. List of Equipment's

Equipment's name	Name of manufacturer and model no.
Digital Balance	Shimadzu AUX 102, Japan
UV visible double beam spectrophotometer	Shimadzu 1601
USP Tablet Dissolution Apparatus Type II	Libindia Disso-2000
Scanning Electronic Microscope	JSM 6360A, JEOL-JFC-1600
pH meter	Rimek minipress-1, Ahmedabad, India
Infra-Red Spectrophotometer	Perkin Elmer Paragon 500
Differential scanning calorimeter	DSC6220 Shimadzu, Japan

PREFORMULATION STUDY

Identification of drug and polymer:

A. Organoleptic properties [6]

The sample of Micronazole Nitrate was studied for organoleptic characters such as color, odor and appearance.

B. Melting point [6]

Melting point was determined by open capillary method using melting point apparatus.

C. FTIR Spectroscopy [7]

The FTIR spectrum of Microinazole Nitrate was recorded using FTIR spectrophotometer (Paragon 500 Perkin Elimer) using KBr pellet technique.

The peaks are is shown in figure no. 7.1

D. UV spectroscopy (Determination of λ Max) [8]

Stock solution (100µg/ml) of Micronazole Nitrate was prepared in 0.1N HCL (pH 1.2). This solution was appropriately diluted with 0.1N HCL to obtain a concentration of 30µg/ml. The solution was kept in a fused silica cuvette 10 mm. The UV spectrum was recorded in the range of 200-400 nm on UV Visible Spectrophotometer (Shimadzu 1600). UV maximum of Micronazole Nitrate was. Found to be 229 nm.

Calibration curve for Miconazole Nitrate

10 mg Miconazole Nitrate was accurately weighted & transferred to 100 ml volumetric flask. It was then dissolved in 0.1N HCL (pH 1.2) and sonicated for 10 min & diluted to volume with 0.1N HCL to give stock solution containing 100µg/ml. This solution was appropriately diluted with 0.1N HCL to obtain a concentration of 5, 10, 15, 20, 25, and 30µg/ml. The above solutions were analyzed by U. V. Spectrophotometer at 229 nm. All the dilutions were made using 0.1N HCL and 0.1N HCL was used as a blank during spectrometric analysis.

E. Differential Scanning Calorimeter studies

Thermal analysis was performed using (DSC6220 Shimadzu) system with a differential scanning calorimeter equipped with a computerized data station. All samples were weighed and heated at a scanning rate of 10°C/min between 30 and 300°C and 40 ml/min of nitrogen flow. The differential scanning calorimetry analysis gives an idea about the interaction of various materials at different temperature. It also allows us to study the possible degradation pathway of the materials.

Determination of solubility [8]

Miconazole Nitrate solubility study was carried out using saturation method.

Preparation of microspheres of Miconazole Nitrate [9]

Microspheres were prepared by a solvent evaporation method. The solvent system acetone/liquid paraffin was used. Agglomeration of micro spheres was prevented by using 1% w/v Span80. Eudragit RSPO was used to

form a matrix of micro spheres and polymer were chosen to produce Chitosan and Hydroxy prropyl methyl cellulose K4M. Eudragit RSPO and Miconazole Nitrate were dissolved in acetone and weighed quantity of Chitosan and Hydroxypropyl methyl cellulose K4M were dispersed it. The total volume of acetone. Was. 12 ml. This homogeneous final dispersion was cooled to 5 °C and poured slowly with stirring (700 rpm) into 80 ml of liquid paraffin containing 1% w/v span 80, which was previously also cooled to 5 °C. The obtained emulsion was stirred at 40 °C for 40 min. The suspension of micro spheres in liquid paraffin was fiiltered and microspheres were washed by petroleum ether and dried in vacuum at room temperature overnight.

Preparation of micro spheres loaded cream:

Oil phase having stearic acid, isopropyl myristate, and aqueous phase having propylene glycol and trietanolamine with water pour in beaker in measured quantity. Transfer to heat in water bath at 62° c, adding aqueous phase in oil phase when temp get touch for the formation of homogenous mixture. Get cool at room temperature for obtaining a white cream. Formulated microspheres are disperse in base of cream evenly with help of continuous stirring till 30 minutes, to getting microspheres loaded cream.

Table : Table showing the Batch codes and polymer content

Batch	Drug	Eudragit RSPO	Eudragit RLPO	Chitosan	HPMC K4M
1	1	1	1	2	-
2	1.5	1 -1/2	½	2	-
3	1	1/2	1- ½	1 -1/2	1/2
4	1.5	2	-	1	1
5	1	-	2	1	1
6	2	1	1	1- 1/2	1/2
7	2	2	-	1- 1/2	1/2
8	1	1- 1/2	½	1 -1/2	1/2
9	1.5	1/2	1 -1/2	-	2

Evaluations of microspheres

The flow properties [3.10.11]

Flow properties of micro spheres were characterized in terms of angle of repose, Carr's index and Hausner's ratio.

(a) Bulk density and tapped density:

Both bulk density, ρb (often called loose or aerated bulk density) and tapped density, ρt were determined. empty measuring cylinder from final weight of measuring cylinder. The cylinder was allowed to fall onto a hard surface from a height of 2 cm at 2 sec intervals. The tapping was continued till no volume change was noted. ρb and ρt were determined by following formulas,

$\rho b = M / Vb$

$\rho t = M / Vt$

b) Carr's Compressibility Index:

An important measure that can be obtained from bulk density determinations is the percent compressibility C, which is defined as follows

$Ic = (\rho t-\rho b)/\rho b \times 100$

c) Hausner ratio: A similar index has been defined by Hausner.

$HR = \rho t / \rho b$

d) Angle of repose:

The angle of. repose of the micro spheres was determined by using funnel method. The accurately weighed powder were taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the microspheres. The diameter of the micro spheres cone was measured and angle of repose was calculated by using the equation.

$\Theta = \tan^{-1} h/r$

where h and r are the height and radius of the micro spheres cone.

Table : Standard values of angle of repose (θ)

Flow ability	Angle of repose
Excellent	<25
Good	25-30
Passable*	30-40
Poor	37-45
Very poor	>45

Percentage yield [11]

The prepared micro spheres of all batches were accurately weighed. The weight quantity of prepared microspheres was divided by the total amount of all the excipients and drug used in the preparation of the micro spheres, which give the total percentage yield of muco adesive microspheres. It was calculated by using following equation.

$$\text{Percentage yield} = \frac{\text{Actual weight of microspheres}}{\text{Total weight of microspheres}} \times 100$$

Particle size determination [11]

Microsphere size was determined by using an optical microscope under regular polarized light, and the mean microsphere size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

$$Xg = \frac{(9ni \times \log Xi)}{N} \times 10$$

where Xg is geometric mean diameter, n is number of particle in range, Xi the midpoint of range and N is the total number of particles.

Scanning electron microscopy

Scanning electron photo micrograph of Miconazole Nitrate loaded microspheres were taken. A small amount of microspheres was spread on glass stub. Afterwards the stub containing the sample was placed in the scanning electron microscope (JSM 6360A, JEOL-JFC-1600) chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 15 KV, chamber pressure of 0.6 mm Hg, at different magnification. The photomicrograph of batch B7 is depicted in Fig. 7.8 and 7.9

Determination of Drug Content [5,12]

To determine the total drug content of microspheres a known amount of microspheres were ground to fine powder. Accurately weighed (50mg) grounded powder of microspheres were soaked in 50 ml of distilled water and sonicated using probe sonicator for 2 h. The whole solution was centrifuged using a tablet of centrifuge to remove the polymeric debris. Then the polymeric debris was washed twice with fresh sollvent (water) to extract any adhered drug. The clear supernatant solution was filtrated through a 0.45 µm whattman filter paper then analyzed for Miconazole Nitrate content by UV/Vis spectrophotometer at 229 nm.

Determination of Drug Entrapment [5,12]

A known amount of microspheres were ground to fine powder. Accurately weighed (50mg) grounded powder of microspheres were soaked in 50 ml of distilled water and sonicated using probe sonicator for 2 h. The whole solution was centrifuged using a tablet of centrifuge to remove the polymeric debris. Then the polymeric debris was washed twice with fresh solvent (water) to extract any adhered drug. The clear supernatant solution was

filtrated through a 0.45 µm whatman filter paper then analyzed for Miconazole Nitrate content by UV/Vis spectrophotometer at 229 nm against blank. The amount of drug entrapped in the microspheres was calculated. The drug entrapment was calculated by the equation

$$DEE = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

In- Vitro Permeation study:

Franz-diffusion cell with receptor volume 22 ml and diffusional area of 3.14 cm2 was used. Dialysis membrane 150 was used as a diffusion membrane and was placed between donor and receptor compartments of the Franz-diffusion cell. The receptor chamber was filled with fresh phosphate buffer, pH 6.8 and 1 g microsphere cream was loaded in the donor compartment. Aliquots (2 ml) of receptor medium were withdrawn and replaced with fresh medium at specified intervals of time i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,12 and 24 h and analyzed by UV.

In vitro drug release studies [5]

Release of Miconazole Nitrate from the microspheres was studied in 0.1N HCL (900 ml) using a USP dissolution testing apparatus II (Paddle type) with a rotating paddle stirrer at 50 rpm and 37° ± 1°C. A 5 ml sample solution was withdrawn from the dissolution apparatus for an every hour for 12 hrs. Samples were replaced by its equivalent volume of dissolution medium. The samples were filtered through Whatman filter paper and solutions were analyzed at 229 nm by UV Spectrophotometer (Libindia Disso-2000). Cumulative percentage drug release was calculated.

Drug release kinetics

Dissolution data of above methods was fitted in Zero order, First order and Higuchi equations.

The mechanism of drug release was determined by using Korsmayer Peppas equation.

RESULT AND DISCUSSION

PREFORMULATION STUDY

Identification of drug and polymer:

A. Organoleptic properties

The sample of Miconazole Nitrate w.as found to be yellow, crystalline powder, odourless.

B. Melting point

Melting point of Miiconazole Nitrate w.as found to be in the range of 172-185^{0}c.

C. FTIR Spectroscopy

The FTIR spectrum is shown in Figure 7.1 and interpretation of FTIR spectra is given in Table 6.1. FTIR spectrum of Miiconazole Nitrate showed all the peaks corresponding to the functional groups present in the stricture of Miconazole Nitrate.

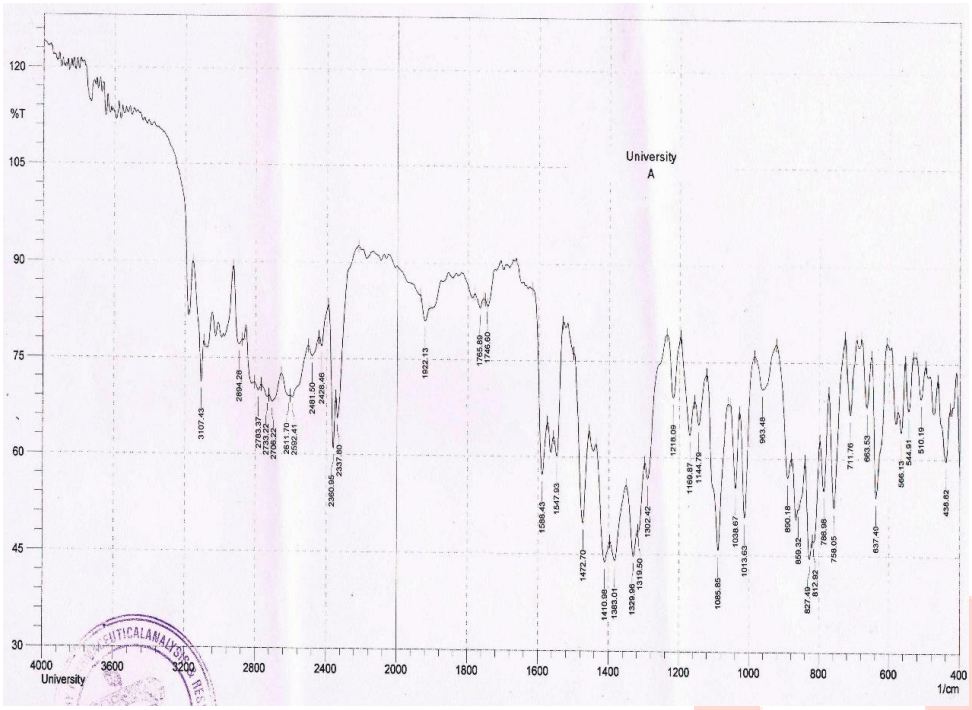


Figure : FTIR spectrum of Miconazole Nitrate

Table : Interpretation of FTIR spectrum of Miconazole Nitrate.

Observed Peaks (cm^{-1})	**Reported Peaks (cm^{-1})**	**Interpretation of Chemical group**
812.92	812	- C – Cl (Stretching)
827.49	827	= C – H (Streching)
1038.67	1038	- CHO (Stretching)
1319.50	1319	Aromatic Amine C – N (Stretching)

D. UV spectroscopy (Determination of λ Max) Wavelength of maximum absorbance (λ_{max}) of Miconazole Nitrate was found to. be 229 nm in 0.1N HCL. The UV

Spectrum is shown in figure 7.2

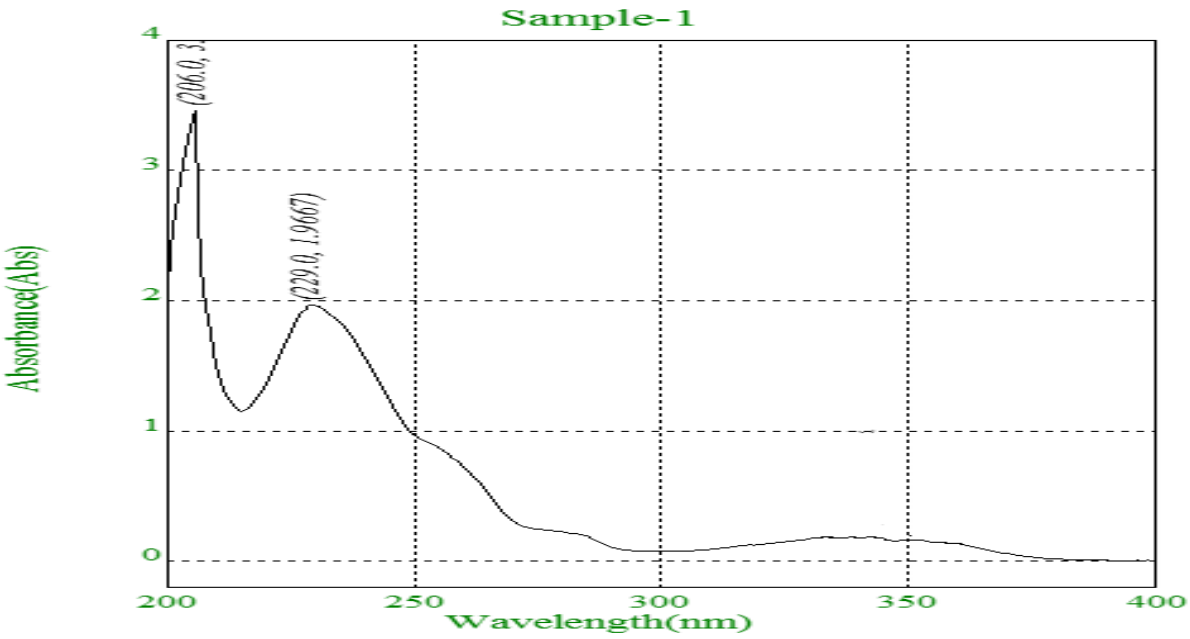


Figure : UV spectrum of Miconazole Nitrate

Calibration curve for Miconazole Nitrate:The calibration curve for Miconazole Nitrate in 0.1N HCL is shown in Figure 6.3. The graph of absorbance vs. concentration for Miconazole Nitrate was found to be linear in the concentration range of 5-30 µg/ml at 229 nm. The r^2 of the calibration curve was. F.ound to be 0.9955

Table : Concentration and absorbance values for Miconazole Nitrate in 0.1N HCl

Sr. no.	Concentration (µg/ml)	Absorbance at 229 nm
1	5	0.339
2	10	0.512
3	15	0.635
4	20	0.804
5	25	0.987
6	30	1.182

B) Drug -Excipients Interaction Study

The drug-excipients interaction study was carried out by using fourier transform infrared spectroscopy (FTIR) and differential scanning colourimetry (DSC).

1) Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of Drug, physical mixture of Drug : HPMC, Drug : HPMC : Eudragit RSPO, Drug : HPMC : Eudragit RSPO: Eudragit RLPO, arre sho.wn in **Figure 13, 14, 15, 16 and 17** respectively and its interpretation in **Table 14**. From FTIR spectra it was observed th.at there is no chemical modification in drug.

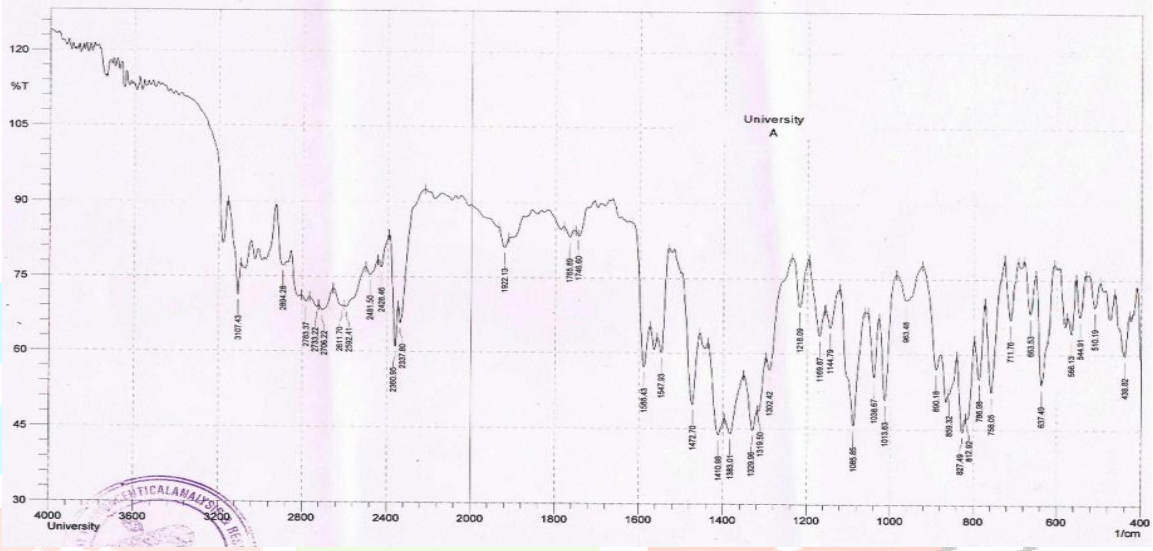


Figure : Infrared specitrum of miconazole nitrate

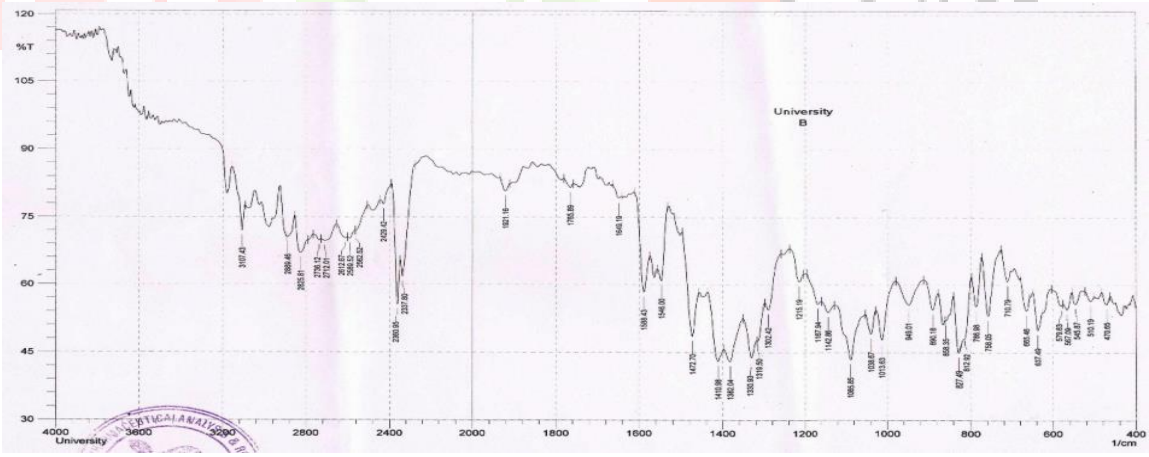


Figure : Iinfrared spectrum of Drug : HPMC

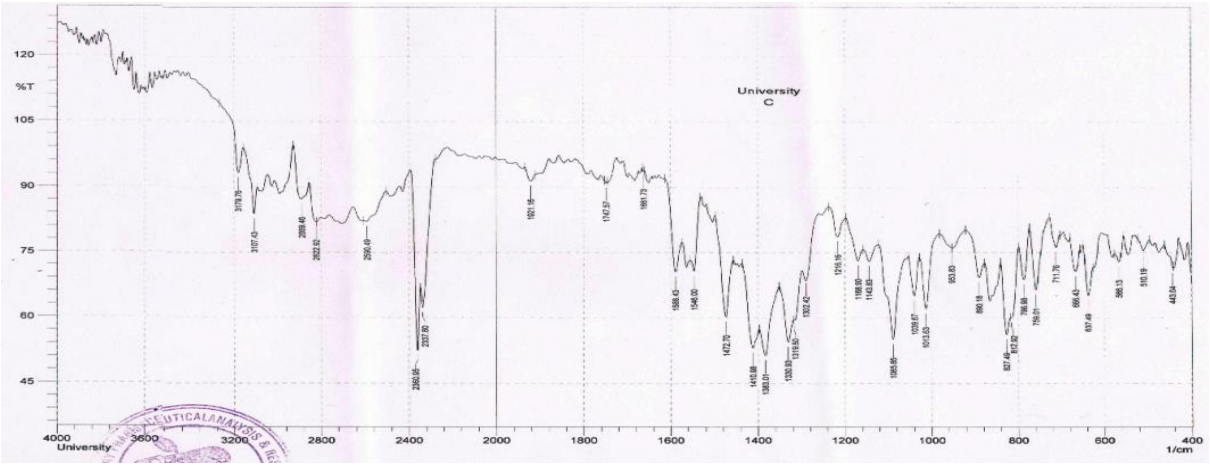


Figure : Inifrared Spectrum of Drug : HPMC : Eudragit RSPO

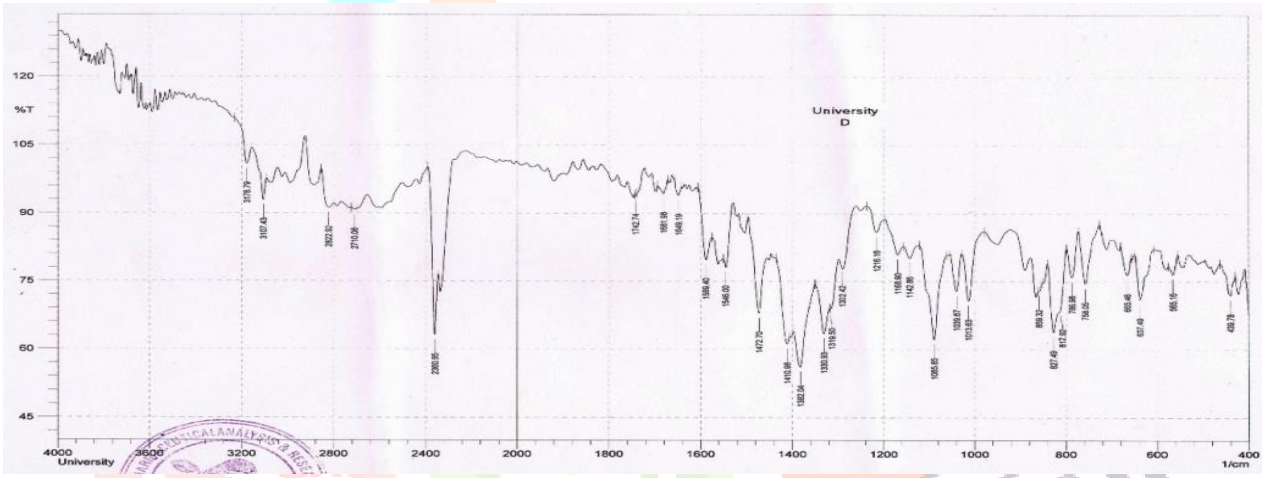


Figure : Infrared Spectrum of Drug : HPMC : Eudragit RSPO : Eudragit RL

Table : Drug-polymer interaction studies by IR spectroscopy

Polymer	Drug Peak	Polymer Peak	Drug + Polymer Peak	Interaction
HPMC	812.92, 827.49, 1038.67, 1319.50	852.56, 944.19,	812.92, 827.49, 858.35, 949.01, 1038.67, 1319.50	No
Eudragit RSPO	812.92, 827.49, 1038.67, 1319.50	1327.07, 1585.18	812.92, 827.49, 1038.67, 1319.50, 1330.93, 1588.85	No
Eudragit RLPO	812.92, 827.49, 1038.67, 1319.50	637.96, 855.46, 1036.92, 1470.27	637.49, 812.92, 827.49, 859.32, 1039.67, 1319.50, 1472.70	No

2) Differential Scanning Calorimetry (DSC)

DSC thermogram showed that there was no any major difference in peak temperature, when compared with pure drug's thermogram **Figure 18**. No interaction was found between drug and polymers. Results are shown. in **Figure 19, 20, 21** and **22.** It was revealed from DCS thermograph of drug polymers that there is no any major difference in the endothermic peak which shows that there is no interaction between drug and polymer.

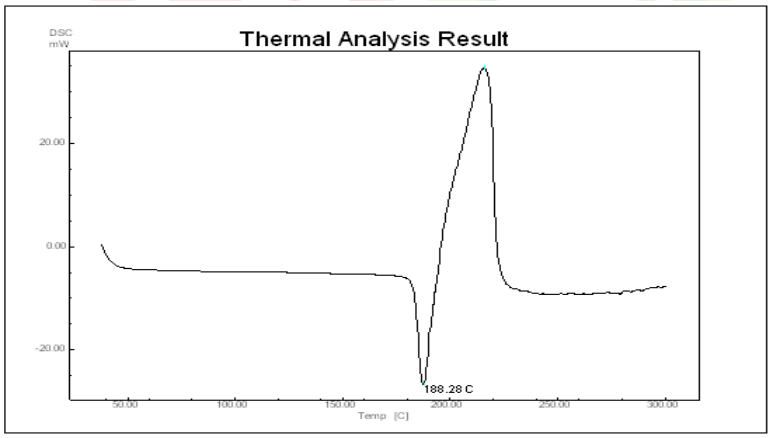


Figure : DSC thermogram of pure miconazole nitrate

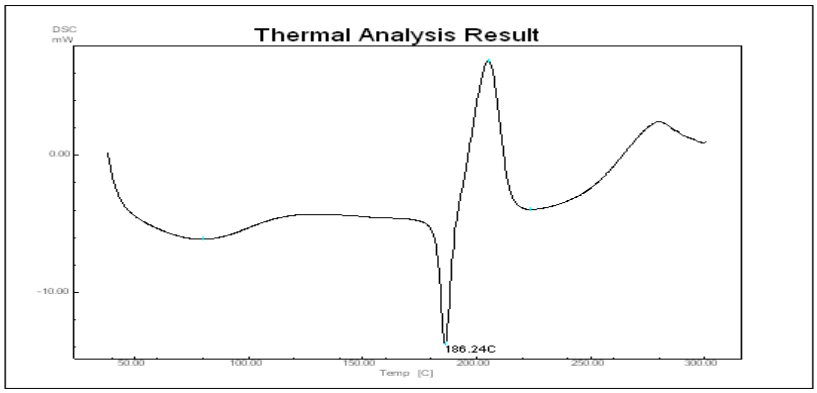


Figure : DSC thermogram of Drug : HPMC

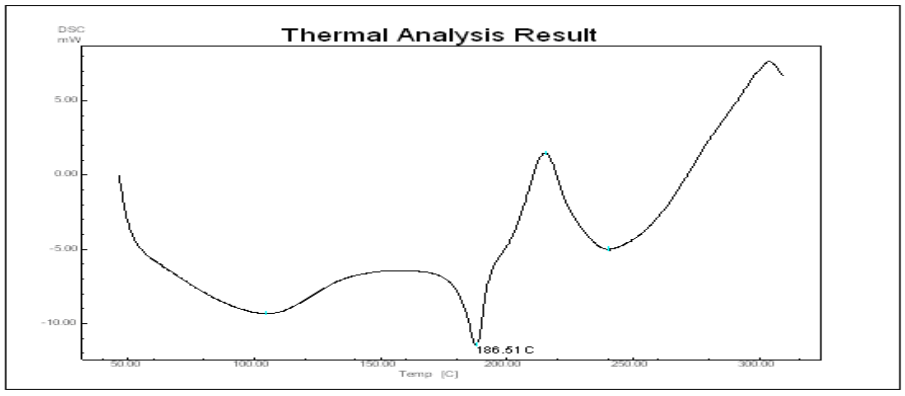


Figure : DSC thermogram of Drug : HPMC : Eudragit RSPO

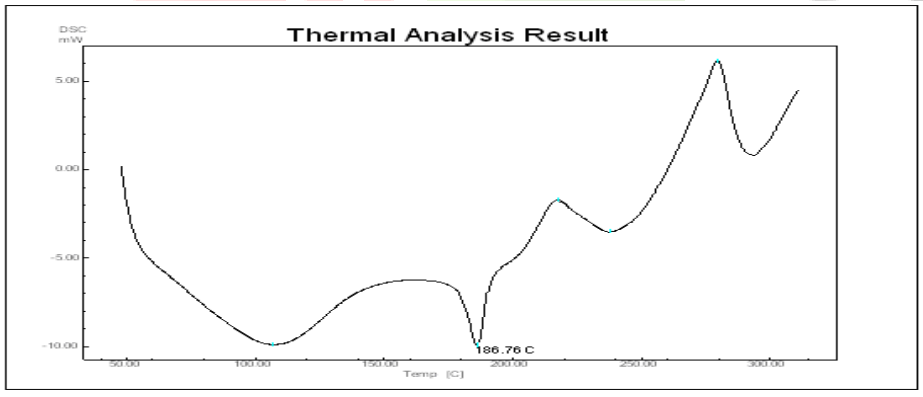


Figure : DSC thermogram of Drug : HPMC : Eudragit RSPO: Eudragit R

C) Analysis of drug

1) Solubility study of miconazole nitrate

The solubility of miconazole nitrate at 25ºC is shown in **Table 15.**

Solvent/ media	**Solubility %**
Water	0.03 ± 0.01
0.1N HCL	97.33 ± 0.58
0.2 M Ammonium Acetate Buffer	34.5 ± 0.46

All values are expressed as mean ± SD, n=3

Evaluation of Muco adhesive Micro spheres

Table : Evaluation of flow properties of microspheres

Batch	**Bulk Density(g/ cc)**	**Tapped Density (g/cc)**	**Carr's compressibility index**	**Hausner's ratio**	**Angle of repose(degree)**
B1	0.302	0.352	16.88	1.16	$29^0$16'
B2	0.309	0.344	11.32	1.11	$30^0$82'
B3	0.389	0.425	8.47	1.09	$30^0$82'
B4	0.467	0.528	11.55	1.13	$28^0$24
B5	0.481	0.525	9.14	1.09	$30^0$82'
B6	0.386	0.442	12.66	1.14	29^O72'
B7	0.489	0.547	10.60	1.11	$30^0$82'
B8	0.584	0.638	8.46	1.09	$30^0$82'
B9	0.504	0.549	8.19	1.08	$27^0$45

Evaluations of Mucoadhesive microspheres

Table. : Evaluations of Mucoadhesive microspheres

Batch	% Yield	Drug content	% DEE	Particle Size (µm)
B1	65.23	54.15	64.21	509
B2	72.41	59.21	72.16	517
B3	68.57	55.64	66.43	421
B4	78.59	62.79	68.38	463
B5	74.63	60.42	71.06	409
B6	72.98	56.83	63.78	486
B7	79.45	64.83	73.45	426
B8	67.51	51.13	61.37	461
B9	73.42	57.94	63.52	487

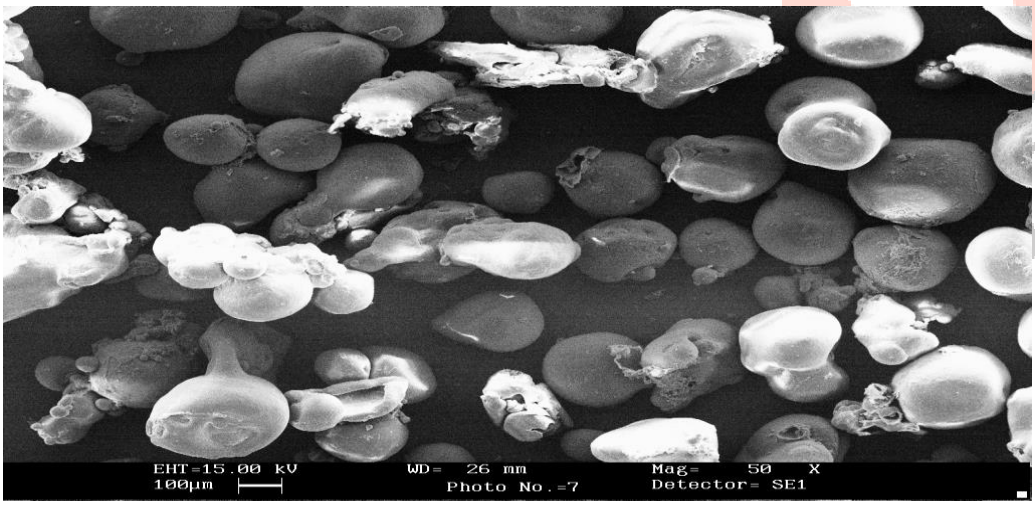


Figure . SEM image of formulation batch B7

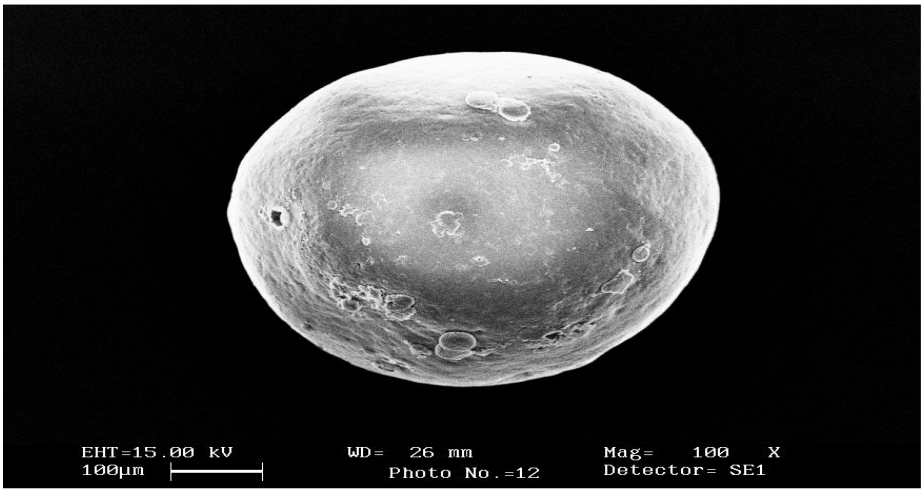


Figure . SEM image of formulation batch B7

In Vitro Dissolution study:

Table : In Vitro Drug Release of Batch B1-B9

TIME	B1	B2	B3	B4	B5	B6	B7	B8	B9
60	5.75	4.96	6.53	7.46	9.43	11.27	11.8	10.75	9.03
120	11.3	9.33	11.57	15.39	16.32	22.26	23.31	21.86	19.35
180	20.44	18.32	22.16	23.37	25.89	31.72	33.31	30.66	31.96
240	27.27	25.27	29.39	34.47	32.74	42.95	44.54	43.07	38.45
300	35.97	33.71	37.71	39.72	42.79	50.16	51.11	50.81	42.48
360	45.25	43.49	46.73	49.02	52.63	57.14	61.65	59.37	48.37
420	51.55	50.04	54.23	58.63	58.84	64.3	69.88	68.52	56
480	57.09	54.92	59.26	65.53	67.06	72.81	78.68	76.65	63.28
540	64.77	61.27	66.03	71.54	74.26	78.47	84.9	81.68	69.41
600	67.1	65.55	70.47	75.22	81.11	83.1	88.91	86.59	77.29
660	71.67	69.86	76.77	82.87	86.95	86.71	94	90.61	84.28
720	79.3	76.94	82.84	87.66	92.42	89.68	96.08	93.07	90.92

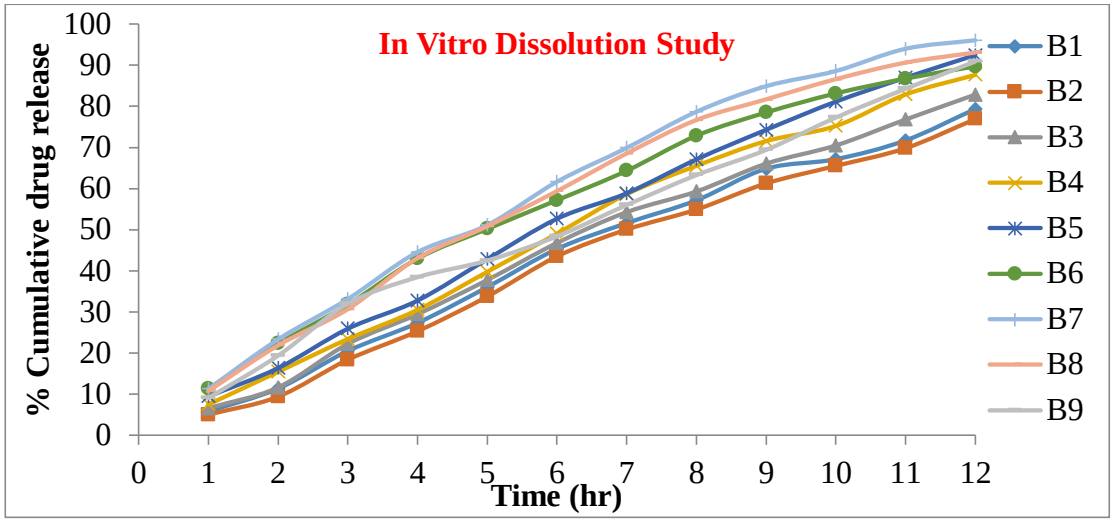


Figure : In Vitro Drug. Release of. Bat.ch B1-B9

From above graph, Zero order release was observed.

SUMMARY AND CONCLUSION

Microspheres were successfully prepared by optimizing various parameters and studying their effects on particle size as well as entrapment efficiency. It was then incorporated into a cream base to form a suitable topical drug delivery system. From the experiments, it was concluded that; as polymer amount increases, particle size and drug entrapment increase; as surfactant concentration increases, particle size and drug entrapment decreases; as stirring speed increases, particle size and entrapment decrease. Microspheres was prepared with aim to deliver the drug which passes through transdermal route as it provides quick onset of action when compared to oral route.

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