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Der Pharmacia Sinica, 2015, 6(12):1-12

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Formulation of azithromycin and chloroquine phosphate FDT by enhancing their solubility using cyclodextrins complex

Pentewar R. S.*, Kshirsagar Nilesh, Sayyed Sarfaraz Ali and Kulkarni Keshav

Department of Pharmaceutics, Channabasweshwar Pharmacy College, Latur, Maharashtra, India

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ABSTRACT

The main aim of the present study was to evaluate Azithromycin and Chloroquine drug in combination therapy used in the treatment of Chloroquine resistant malaria by increasing the solubility and dissolution rate of Azithromycin and Chloroquine phosphate by complexation techniques using β-Cyclodextrins with varying concentrations and after selection of proper ratio with carries an attempt was made to develop and evaluate fast dissolving tablet of Azithromycin Chloroquine phosphate using synthetic super disintegrates. The main objective of the present investigation is to explore the possibility of improving low solubility and hence dissolution profile. Fast dissolving drug delivery system of Azithromycin and Chloroquine phosphate with an aim of improving dissolution profile, patient compliance better therapeutic efficacy, less side effect and reduce dosage regimen with less toxicity for treatment for many acute and chronic disease. Evaluation of solubility profile, the pre and post compression parameters of the tablets, drug content, % drug release, drug content of all the formulations were with its range and the disintegration time 19-29 Sec. The dissolution study shows that the formulation containing β-Cyclodextrins from Azithromycin FDT shows 81.55 to 91.61 at the end of 45 min and Chloroquin phosphate shows 80.0 to 90.0% drug release at the end of 45 min.

Key words: Malaria**,** Fast dissolving tablet, Azithromycin, Chloroquine phosphate, Beta Cyclodextrins, Solubility.

INTRODUCTION

Now a day's efforts made by pharmaceutical companies to focus on development of new pharmaceutical dosage form. For the most therapeutic agents used to produce systemic effect, the oral routes still represent the preferred route of administration, owing to its several advantages and high patient compliance compared to many other routes.

Fast dissolving drug delivery systems have started gaining popularity and acceptance as new drug delivery systems, because they are easy to administer and lead to better patient compliance. Fast dissolving drug delivery systems (FDDDS) are a new generation of formulations which combine the advantages of both liquid and conventional tablet formulations and at the same time, offer added advantages over both the traditional dosage forms. Fast dissolving tablets are the solid dosage form, which disintegrates rapidly in the oral cavity without the need of water. Not all fast dissolving technologies actually dissolve; some use different disintegration mechanisms such as high levels of disintegrates or effervescent agents that cause the dosage form to disintegrate rapidly in the patient's mouth within a minute and can be gulped easily without the need of water. The bioavailability of some drugs may be increased due to absorption of drugs in oral cavity and also due to pre-gastric absorption of saliva containing dispersed drugs that pass down into the stomach[1-3].

Difficulties Associated with Existing Oral Dosage Forms.[4-6]

- Swallowing powder and liquids may become difficult in patients who may suffer from tremors.
- There may be gastrointestinal ulceration due to adherence to an esophagus, dysphasia and obstacles.
- Due to the swallowing of solid forms of tablets or capsules dosage forms may produce the difficulty in young adult patients due to incomplete development of nervous system muscular systems.
- The liquid medicaments like, syrups and suspensions stored in multidose bottles, uniformity of the each dose is difficult.

Advantages of Fdts.[7-10]

- Ease of administration to geriatric, pediatric, mentally disabled, and bed-ridden patients, who have difficulty in swallowing the tablet.
- Improved compliance / added convenience.
- Adaptable and amenable to existing processing and packaging machinery.
- No water needed.
- Allow high drug loading.
- Provide new business opportunities in the form of product differentiation, patent-life extension, uniqueness, line extension, and lifecycle management, and exclusivity of product promotion.

The Need for Development of Fdts.[11-12]

1. Patient factors: Fast dissolving dosage forms are suitable for those patients (particularly pediatric and geriatric patients) who are not able to swallow traditional tablets and capsules with an 8-oz glass of water.

- Patients who have difficulty in swallowing or chewing solid dosage forms.
- Patients incompliance due to fear of choking.
- Very elderly patients of depression who may not be able to swallow the solid dosage forms.
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2. Effectiveness factor

- Dispersion in saliva in oral cavity causes pre-gastric absorption of drug which dissolves. Buccal, pharyngeal and gastric regions are all areas of absorption for many drugs. Any pre-gastric absorption avoids first pass hepatic metabolism which increase the bioavailability.

3. Manufacturing and marketing factors

- As a drug nears the end of its patent life, it is common for pharmaceutical manufacturers to develop a given drug entity in a new and improved dosage form. A new dosage form allows a manufacturer to extend market exclusivity, unique product differentiation, value-added product line extension, and extend patent protection, while offering its patient population a more convenient dosage form This leads to increased revenue, while also targeting underserved and under-treated patient populations.
- FDDTs, as a novel dosage form, have several characteristics to distinguish them from the more traditional dosage forms.

The purpose of this work was to prepare solid dispersion of Azithromycin and Chloroquine phosphate to increase its solubility and its oral bioavailability by solid dispersion. For getting the rapid absorption and formulate fast dissolving tablets of Azithromycin and Chloroquine phosphate using Superdisintegrants. Solid dispersion by kneading method using Beta cyclodextrins.

MATERIALS AND METHODS

Azithromycin and Chloroquine phosphate obtained from Research lab chem. Industry, Mumbai.

Beta cyclodextrins obtained Gangwal chemical Pvt. Limited. Crosscamelose and Crosspovidone are obtained Research lab chem. Industry, Mumbai. Aerosol pharma200 and sucralose is obtained from Research lab chem. Industry, Mumbai.

Complexation processes.[13]

Various approaches of complexation with cyclodextrins has gained good acceptance in recent years in industry for enhancing the solubility and dissolution rate of poorly soluble drugs. Cyclodextrins (CDs) are cyclic torus-shaped molecules with a hydrophilic outer surface and lipophilic central cavity which can accommodate a variety of

lipophilic drugs. As a consequence of inclusion process, many physicochemical properties such as solubility, dissolution rate, stability and bioavailability can be favorably enhanced. Cyclodextrins are being increasingly applied in various pharmaceutical formulations in recent years due to their approval by various regulatory agencies. Cyclodextrins are produced from starch by means of enzymatic conversion. The mechanism of complexation processes were discussed as CDs can be regarded as cylinders with hydrophilic outside and hydrophobic inside. The hydrophobic cavity forms an ideal space in which poorly water soluble molecules are to be protected from the surrounding atmosphere shelter their most hydrophobic parts or whole molecules. These hydrophobic molecules which can fit in the CD cavity are included in it in the presence of water. In aqueous solution the polar CD cavity is occupied by water molecules that are in an energetically unfavoured state (Polar-a polar repulsion) and are therefore, readily replaced by an appropriate guest molecules that is less polar than water and forms an inclusion complex. The degree of complexation with CD depends upon the dimensions and lipophilicity of the guest molecules. The guest molecule or as part of it must fit into the CD cavity. For many drugs γ-CD offers the most interesting cavity size. Its dimensions are comparable to those of the substituted phenyl groups. Such groups are often most hydrophobic parts of drug and are therefore responsible for their poor solubility in water. Hiding these groups in CDs will markedly increase their overall aqueous solubility. There are a few energetically favorable interactions that helps shift the equilibrium towards complex formation.

- Displacement of polar water molecule from the a polar cyclodextrin cavity.
- The increase number of hydrogen bond formed as the displaced water returns to the larger pool. A reduction of the repulsive interaction between hydrophobic guest and the aqueous environment.
- An increase in hydrophobic interaction as the guest inserts itself into the polar cyclodextrin cavity.

Table no 1: Formulation table

Ingredients	F1	F2	F3	F4	F5	F6
Azithromycin	250	250	250	250	250	250
Chloroquine phophate	150	150	150	150	150	150
beta cyclodextrin	25	50	75	100	100	150
Crosscarmalose sodium	50	50	50			50
crosspovidone				50	60	
fenugreek						
Mannitol	200	200	200	100	100	200
Aerosol pharma 200	20	20	20	20	20	20
sucralose	20	20	20	10	10	20

In Vitro Disintegration test:
The USP device to rest disintegration was six glass tubes that are “3 long, open at the top, and held against 10” screen at the bottom end of the basket rack assembly.

One tablet is placed in each tube and the basket rack is poisoned in 1 liter beaker of distilled water at 37± 2^{o}C, such that the tablets remain below the surface of the liquid on their upward movement and descend not closer than 2.5cm from the bottom of the beaker.

In-vitro drug release studies:
In Vitro release studies of Azithromycin and Chloroquine from different formulations were performed according to USP XVIII apparatus II, paddle method. Paddle speed was maintained at 50 rpm and 900 mL of 0.1N HCl was used as the dissolution medium. Samples (10 mL) were collected at predetermined time intervals (5, 10, 15, 30 and 45 min) and replaced with equal volume of fresh medium, filtered through a 0.45 μm filter and analyzed with a UV—Visible spectrophotometer at nm. Drug concentration was calculated from a standard calibration plot and expressed as cumulative % drug dissolved.

In vitro drug release studies details:
Apparatus used : USP XXIII dissolution test apparatus, Dissolution medium : 0.1 N HCL, Dissolution medium volume : 900 ml, Temperature : 37 ± 0.5°C, Speed of basket paddle : 50 rpm, Sampling intervals : 5 min, Sample withdraw : 10 ml.

RESULTS AND DISCUSSION

Table no 2: In-vitro dissolution study of Azithromycin

Time (min)	% Drug release					
	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
5	44.00	40.77	41.65	41.2	26.22	30.26
10	71.2	71.64	58.70	59.64	51.92	56.56
15	74.23	83.94	61.45	76.25	66.90	68.39
30	78.05	85.38	73.11	82.25	76.61	80.32
45	81.55	91.61	82.14	91.25	88.21	89.32

Table no 3: In-vitro dissolution study of Chloroquine phophate

Time (min)	% Drug release					
	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
5	47.6	40.77	26.89	29.98	26.37	32.44
10	62.23	65.22	45.36	49.65	55.51	50.31
15	76.3	83.94	66.27	64.25	71.82	67.31
30	83.79	89.32	76.68	85.53	77.55	76.25
45	87.78	91	80	93	89	86

In present work, an attempt has been made to increase the solubility and dissolution rate of Azithromycin and Chloroquine phosphate by complexation techniques using β- Cyclodextrins with varying concentrations and after selection of proper ratio with carries an attempt was made to develop and evaluate fast dissolving tablet of Azithromycin Chloroquine phosphate using synthetic super disintegrates. The standard calibration curves of Azithromycin: Calibration curve for the estimation of Azithromycin was constructed in 0.1 N HCl. at 240 nm the method obeyed Beer's Lambert law in the range of 2-12µg/ml.

The standard calibration curves of Chloroquine phosphate: Calibration curve for the estimation of Chloroquine phosphate was constructed in Distilled water and 0.1 N HCl at 254 nm the method obeyed Beer's Lambert law in the range of 10 60µg/ml.

Precompressional Parameters Study:
Flow properties: The bulk density obtained for all the formulations in the range of 0.52 to 0.58 (g/ml) and the tapped density in the range of 0.55 to 0.61(g/ml).

The Angle of repose of the powder blend of all the formulations was found in range of 28.03 to 29.41 which is in the excellent or in the acceptable range means showing the good flow ability necessary for proper flow of powder blend into the die cavity.

The Carr's index of the powder blend of all the formulations was found in the range of 1.18 to 10.17%, which is good, or in the acceptable range means showing good or fair flow ability for proper flow of powder blend.
The Hausner's ratio was found to be in the range. All these results indicated that, the powder mixture possess good flow of powder blend into the die cavity and compressibility properties.

Angle of Repose of Granules: All batches were evaluated for flow property. The value of bulk density, tapped density of formulation F1 to F6 was in range. The value of hausners ratio of formulation F1 to F6 was in the range. The value of Carr's index of formulation F1 to F6 was in the range. Which indicates the good flow property; the values of Carr's index and angle of repose indicate excellent flow properties.

Post-Compression Parameters Study: The powder blend was compressed using 4 station compression machine. Tablets prepared by using mentioned formula have found to be good without any chipping, capping and sticking. Various physical parameters like thickness, hardness, weight variation, friability, hardness, disintegration time were measured to evaluate tablets. All the formulations have therefore thought to show the acceptable physical parameters of tablets. As per the pharmacopoeial requirement, formulation of oral disintegrating tablet exhibited disintegration in time F1 to F6 batches passes the disintegration time requirement. F6 batch exhibited the least disintegration time

i.e. 29 seconds and acceptable mouths feel. So from above observation it is concluded that the optimized formulations (batch F6) contains hydroxyl properly betacyclodextrin is sufficient to mask the Azithromycin taste with acceptable DT.

Hardness: The hardness of the tablets prepared was determined by Monsanto Hardness tester and found to be within the range of 2.7 kg/cm2 to 3.8 kg/cm2.

Friability test: The friability was found in all designed formulations in the range. to be well within the approved range (<1%).

Weight variation test: The weight variation was found in all designed formulations in the range.and % deviation were in a range. All the tablets passed weight variation test as the average percentage weight variation was within 7.5 % i.e. in the pharmacopoeia limits.

Thickness: The mean thickness was (n=3) almost uniform in all the formulations and values ranged from 3.0 mm. to 3.69 mm. The standard deviation values indicated that all the formulations were within the range.

In- vitro disintegration time: The in-vitro disintegration time was measured by the time taken to undergo complete disintegration. Rapid disintegration within 3 minutes was observed in all the formulations. The disintegration time of all the formulations is checked & is found within the range of 30 sec. to 60 sec.

Wetting time: Wetting time is closely related to the inner structure of the tablet. The wetting time of tablets prepared were found to be in the range of 20 to 37 sec.

Drug Content: In vitro drug release study was performed using USP II dissolution apparatus at 100rpm using 900ml of 0.1N HCl maintained at 37±0.50 C as the dissolution medium. From the data it is evident that as the proportion of the super disintegrating agents in the formulation of Batch No. F2 increases cumulative percent drug release in 45min. The drug content uniformity was performed for all the formulations. The average value and standard deviations of all the formulations were calculated. The percentage drugs content of the tablets were found to be between 99.12% to 99.99%.

IR Studies: FTIR studies were carried out for detection of drug – polymer interaction. In the present study the IR study of pure drug Azithromycin Chloroquine phosphate, drug with Beta-cyclodextrin and Crosscarmalose Sodium, crosspovidone and mixture were carried out to study the compatibility between them.

UV-Visible spectrophotometric study:
UV-Visible spectrophotometric Azithromycin
λ max determination:-The UV spectrum of Azithromycin in 0. N HCl scanned in the range of 400-200 nm. The spectrum indicated that the observed λ_{max} of azitromycin was 240 nm which is matched with pharmacopoeial value.

UV-Visible spectrophotometric Chloroquine phosphate
λ max determination:-The UV spectrum of Chloroquine phosphate in 0. N HCl scanned in the range of 400-200 nm. The spectrum indicated that the observed λ_{max} of Chloroquine phosphate was 254 nm which is matched with pharmacopoeial value.

Preparation of standard calibration curve of Azithromycin:-
Azithromycin showed maximum absorption at wavelength 240 nm in 0.1 N HCl. Standard curve was plotted by taking absorption of diluted stock solutions (2, 4, 6, 8,10,12 µg/ml) at wavelength 240 nm.

Preparation of standard calibration curve of Chloroquine phophate:-
Azithromycin showed maximum absorption at wavelength 254 nm in 0.1 N HCl. Standard curve was plotted by taking absorption of diluted stock solutions (2, 4, 6, 8,10,12 µg/ml) at wavelength 240 nm.

Precompression parameter study:

Table no 4: Precompression parameter study

Formulation code	Angle of repose	Bulk density (wt/ml)	Taped density (wt/ml)	Hausner ratio (%)	Compressibility index(%)
F1	29.29±1.25	0.53±0.02	0.55±0.01	1.03±0.04	3.63±0.23
F2	28.29±0.89	0.55±0.02	0.56±0.02	1.08±0.06	1.57±0.23
F3	28.22±0.49	0.52±0.03	0.59±0.03	1.13±0.07	1.18±0.46
F4	29.41±0.51	0.56±0.02	0.60±0.04	1.07±0.06	6.66±0.58
F5	28.39±1.25	0.53±0.03	0.59±0.03	1.11±0.04	10.16±0.46
F6	29.34±1.12	0.52±0.01	0.61±0.01	1.17±0.04	4.75±0.23

Table no 5: Precompression parameter study

Formulation code	Hardness (kg/cm^2)	Friability (%)	Weight variation (mg)	Thickness (mm)
F1	2.8 ±0.121	0.112±0.025	715±0.220	3.20±0.121
F2	3.1±0.121	0.185±0.094	740±0.331	3.00±0.134
F3	2.9±0.151	0.123±0.045	765±0.232	3.20±0.161
F4	3.0±0.114	0.254±0.110	680±0.235	3.25±0.112
F5	2.7±0.095	0.263±0.065	690±0.165	3.30±0.112
F6	2.9±0.135	0.126±0.056	840±0.230	3.35±0.023

The values represents mean±SD, n = 3

Post compression parameter study

Table no 6: Post compression parameter study

Formulation code	Water absorption ratio (%)	Average Wetting time (sec)	Disintegration time (sec)
F1	75.12±0.124	32±1.25	19±0.122
F2	76.12±0.236	21±1.56	20±0.123
F3	77.36±0.321	20±1.15	22±0.214
F4	74.36±0.123	36±2.03	23±0.321
F5	77.25±0.098	21±1.86	25±0.126
F6	76.65±0.235	37±1.19	29±0.236

The values represents mean±SD, n =3

Table no 7: In-vitro dissolution study Azithromycin of batches F1-F6.

Time (min)	% Drug release					
	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
5	44.00	40.77	41.65	41.2	26.22	30.26
10	71.2	71.64	58.70	59.64	51.92	56.56
15	74.23	83.94	61.45	76.25	66.90	68.39
30	78.05	85.38	73.11	82.25	76.61	80.32
45	81.55	91.61	82.14	91.25	88.21	89.32

Table no 8: In-vitro dissolution study Chloroquine phophate of batches F1-F6

Time (min)	% Drug release					
	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
5	47.6	40.77	26.89	29.98	26.37	32.44
10	62.23	65.22	45.36	49.65	55.51	50.31
15	76.3	83.94	66.27	64.25	71.82	67.31
30	83.79	89.32	76.68	85.53	77.55	76.25
45	87.78	91	80	93	89	86

Table no 9: Kinetic Order of drug release profile Azithromycin

Formulation	Zero order (R)	First order (R)	Higuchi's (R)
F1	0.5332	0.9553	0.9238
F2	0.6971	0.8182	0.8549
F3	0.6862	0.8913	0.9211
F4	0.6971	0.9553	0.9238
F5	0.7889	0.9636	0.9533
F6	0.7637	0.9636	0.9525

Table no 10: Kinetic order drug release profile chloroquine phosphate

Formulation	Zero order (R)	First order (R)	Higuchi's (R)
F1	0.6331	0.8661	0.8905
F2	0.6342	0.831	0.88
F3	0.7575	0.8839	0.9368
F4	0.8387	0.9953	0.9806
F5	0.7475	0.9299	0.928
F6	0.7691	0.9439	0.9558

FTIR OF DRUG, EXCIPIENT

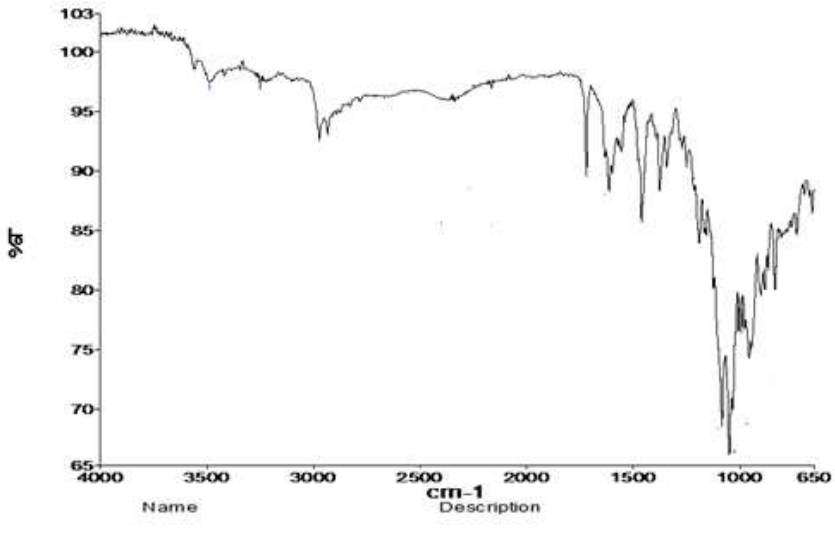

Figure No. 1: FTIR Spectra of Azithromycin

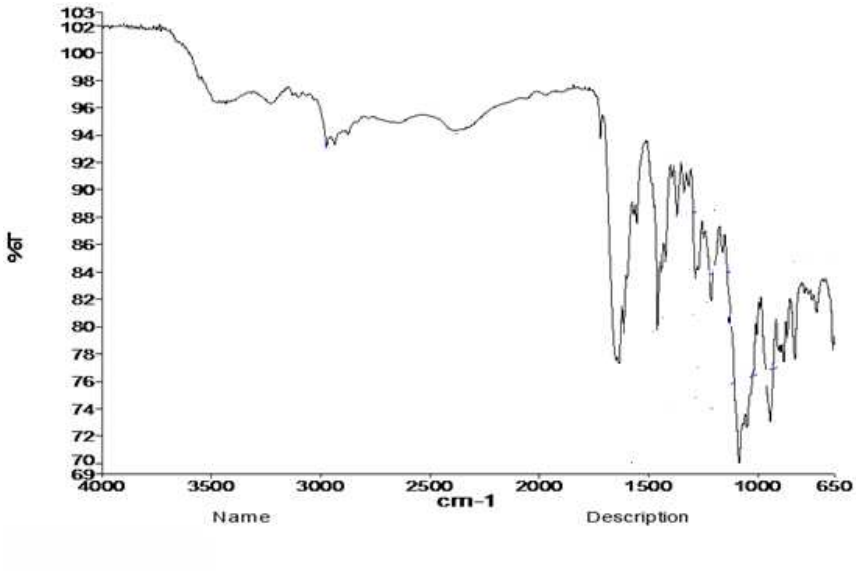

Figure No. 2: FTIR Spectra of Chloroquine phosphate

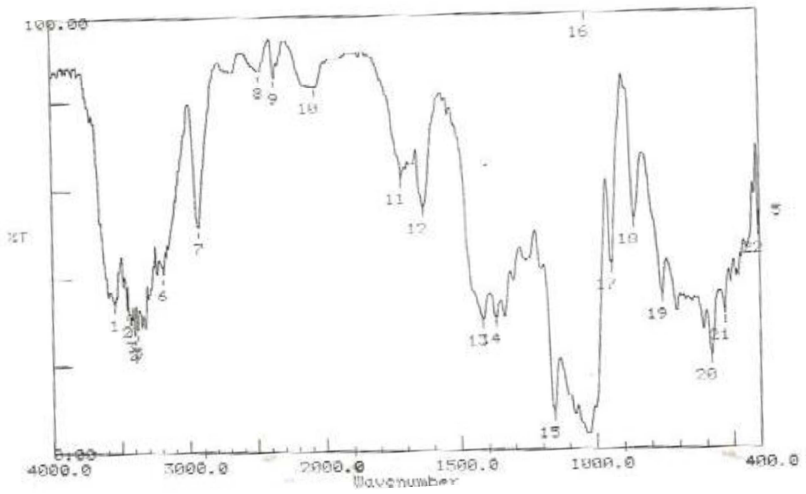

Figure No. 3: FTIR Spectra of β- Cyclodextrin

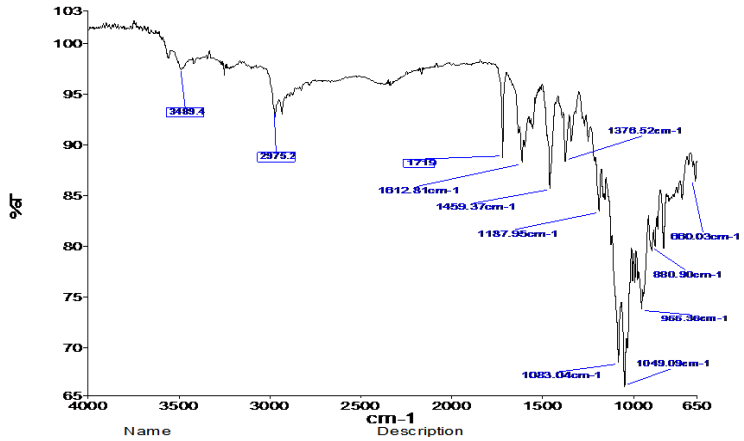

Figure No. 4: FTIR Spectra of Azithromycin and Chloroquine phosphate

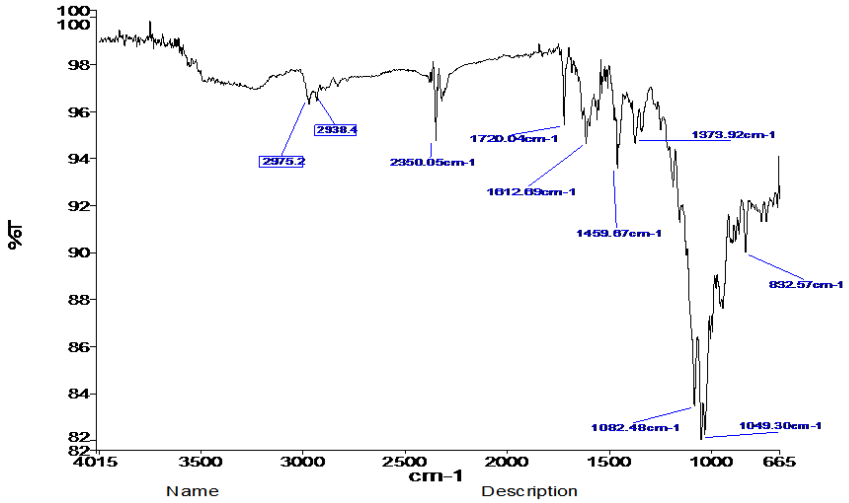

Figure No. 5: FTIR Spectra of Azithromycin, Chloroquine phosphate, β- Cyclodextrins

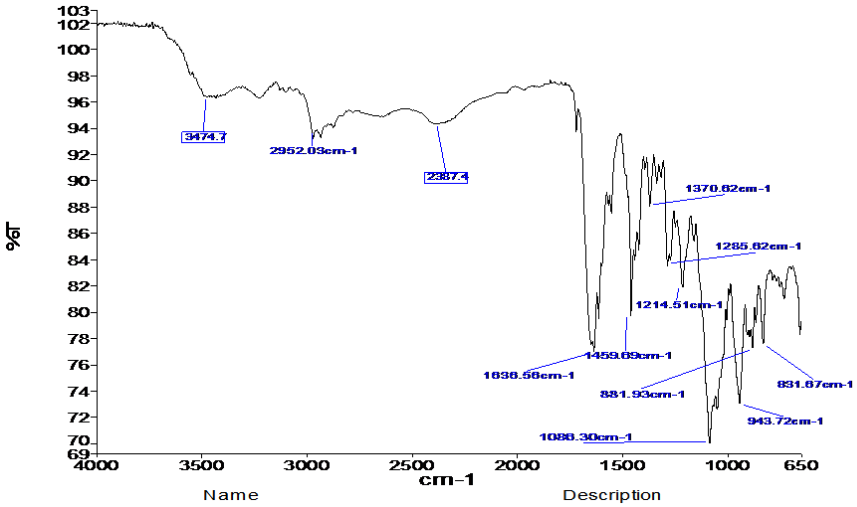

Figure No. 6: FTIR Spectra of Azithromycin, Chloroquine phosphate, crosspovidone

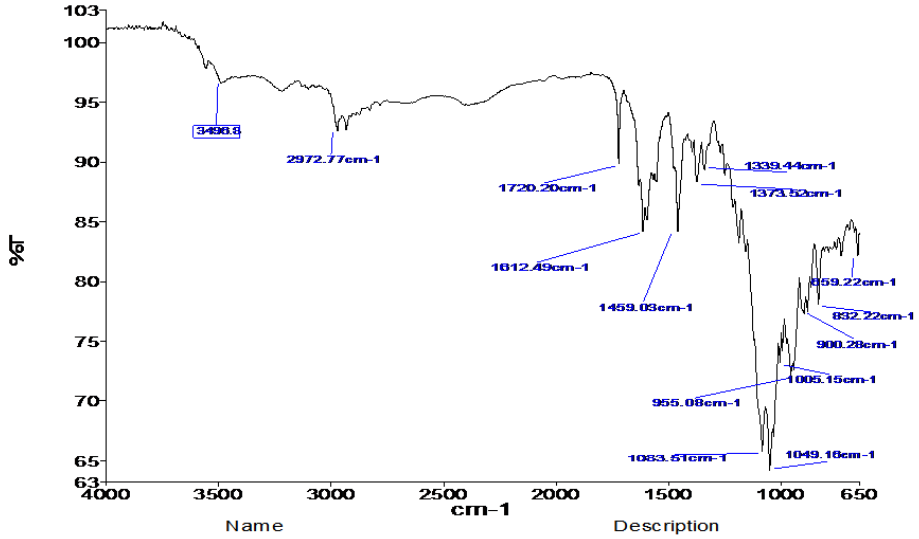

Figure No. 7: FTIR Spectra of Azithromycin, Chloroquine phosphate, Crosscarmalose sodium

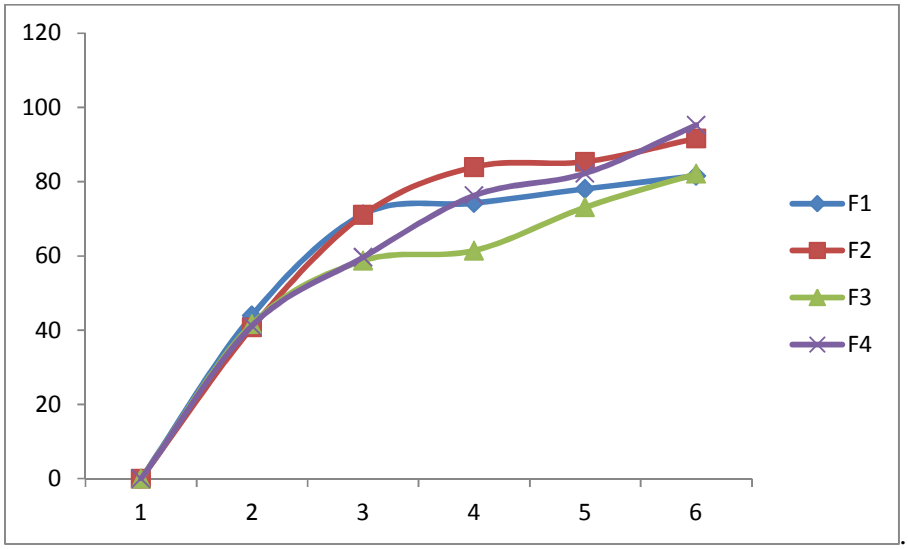

Figure No.8:-In-vitro dissolution study Azithromycin of batches F1 to F4

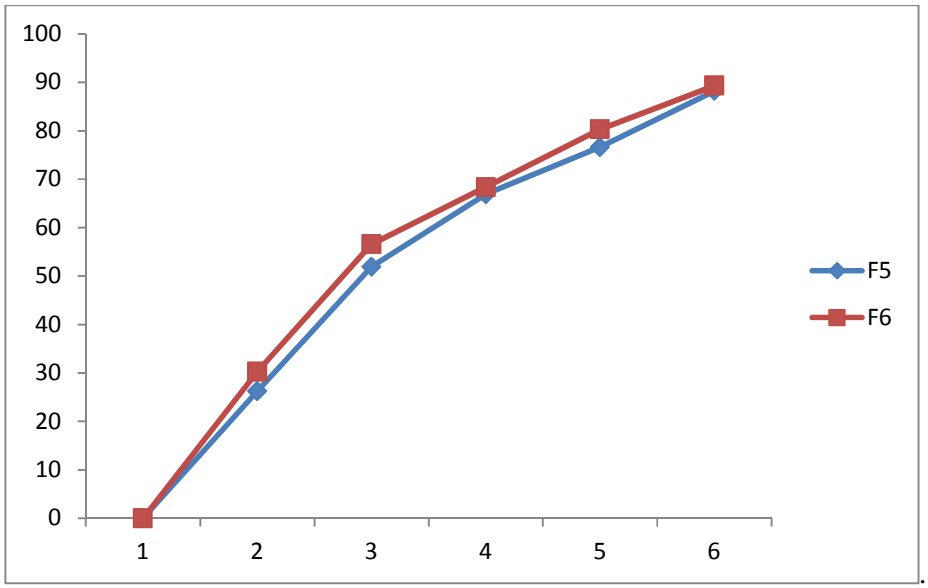

Figure No. 9:-In-vitro dissolution study Azithromycin of batches F5 to F6

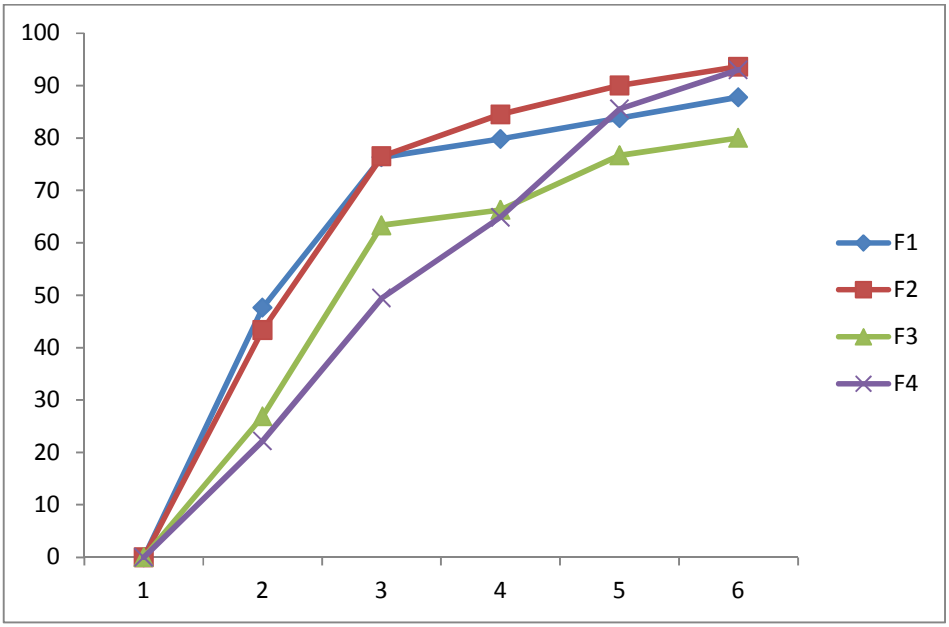

Figure No.10:-In-vitro dissolution study Chloroquine phophate of batches F1 to F4

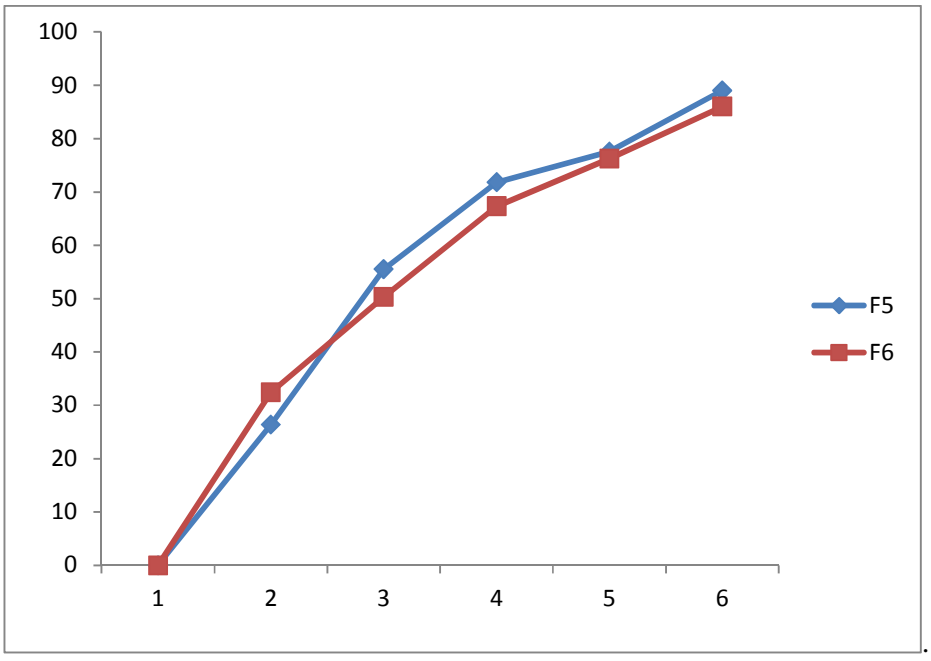

Figure No. 11:-In-vitro dissolution study Chloroquine phophate of batches F5 to F6

CONCLUSION

At the end, from the experiments carried out and results obtained, it can be concluded that the developed formulations achieved the objective of the investigation. The data obtained from the study of "Formulation of Azithromycin and Chloroquine Phosphate FDT by enhancing their Solubility using cyclodextrins complex" it can be concluded that FTIR, % drug content, % drug release, friability , weight variation test of all the formulations were within its range and the disintegration time was from 19-29 Sec. The dissolution study shows that the formulation

containing β-Cyclodextrins from Azithromycin FDT shows 81.55 to 91.61 at the end of 45 min and Chloroquin phosphate shows 80.0 to 90.0% drug release at the end of 45 min.

The study conclusively demonstrated Azithromycin and Chloroquine using beta cyclodextrin comlex and superdisintegrants with varying concentrations showed rapid disintegration and dissolution of FDT. Rapid disintegration of tablets formulated in this investigation may possibly help in administration of bitter drugs in a more palatable form without water. Thus, the "patient-friendly dosage form" of bitter drugs, especially for pediatric, geriatric, bedridden, and non-cooperative patients, can be successfully formulated using this technology.

Acknowledgement
The author wish to express his gratitude to Gangwal Chemical Pvt Ltd. The authors are thankful to the Principal of Channabasweshwar Pharmacy college, Latur, India for providing necessary facilities to carry out this work.

REFERENCES

[1]Divyangi Patel, Jaydeep M Rathod, Dr. K.R Patel, Dr. M.R Patel, *international journal of universal pharmacy and bio* sciences 3(3): mayjune.338-360.**2014**
[2]Md. Nehal Siddiqui, Garima Garg, Pramod Kumar Sharma, *international journal of pharmaceutical sciences review and research*, volume 4, issue 2, september – october **2010**; article 015.
[3]Ujjwal Nautiyal, Satinderjeet Singh, Ramandeep Singh, Gopal, Satinder Kakar, *journal of pharmaceutical, chemical and biological sciences* ISSN: 2348-7658, may **2014**; 2(1):05-26.
[4]k.Kavitha, Kumutha Subramaniam, Boey Jia Hui, K. Santhi, Sa Dhanaraj, And M Rupesh Kumar,; *research journal of pharmaceutical, biological and chemical sciences* october-december **2013** volume 4 issue 4 page no. 1510-1537.
[5]Alok Kumar Gupta, Anuj Mittal And Prof. K. K. Jha, *the pharma innovation*, vol. 1 no. 1.1-8. **2012**
[6]Liang ac and Chen lh, fast-dissolving intraoral drug delivery systems, *expert opin.ther. Patents*, , 11(6). **2001**
[7]Modi a et al. *Pharmacia*: 1(3)95-101. **2012**
[8]Chang rk, guo x, burnside ba, couch ra. fast dissolving tablets. pharm tech,; 24(6):52-58.**2000**
[9] Bonger rah, wilkosz mf, fast dissolving tablet. Accessed **2008**.
[10]Kearney p. process for preparing fast dispersing solid dosage form. Us patent 5,976,577. **1999** nov2; **1998**
[11]Lawrence j, posage g. bicovex rapidly disintegrating dosage form. us patent 6,224,905. dec 3;
[12]Dobetti l. *pharm technol drug delivery*;(suppl):44–50,**2001**
[13]Saravana Kumar K, Sushma M, Prasanna Raju Y, *j. pharm. sci. & res*. vol.5(5),120 – 124. (**2013**).
Government official site References
[1]National Drug Policy for Malaria 2010, NVBDCP, MoH&FW, Govt. of India, **2010**.
[2]Operation Manual for Malaria Control for District level Officers. NVBDCP, **2008**.Website of National Vector Borne Disease Control Programme http://www.nvbdcp.gov.in/malaria-new.html
[3]World Health Organization, WHO Guidelines for the Treatment of Malaria. Geneva. (**2006**).
[4]World Health Organization.
[5]National Institute of Malaria Research Sector 8, Dwarka,New Delhi, NIMR/TRS-06/APR-**2009**.
[6]http://www.malariajournal.com/content/13/1/36.