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FORMULATION AND EVALUATION OF AZITHROMYCIN MICROSPHERES

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ABSTRACT

In the present work, bioadhesive microspheres of Azithromycin using Sodium alginate along with Carbopol 934, HPMC K4M as copolymers were formulated to deliver Azithromycin via oral route. The results of this investigation indicate that solvent removal cross linking method can be successfully employed to fabricate Azithromycin microspheres. The technique provides characteristic advantage over conventional microspheres method, which involves an "all-aqueous" system, avoids residual solvents in microspheres. FT-IR spectra of the physical mixture revealed that the drug is compatible with the polymers and copolymers used. Micromeritic studies revealed that the mean particle size of the prepared microspheres and bulk density and tapped density were conducted are suitable for bioadhesive microspheres for oral administration. The *in-vitro* mucoadhesive study of Azithromycin using sodium alginate along with Carbopol 934 and HPMC K4M as copolymer was performed. The *invitro* drug release increased with increase in the polymer and copolymer concentration. The *invitro* drug release of optimized formulation is compared with marketed formulation and optimized formulation exhibited good drug release. Stability studies of microspheres were conducted for 1 weak 1 month and 2months. Analysis of drug release mechanism showed that the drug release from the formulations followed non-Fickian diffusion And the best fit model was found to be Korsmeyer-Peppas. Based on the results of evaluation tests formulation coded T_4 was concluded as best formulation.

KEYWORDS: Azithromycin, Carbopol, Microspheres, Copolymers.

INTRODUCTION

The oral route for drug delivery is the most popular, desirable and most preferred method for administering therapeutically agents for systemic effects because it is a natural, convenient and cost effective to manufacturing process. Oral route is the most commonly used route for drug administration Even for sustained release systems the oral route of administration has been investigated most because of flexibility in designing dosage forms. present controlled release drug delivery systems are for a maximum of 12 hours clinical effectiveness. such systems are primarily used for the drugs with short elimination half life.

Today these conventional drug delivery systems are widely used. The term drug delivery can be defined as techniques that are used to get the therapeutic agents inside the human body conventional drug therapy require periodic doses of therapeutic agents. These agents are formulated to produce maximum stability, activity and bioavailability.

ADVANTAGES OF CONTROLLED DRUG DELIVERY SYSTEM

- Improved patient convenience and compliance due to less frequent drug administration.
- Reduction in fluctuation in steady state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects.
- Increased safety margin of high potency drugs due to better control of plasma levels. Maximum utilization drug enabling reductin in total amount of dose administered.
- Reduction in health care costs through improved therapy, shorter treatment period, less frequency of dosing and reduction personal time to dispense, administer and monitor patients.

INTRODUCTION TO MICROSPHERES

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 µm. They are made of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems.

ADVANTAGES OF MICROSPHERES

- Taste and odor masking
- Conversion of oils and other liquids to solids for ease of handling
- Protection of drugs against the environment (moisture, light, heat and/or oxidation)

Delay of volatilization, Separation of incompatible materials (other drugs or excipients such as buffers

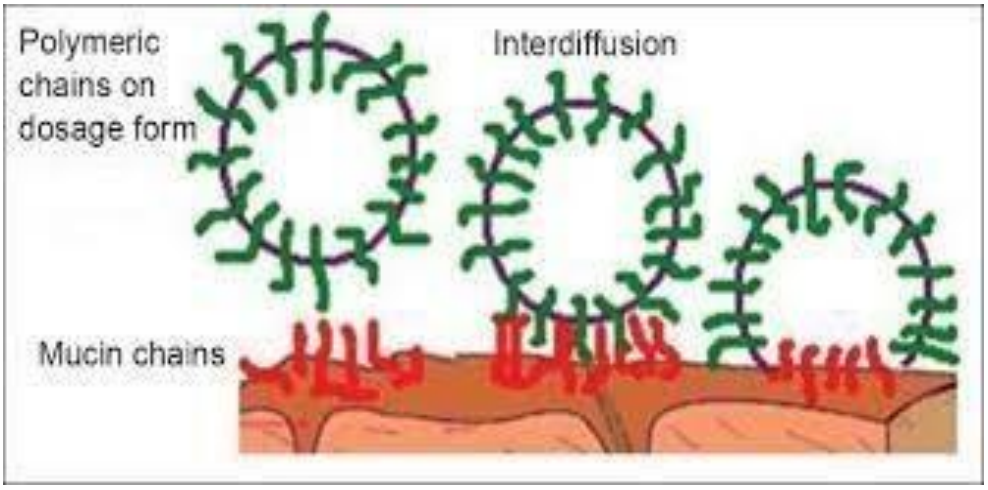
- Improvement of flow of powders
- Production of sustained-release, controlled-release and targeted medications with Reduced dose dumping potential compared to large implantable devices
- Microencapsulation has also been used medically for the encapsulation of live cells and vaccines.

MUCOADHESION / BIOADHESION

Bioadhesion can be defined as the state in which two materials, at least one of which is biological in nature, are maintained together for a prolonged time period by means of interfacial forces.

Diffusion theory

According to this theory, the polymer chains and the mucus mix to a sufficient depth to create a semi permanent adhesive bond. The exact depth to which the polymer chain penetrates the mucus depends on the diffusion coefficient and the time of contact. The diffusion coefficient in terms depends on the value of molecular weight between cross linking and decreases significantly as the cross linking density increases.



Methods of preparation of mucoadhesive microspheres

1) Air suspension
2) Spray drying
3) Polymerization
4) Coacervation
5) Solvent evaporation
6) Wet inversion technique
7) Hot melt microencapsulation
8) Ionotropic gelation technique
9) Orifice ionic gelation method

Mechanism of Action

Azithromycin prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome, thus inhibiting translation of mRNA. Nucleic acid synthesis is not affected.

MATERIALS AND METHODS

Azithromycin, Acetic acid from Pellets India Private Ltd. Sodium chloride from Merch Specialities Pvt Ltd. Mumbai. Methanol, Paraffin, Span-60 and Potassium bromide from Chaitanya Scientifics Vijayawada. Hydrochloric acid, Glutaraldehyde, Petroleum, ether and Chitosan from Srinivasa Scientifics Hyderabad.

Preparation of microspheres by solvent removal method

It is a non-aqueous method of microencapsulation, particularly suitable for water labile polymers such as the polyanhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mixture is then suspended in silicone oil containing span 85 and methylene chloride. After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vacuum.

Swelling Index

Swelling ratio of different dried microspheres were determined gravimetrically in simulated gastric fluid pH 1.2. The microspheres were removed after 24 hours from the solution, blotted to remove excess surface liquid and weighed on balance.41 Swelling ratio (% w/v) was

determined from the following relationship:

$$\text{Swelling ratio} = \frac{(Wt - W0)}{(W0)} \times 100$$

Where

W0 & Wt are initial weight and Final weight of microspheres respectively.

Buoyancy studies

Microspheres (300mg) were spread over the surface of a USP XXIV dissolution apparatus type II filled with 900 mL of 0.1 N HCl containing 0.02% tween 80. The medium was agitated with a paddle rotating at 50rpm for 1 hr. After agitation for a predetermined time interval, the microspheres that floated over the surface of the medium and those settled at the bottom of the flask were recovered separately.42 The microspheres were dried and weighed.

Buoyancy % = Qr/Qr+Qs X 100

Where Qr and Qs are the weight of the floating and the settled microspheres.

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 onto 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 1.2 at 37^0C contained in a 1-litre vessel of the apparatus. At an interval of 1 hour the machine is stopped and number of microspheres still adhering to mucosal surface was counted.

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microspheres adhered}}{\text{Number of microspheres applied}} \times 100$$

Particle size analysis

Samples of the microparticles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 Microparticles sizes were calculated under 45x magnification.

The average particle size was determined by using the Edmondson's equation:

D mean = nd

Where,

n – Number of microspheres observed

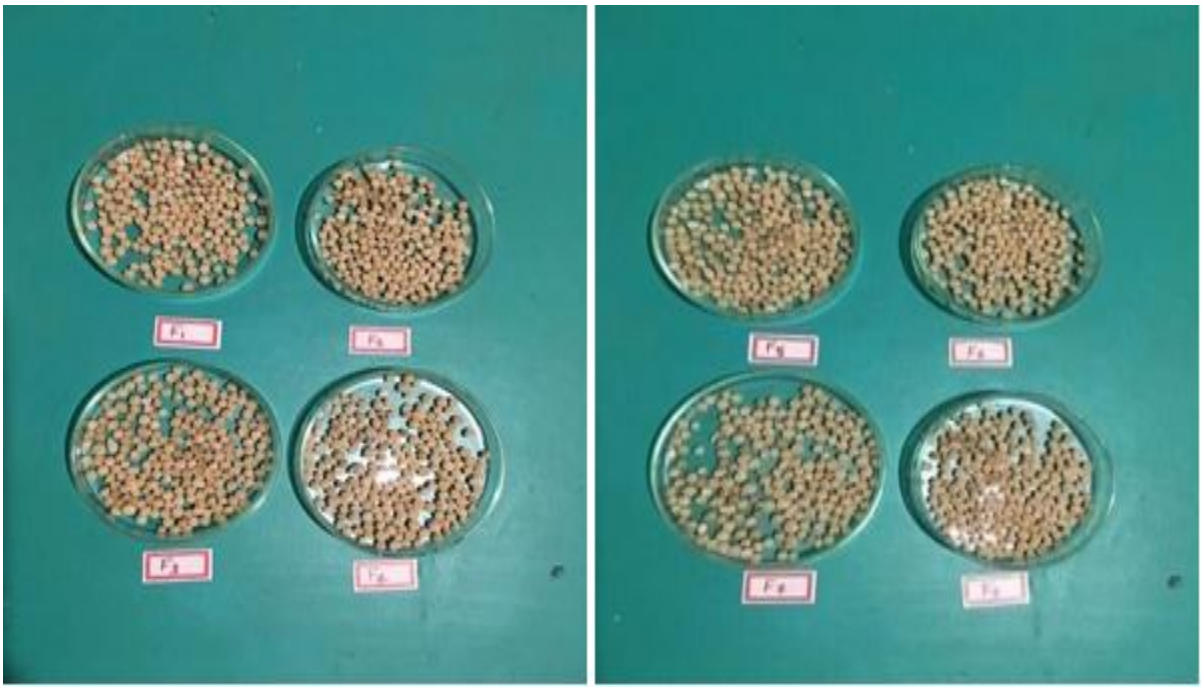
d – Mean size range

RESULTS AND DISCUSSION

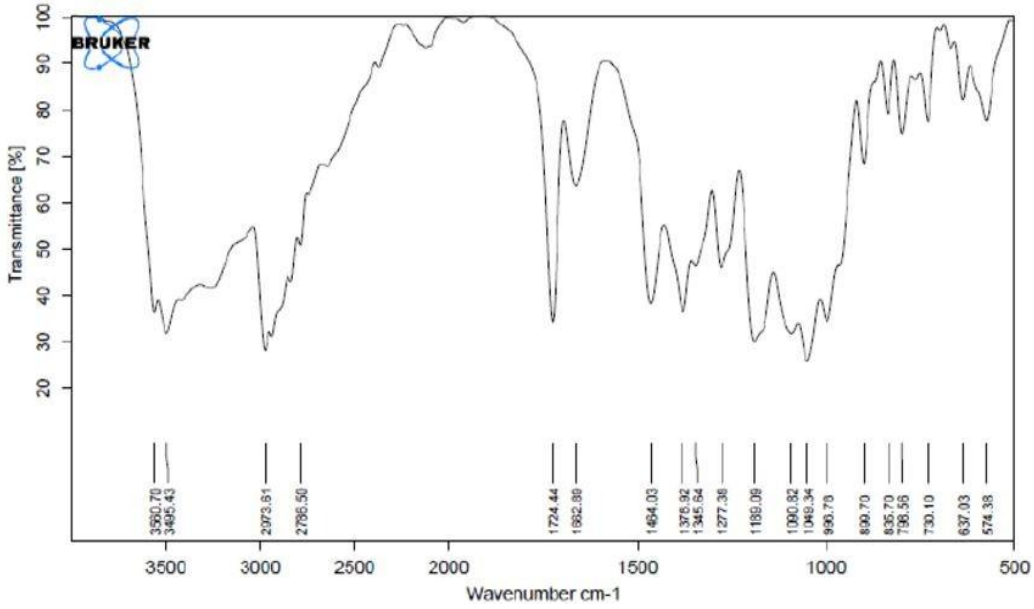
Prepared Formulation Of Bioadhesive Microspheres

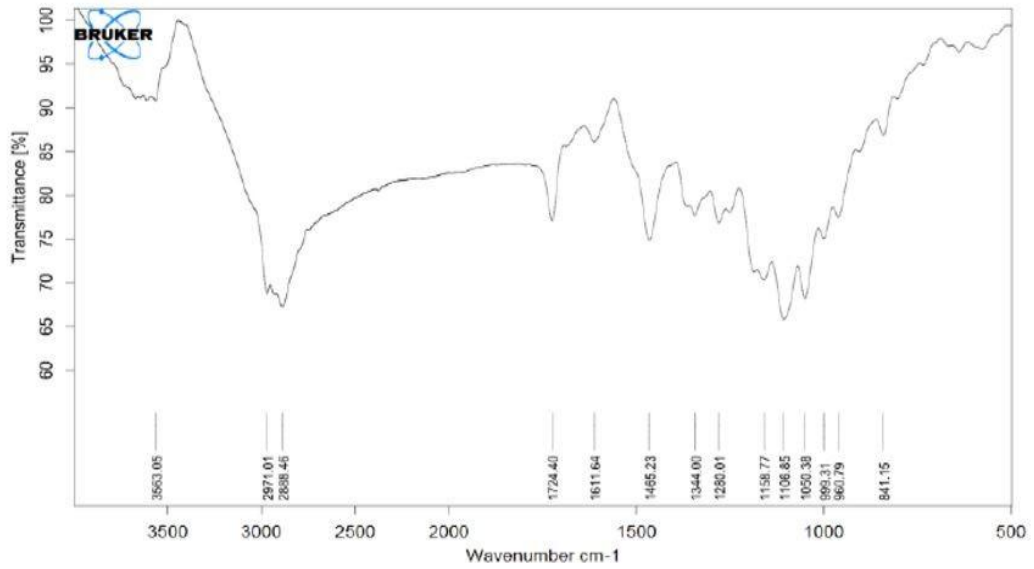
S.NO 1	FORMULATION CODE F1	Drug (mg)	Polymer ratio			Chitosan	Petrolium ether
			Sodiu m alginat e	Carbopo l (934)	HPM C		
		0.3	3	-	-	3	5
2	F2	0.3	4	-	-	3	5
3	F3	0.3	3	2	-	3	5
4	F4	0.3	3	4	4	3	5
5	F5	0.3	3	3.5	-	3	5
6	F6	0.3	3	-	2	3	5
7	F7	0.3	3	-	3.5	3	5
8	F8	0.3	3	2	2	3	5

Photograph of Prepared Microspheres



FTIR of pure drug of Azithromycin and FTIR of optimized formulation(F4)





Micrometric properties of formulated microspheres.

FORMULATIO N CODE	BULK DENSIT Y g/cm^3	TAPPED DENSIT Y	HAUSNER' S RATIO	ANGLE OF REPOS E	CARR' S INDEX
F1	0.45	0.81	0.5505	1.2	44.44
F2	0.492	0.802	0.0427	1.56	18.85
F3	0.502	0.84	0.0418	1.5	24.23
F4	0.562	0.857	0.0458	1.46	20.12
F5	0.42	0.79	0.5316	1.37	25.83
F6	0.411	0.75	0.0217	1.38	20.2
F7	0.35	0.8	0.0307	1.2	36.25
F8	0.53	0.877	0.0421	1.35	26.25

Percentage yield and percentage drug entrapment efficiency of the prepared microspheres.

S.No	Formulatio n code	% yield	Drug Content (mg)	% Drug entrapment efficiency
1	F1	80	12.40	82.66
2	F2	83.33	12.66	84.4
3	F3	85	12.70	84.66
4	**F4**	**88**	**13.29**	**88.66**
5	F5	62.22	8.07	53.2
6	F6	80	8.25	55
7	F7	80	10.33	68.86
8	F8	87	11.5	76.66

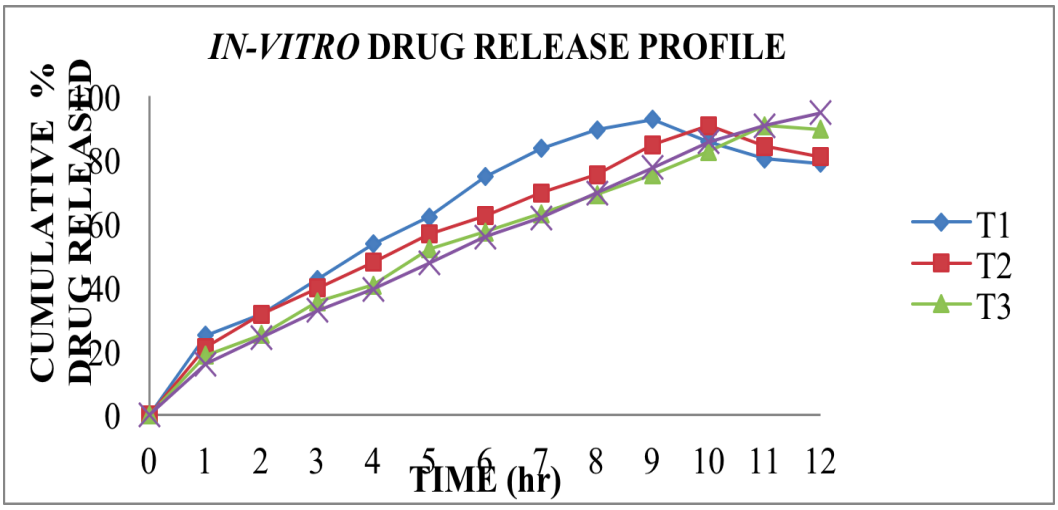
Percentage mucoadhesion, percentage swelling, buoyancy % and average particle size of the prepared microspheres(f1-f8).

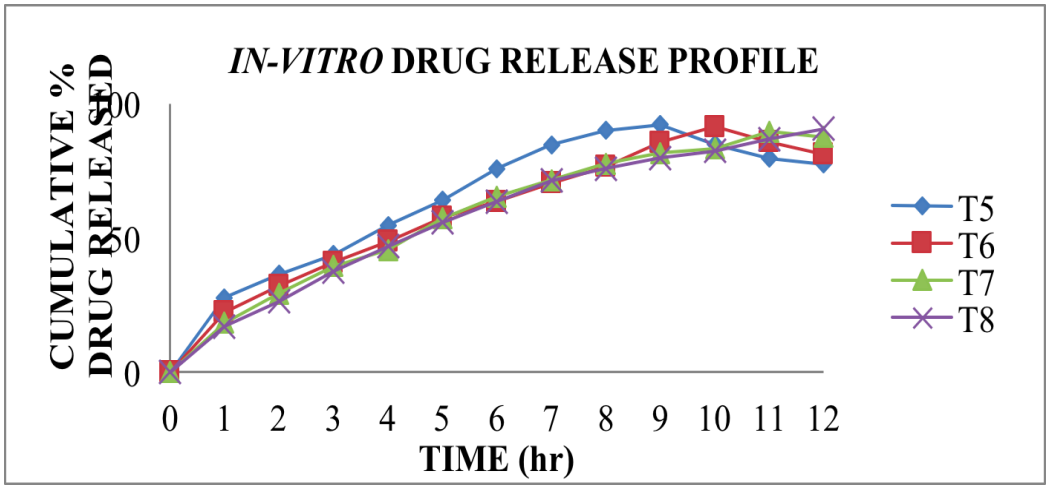
S.NO.	FORMULATION code	PERCENTAGE MUCOADHESION	PERCENTAGE SWELLING	Buoyanc y %	AVERAG E PARTICL E SIZE
1	F1	65	28	52.349	512
2	F2	70	42	59.375	617
3	F3	75	62	64.024	711
4	F4	**85**	**85**	**82.386**	**834**
5	F5	60	24	75.439	517
6	F6	65	39	79.781	642
7	F7	70	55	81.386	792
8	F8	75	64	70.833	826

***In-Vitro* drug release data of Azithromycin encapsulated microspheres (F1-F8).**

TIME (hrs)	CUMULATIVE PERCENT OF DRUG RELEASE							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
0	0	0	0	0	0	0	0	0
1	24.88	21.11	18.66	15.88	27.77	22.44	18.44	17.11
2	31.55	31.55	25.11	24.22	36.44	32.22	29.33	26.44
3	42.44	39.77	35.44	32.66	43.77	40.88	39.55	37.55
4	53.55	47.77	40.66	39.33	54.66	48.66	45.55	46.88
5	62	56.66	52	47.55	64.01	57.55	57.33	55.77
6	74.66	62.44	57.33	55.77	75.77	63.55	65.33	63.55
7	83.55	69.55	63.11	61.77	84.65	70.44	71.55	71.33
8	89.33	75.33	69.11	69.55	90	76.55	77.56	75.77
9	92.66	84.66	75.33	77.55	92.22	85.55	81.55	79.77
10	85.55	90.66	82.66	85.55	84.88	91.33	83.33	82.44
11	80.22	84.22	90.66	87.42	79.55	85.77	89.55	86.88
12	78.88	80.88	89.55	92.66	77.55	81.11	87.55	90.66

Comparison of *In-Vitro* drug release profile of Azithromycin encapsulated microspheres

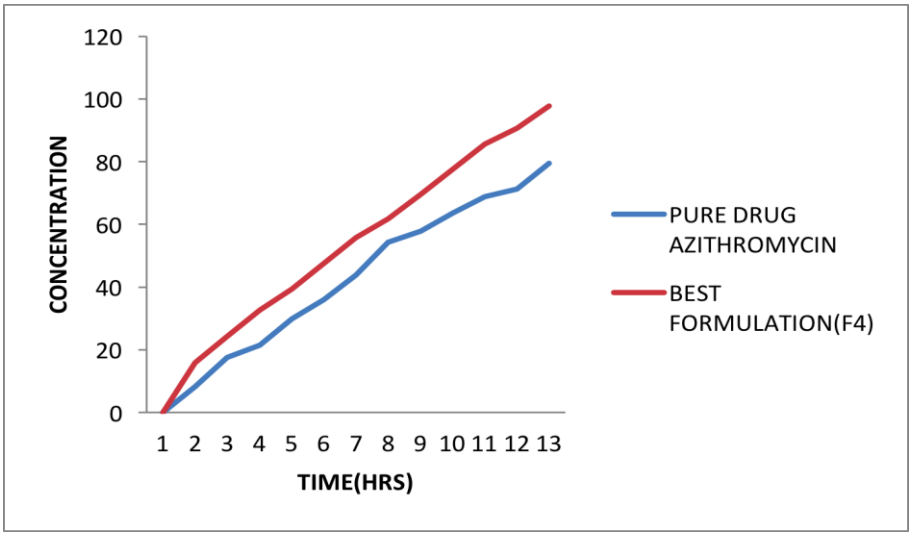




Comparison of Drug dissolution studies between optimised formulation and marketed formulation drug of Azithromycin.

TIME(hr)	MARKETED FORMULATION AZITHROMYCIN	BEST FORMULATION(F4)
0	0	0
1	8.16	15.88
2	17.56	24.22
3	21.42	32.66
4	29.85	39.33
5	36.01	47.55
6	43.88	55.77
7	54.31	61.77
8	57.73	69.55
9	63.63	77.55
10	68.9	85.55
11	71.22	87.42
12	79.52	92.66

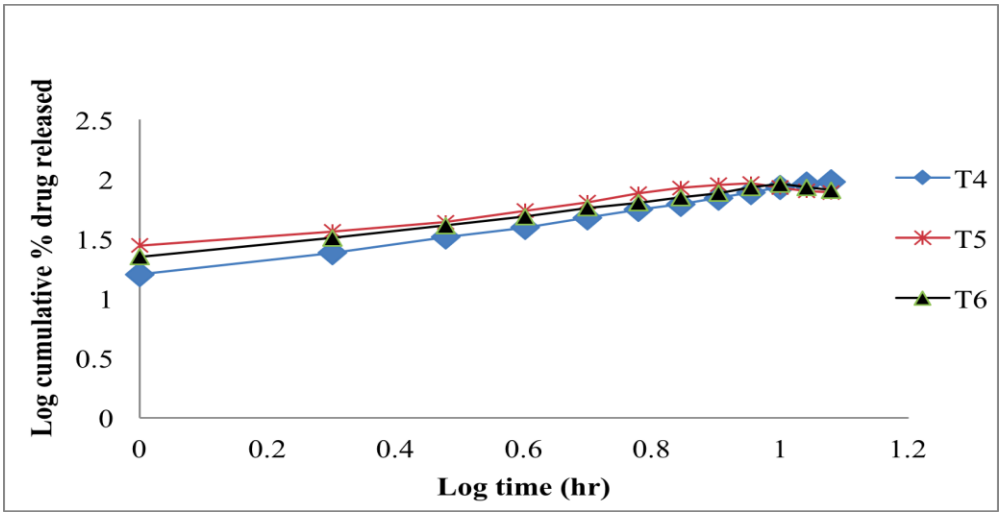
Comparison of Drug dissolution studies between optimised formulation and marketed formulation drug of Azithromycin



RELEASE KINETICS STUDIES OF THE PREPARED FORMULATIONS

Formulation code	Release model								
	Zero order		First order		Higuchi matrix		Koresmeyer-peppas		
	K	R^2	K	R^2	K	R^2	N	K	R^2
F1	21.6	0.797	1.923	0.720	-0.313	0.912	0.556	1.388	0.925
F2	16.39	0.908	1.991	0.890	-3.945	0.970	0.595	1.326	0.983
F3	10.45	0.976	2.062	0.945	-8.966	0.975	0.673	1.233	0.991
F4	7.434	0.990	2.118	0.914	-12.25	0.962	0.743	1.171	0.996
F5	24.34	0.768	1.897	0.689	2.624	0.903	0.498	1.442	0.914
F6	17.19	0.904	1.990	0.885	-3.333	0.971	0.579	1.346	0.981
F7	14.53	0.936	2.018	0.985	-6.239	0.983	0.655	1.278	0.990
F8	13.06	0.948	2.032	0.991	-7.587	0.984	0.690	1.241	0.991

Korsmeyer-Peppas plots of Azythromycin microspheres formulations T_4, T_5 and T_6



Stability studies of optimized formulation of microspheres

Formu lation code	Parameters	Observation on storage for			
		INITIAL	AFTER 1 WEEK	AFTER 2 MONTHS	AFTER 3 MONTHS
F4	Drug release	97.66	97.56	97.41	97.4
F8		90.66	90.63	90.53	90.43
		89.55	89.51	89.47	89.43

CONCLUSION

It was concluded that F4 was selected as best formulation based on results of evaluation tests Azithromycin using sodium alginate, carbapol 934 along with HPMC K4M as copolymers in the ratio of 3:4:4 was selected as optimised formulation upon the observation of micrometric properties which are within the range and increase in the polymer concentration leads to the increase of percentage yield drug entrapment efficiency, muco adhesion and buoyancy. invitro drug release was found to be 92% compared with the marketed formulation i.e, 79% upon observation on storage for one week, two months and three months they were found to be stable by exhibiting the drug release.

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REFERENCES

1. Mulugeta Fentie*, Anteneh Belete and Tsige Gebre-Mariam Formulation of Sustained Release Floating Microspheres of Furosemide from Ethylcellulose and Hydroxypropyl Methylcellulose Polymer Blends J Nanomed Nanotechnol, 2015; 6: 1 http://dx.doi.org/10.4172/2157-7439.1000262
2. Abhijeet A. Durgavale*, Archana R. Dhole, Shrinivas K. Mohite, Chandrakant S. Magdum Formulation and Evaluation of Floating Microsphere of Captopril using Different Gas Forming Agents. Am. J. Pharm Tech Res. 2012; 2(2).
3. A Salomy Monica Diyya*, V Rakesh Kumar Formulation and In Vitro Evaluation of Floating Microspheres of Glipizide, 2015; 3(2): Page-606-615 606 IIIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved CODEN (USA)-

IJPRUR, e-ISSN: 2348-6465.

4. Megha Sharma,[a,c] Seema Kohli,[b], Agnimitra Dinda *In-vitro* and *in-vivo* evaluation of repaglinide loaded floating microspheres prepared from different viscosity grades of HPMC polymer Saudi Pharmaceutical Journal Available online 27 February 2015.
5. Pavan Kumar Perumandla*, Sree Priya Formulation And In Vitro Evaluation Of Floating Microspheres Of Dextromethorphan Hydrobromide International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 6, Issue 4, 2014.
6. S.K.Senthilkumar1*, B. Jaykar 2, S.Kavimani3 Formulation, Characterization and Invitro Evaluation of Floating Microsphere Containing Rabeprazole Sodium /JITPS 2010; 1(6): 274-282.
7. V. Sai Kishore*, B. Tejeswara Rao, K. Pavani, D. Nagasen, K. Rakesh Varma and D. Gowtham Plantago Ovata Seeds And Bhringaraj Leaves As Superdisintegrants: Formulation And Evaluation Of Sotalol Hydrochloride Orodispersible Tablets IJPCBS, 2013; 3(4): 1040-1046.
8. Sanjeev Kumar 1*, Tara Chand 1 Formulation and Development of Floating and Mucoadhesive Microspheres of Clarithromycin The Pharma Innovation – Journal Vol. 2 No. 5 2013.
9. Mukesh P. Ratnaparkhi*, Suresh B. Dhiwar, Kiran E. Dhage, Sharvari S. Bhore, Pratiksha M. Kadam and Pradeep S. Patil Formulation and in-vitro characterization of floating microspheres of Metfomin HCl Scholars Research Library Der Pharmacia Lettre, 2012; 4(5): 1390-1400.
10. Kapil Kumar1* and AK Rai2 Development and Evaluation of Floating Microspheres of Curcumin Tropical Journal of Pharmaceutical Research, October 2012; 11(5): 713-719.
11. Parmar Kunal Vinodbhai a, Dr. M.C. Gohel b, Dr. R.K. Parikh b, Dr. Shital Bariyac Ms. Rajeshvari N. Suthar Sustained Release Floating Microspheres Of Acyclovir: Formulation, Optimization, Characterization And In Vitro Evaluation International Journal of Drug Development & Research | Jan-March 2011 | Vol. 3 | Issue 1 | ISSN 0975-9344 | Available online http://www.ijddr.in