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FORMULATIONANDEVALUATIONOFMETRONIDAZOLETABLETED MICROSPHERE FOR COLONIC DELIVERY

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Abstract:
The objective of this study was to formulate and evaluate metronidazole tableted microspheres aimed at targeted colonic delivery, enhancing the therapeutic efficacy and reducing systemic side effects. Metronidazole, an antimicrobial agent widely used in the treatment of colonic infections and inflammatory bowel diseases, benefits significantly from site-specific delivery. Microspheres were prepared using an emulsion-solvent evaporation method, incorporating metronidazole into a biodegradable polymer matrix of Eudragit S100, designed to release the drug specifically in the colonic environment.Characterization of the microspheres included particle size analysis, surface morphology via scanning electron microscopy (SEM), encapsulation efficiency, and in-vitro drug release studies. The particle size of the microspheres ranged between 50-150 µm with a smooth surface, ensuring uniform distribution. Encapsulation efficiency was determined to be 85%, indicating effective drug loading within the microspheres. In-vitro release studies in simulated gastrointestinal fluids showed minimal drug release in the acidic environment of the stomach and the neutral pH of the small intestine. However, a significant release was observed in the colonic pH environment, confirming the targeted delivery potential of the formulation.Tableting of the microspheres was performed using direct compression with suitable excipients, maintaining the integrity of the microspheres. The tableted microspheres demonstrated acceptable hardness, friability, and disintegration time, aligning with pharmacopeial standards.In conclusion, the formulated metronidazole tableted microspheres exhibit promising characteristics for colonic delivery, providing a potential approach for enhancing the treatment of colonic diseases while minimizing systemic exposure and side effects. Further in-vivo studies are warranted to corroborate these findings and optimize the formulation for clinical application.

Keywords: metronidazole tableted microspheres, targeted colonic delivery, antimicrobial agent

INTRODUCTION

Colonic drug delivery refers to the targeted release of pharmaceutical substances specifically in the colonregionofthe gastrointestinaltract.Thismethod isemployed to improve thetherapeutic efficacy and reduce the side effects of certain drugs by delivering them directly to the colon.
Therareseveralreasonswhydrugsmightbetargetedtothecolon:[1]

- Treatment of Colonic Diseases: Drugs designed to treat conditions such as inflammatory boweldisease(IBD), colitis, or colorectalcancer can benefit fromtargeted deliveryto the colon.
- LocalAction: Some drugsact locally inthe colon, suchasdrugsfortreating coloncancer or localized infections.
- Systemic Absorption: Certain drugs are absorbed more efficiently in the colon due to its unique physiology, which can help in achieving desired therapeutic levels while minimizing systemic side effects.

Varioustechniquesareemployedtoachievecolonicdrugdelivery:[2]

1. **pH-Sensitive Coatings:** A lot of formulations include pH-sensitive coatings, which dissolve and release the medicationwhentheyreachthe more neutralpH ofthe colonbut do not break down in the acidic environment of the stomach and small intestine.
2. **Time-Release** Formulations: These formulations make sure the medication reaches the colon undamaged by releasing the drug after a certain amount of time.
3. **Microbial Degradation**: Whena medication isconjugated withpolysaccharides, colonic bacteria break them down and release the active ingredient.
4. **Prodrug** Approach: Prodrugs are inert substances that the colon metabolizes to produce the active medication.

Colonic drug deliverysystemsoffer severaladvantages, including enhanced drug bioavailability, reduced systemic side effects, and improved patient compliance. However, challenges such as variability in colonic transit time and potential interindividual differences in colonic physiology need to be addressed in the design and development of these delivery systems.[3]

Typesofcolonicdrug delivery

There are various types of colonic drug delivery systems designed to target pharmaceutical substances specifically to the colon. Here are some common types:[4]

1. **pH-Dependent Systems**: These systems take use of the variations in pH throughout the digestive system. The purpose of coatings and formulations is to release the medication bydissolving inthe more neutralpH environment ofthe colonwhile resisting breakdown in the acidic environment of the stomach and small intestine.
2. **Time-Controlled Release Systems**: These systemsare designed to release the drug after a predetermined period, ensuring that it reaches the colon intact. Various mechanisms such as erosion, diffusion, or osmosis control the release of the drug.
3. **Microbially Triggered Systems**:Thesesystemstakeadvantageoftheenzymaticactivity of colonic bacteria. The drug is conjugated with polymers or other materials that are degraded by colonic bacteria, releasing the active drug at the desired site.
4. **Coating with Enteric Polymers**: Enteric coatings are used to protect the drug from degradation in the stomach and small intestine and ensure its release in the colon. These coatings can be pH-dependent or time-dependent.
5. **Prodrug Approach**: Prodrugs are inactive compounds that are metabolized in the colon to release the active drug. This approach can enhance colonic drug delivery by utilizing

enzymatic activity in the colon to convert the prodrug into its active form.[5]

6. **Microbial-Triggered Delivery**: Some systems utilize bacteria-specific enzymes to trigger drugrelease. These systemsrelyonthe presence ofspecific bacterialstrains inthe colon to activate drug release.
7. **Multi-Particulate Systems**: In these systems, drugs are encapsulated in microspheres or nanoparticles, allowing for controlled release and targeting to specific regions of the colon.
8. **Bioresponsive Systems**: These systems respond to changes in physiological parameters such as pH, enzymes, or bacterial activity in the colon to trigger drug release. They canbe designed to release the drug in response to specific colonic conditions.

Eachofthese colonic drug deliverysystems has its advantages and limitations, and the choice of system depends on factors such as the physicochemical properties of the drug, desired release kinetics, and patient-specific considerations.

Advantagesofcolonicdrug delivery[6-15]

Colonicdrug deliveryoffersseveraladvantagesoverconventionaldrug deliverymethods:

- **Targeted Drug Delivery**: Colonic drug delivery systems allow for the targeted release of drugs specifically in the colon region of the gastrointestinal tract. This targeted delivery can enhance the therapeutic efficacy of drugs intended to treat colonic diseases or conditions localized in the colon.
- **Reduced Systemic Side Effects**: By delivering drugs directly to the colon, colonic drug delivery systems can minimize systemic exposure to the drug, reducing the risk of systemic side effects. This is particularly beneficial for drugs with potential adverse effects on other organs or systems in the body.

FORMULATIONDEVELOPMENT

- **Preparation of microspheres**: Solvent evaporation was used to createthe enteric coated microspheres. Table provided the medication to polymer ratio used in the preparation of theentericcoated microspheres.Usingamagneticstirrer,thepolymerwasdissolvedin10 milliliters of acetone to create the solution. After then, the medication was scattered throughout the polymer solution. After that, the resultant dispersion was added to a 250 ml vesseltogether with30 mlof liquid paraffin, and it was stirred at a minimumspeed of 1000 rpm. After two hours of stirring, all of the acetone evaporated. The microspheres that were created after the acetone evaporated were filtered and given four or five hexane washes. The cleaned microspheres were then collected after drying at room temperature.
- **Preparation of tabletted microspheres:** Using Mg stearate 27 as lubricant, cross-povidoneasbinder, andmicrocrystallinecelluloseasdiluents,theoptimizedMNZ loaded microspheres were compressed to create tablets. A 250 mg tablet was made, and the formulations of batches 1, 2, and 3 (F5, F8, and F14) were optimized to compress a 10mg tablet of medication. The tablets were coded T1, T2, and T3 for each batch. Table shows the quantity of excipients needed to make a 250 mg tablet and the quantity of microspheres comparable to a 10 mg medication.[16-29]

Table1:FormulationTableofTablettedMicrospheres

S.No.	Formulation Code	MNZ Microspheres (mg)	Cross Povidone (mg)	Mg Stearate (mg)	Microcrystalline cellulose (mg)
1.	**T1**	142.5	11.40	2.85	93.25
2.	**T2**	88	7.04	1.76	153.2
3.	**T3**	122.5	9.80	2.45	115.25

Table2. FormulationTableforMicrospheres

S.No.	Batch	Formulation	Drug (mg)	Polymer(mg)			Liquid Paraffin (ml)	Acetone (ml)
				CAP	HPMCP	EudragitS 1 00		
1.	Batch1	**F1**	100	100	---	---	25	10
2.		**F2**	100	200	---	---	25	10
3.		**F3**	100	300	---	---	25	10
4.		**F4**	100	400	---	---	25	10
5.		**F5**	100	500	---	---	25	10
6.	Batch2	**F6**	100	---	100	---	25	10
7.		**F7**	100	---	200	---	25	10
8.		**F8**	100	---	300	---	25	10
9.		**F9**	100	---	400	---	25	10
10.		**F10**	100	---	500	---	25	10
11.	Batch3	**F11**	100	---	---	100	25	10
12.		**F12**	100	---	---	200	25	10
13.		**F13**	100	---	---	300	25	10
14.		**F14**	100	---	---	400	25	10
15.		**F15**	100	---	---	500	25	10

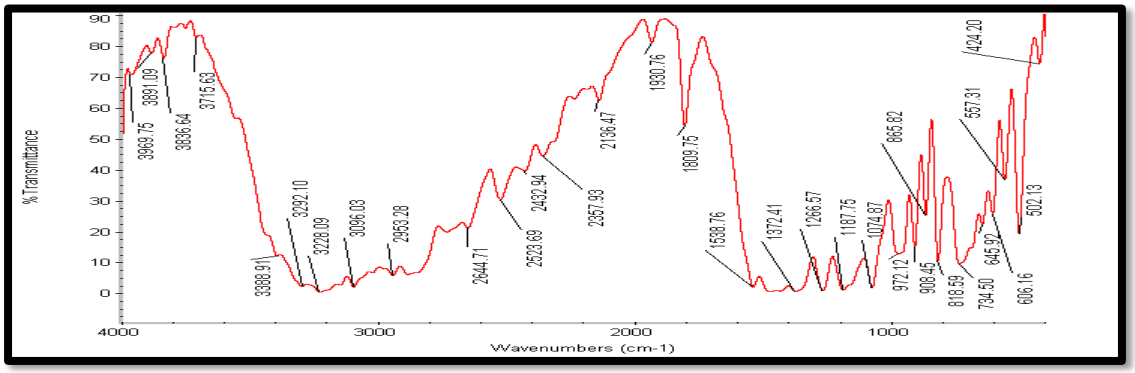
IDENTIFICATION OF DRUG

- **Determination of melting point:** The melting point of MNZ was found to be 161.33°C±0.577. The reported valve of melting point is 159°C-163°C.
- **Fourier Transforms Infrared (FT-IR) Spectroscopy Analysis:** Identification of MNZ was carried out by the FT-IR spectroscopy. The following peaks were found which are given in Table and the FT-IR spectra are shown in Figure which revealed that given drug is MNZ

Table3 Major in frared band assignments of MNZ

S.No.	Assignments	ReportedBandPosition(cm^{-1})	Observed BandPosition(cm^{-1})
1.	-OH(str)	3230	3228.09
2.	-C-CH(str)	3105	3096.03
3.	-N-O(str)	1538&1375	1538.76&1372.41
4.	-C-O(str)	1078	1074.87
5.	-C-N(str)	830	818.59

Fig1. FT-IRspectraofDrug



PREFORMULATIONSTUDIES

Determination of Solubility: The solubility of MNZ was determined in the different media mainly water, 0.1N HCl, phosphate buffer pH 6.8and 7.4. The solubilityprofile indifferent media is given in table 6.7 with their reported valve.

Table5 Table for solubilityprofile of MNZ in different solven

S.No.	Solvent	Solubility(mg/ml)				
		Observedvalue				Reported value[70]
		S1	S2	S3	mean±S.D.	
1.	Water	10.3	10.2	10.09	10.19±0.109	10.2
2.	0.1NHCl	37.1	36.9	37.09	37.03±0.112	32.9
3.	PhosphatebufferpH6.8	12.1	12.4	12.2	11.8±0.152	12.3
4.	PhosphatebufferpH7.4	11.2	11.1	11.2	11.16±0.057	11.63

DeterminationofPartition coefficient

ThepartitioncoefficientofMNZwasfoundtobe-0.212±0.0005.Thereportedvalveofpartition coefficient is -0.27. 6.3.3

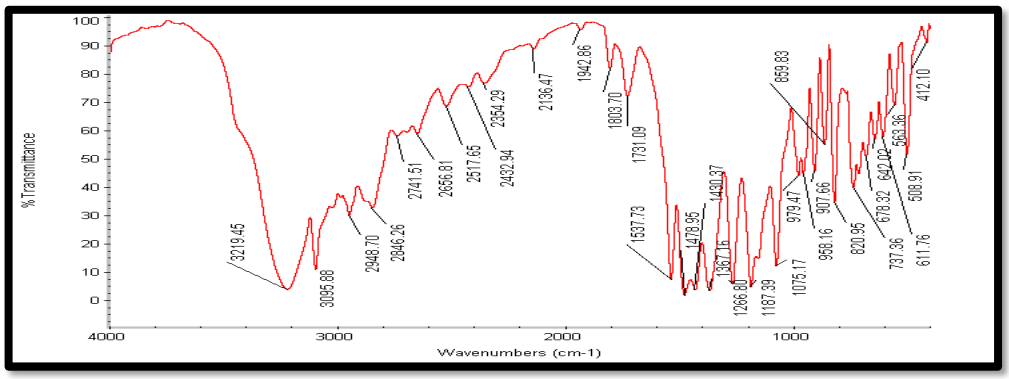
DeterminationofDissociationcoefficient

ThedissociationcoefficientofMNZwasfoundtobe2.606±0.005. Thereportedvalueis2.63.6.3.4

Drug-Excipientcompatibilitystudies

TheFT-IRspectraofphysicalmixture ofdrug-polymeraregiven inTableand represented inFigures

Fig2.FT-IRspectraofphysicalmixtureofDrugand CAP



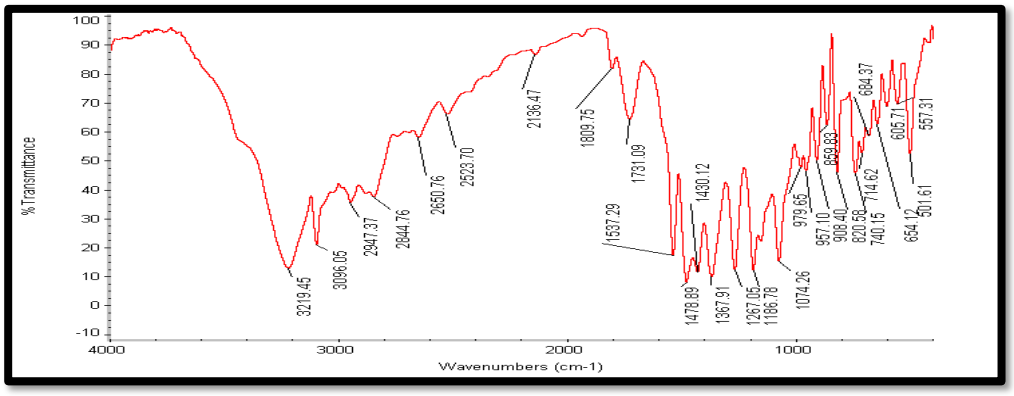


Fig3.FT-IRspectraofphysicalmixtureofDrugandHPMCP

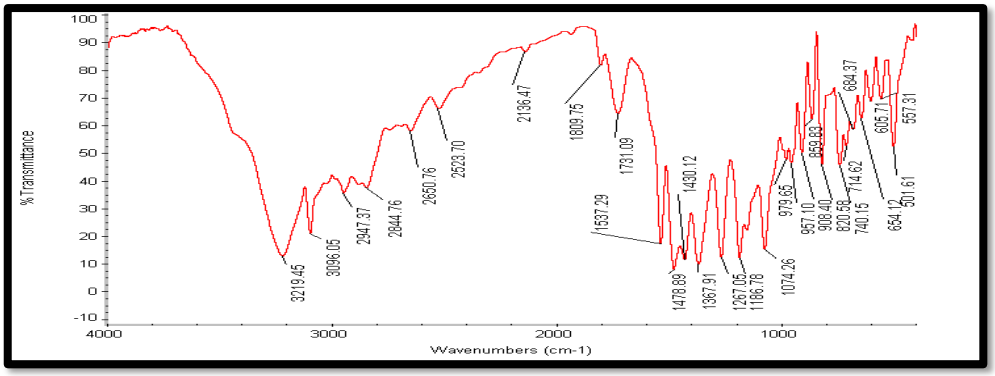


Fig4.FT-IRspectra ofphysicalmixtureofDrugand EudragitS 100

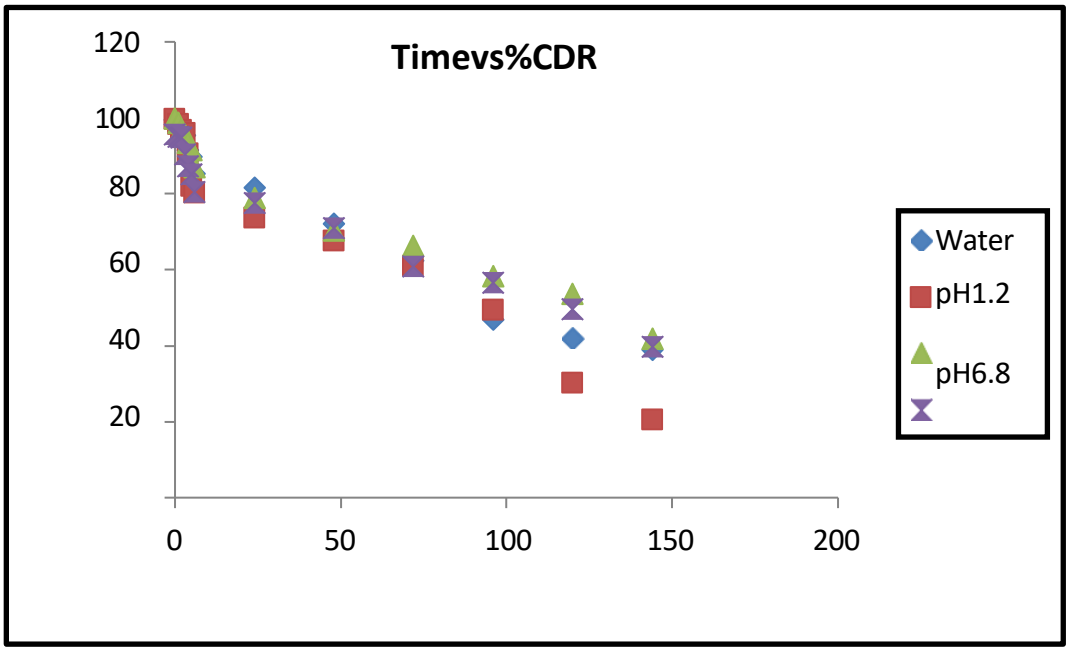
Table6.Majorinfraredbandassignmentsofdrugin physicalmixtureofdrugpolymers

Assignments	Band Position in Drug(cm^{-1})	BandPositioninphysicalmixtureofdrugand polymers (cm^{-1})		
		CAP	HPMCP	Eudragit S 100
-OH(str)	3228.09	3219.45	3219.45	3221.55
-C-CH(str)	3096.03	3095.88	3096.05	3097.03
-N-O(str)	1538.76 & 1372.41	1537.73 & 1367.16	1537.29 & 1367.91	1543.53 & 1376.30
-C-O(str)	1074.87	1075.81	1074.26	1072.81
-C-N(str)	818.59	820.95	820.58	829.58

After comparing the FT-IR spectra of given drug and physical mixture of drug polymer it was found that there were prominent peaks of drug MNZ in physical mixture those can be identified in the pure drug spectra.This revealed that there is no interaction between drug and polymers used to prepare the microspheres. The peaks of MNZ found in physical mixture were similar to the spectra of pure drug MNZ. The peaks of various functional groups (as describedin the spectra of MNZ were alsopresent in the spectra of physical mixture of drug and polymer.

Stability studies: The stability study of different drug was done at room temperature at different pH. The studywas performed in distilled water, pH 1.2, phosphate buffer pH 6.8 and 7.4. The results of 7 days study in different media are given in Figures.

Fig5.StabilitystudyofDrugindifferent pH



The liquid state stability study of drug in the different media indicates that drug is stable. The drugwas more stable in phosphate buffer pH 6.8, showed 41.78percentage remaining drug in the solution. Thedrugconcentrationremained38.865indistilledwater,20.39percentagein0.1NHCland 39.66percentageinphosphatebufferpH7.4.

EVALUATIONOFMICROSPHERES 6.4.1

Percentage yield: The percentage yield of different formulation is shown in table 6.9. 6.10. 6.11 and the graphical representation of different formulations is also shown in Fig The percentage yield of MNZ microspheres in Batch 1 (different CAP formulations F1-F5) ranges 74.766±0.152 to 95.666±0.585 percentage. The highest yield was found in F5 formulation (1:5), it was 95.666±0.585 percentage and the lowest was in F1 formulation i.e. 74.766±0.152percentage. (Figures and Appendices II)

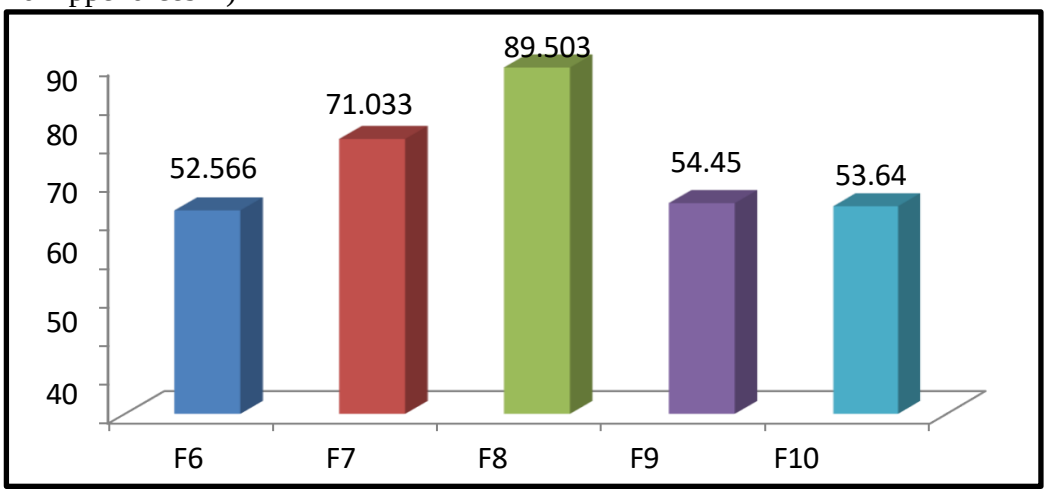


Fig 6. Comparative graph showing percentage yield distribution of various formulations of HPMCP

The percentage yield of MNZ microspheres in Batch 3 (different Eudragit S 100 formulations F11- F15) ranges 63.056±1.154 to 98.253±0.351 percentage. The highest yield was found in F14 formulation (1:4), it was 98.253±0.351 percentage and the lowest was in F11 formulation i.e. 63.056±1.154percentage. The graphical representation of different formulations of Eudragit S 100 is shown in (Figures and Appendices II)

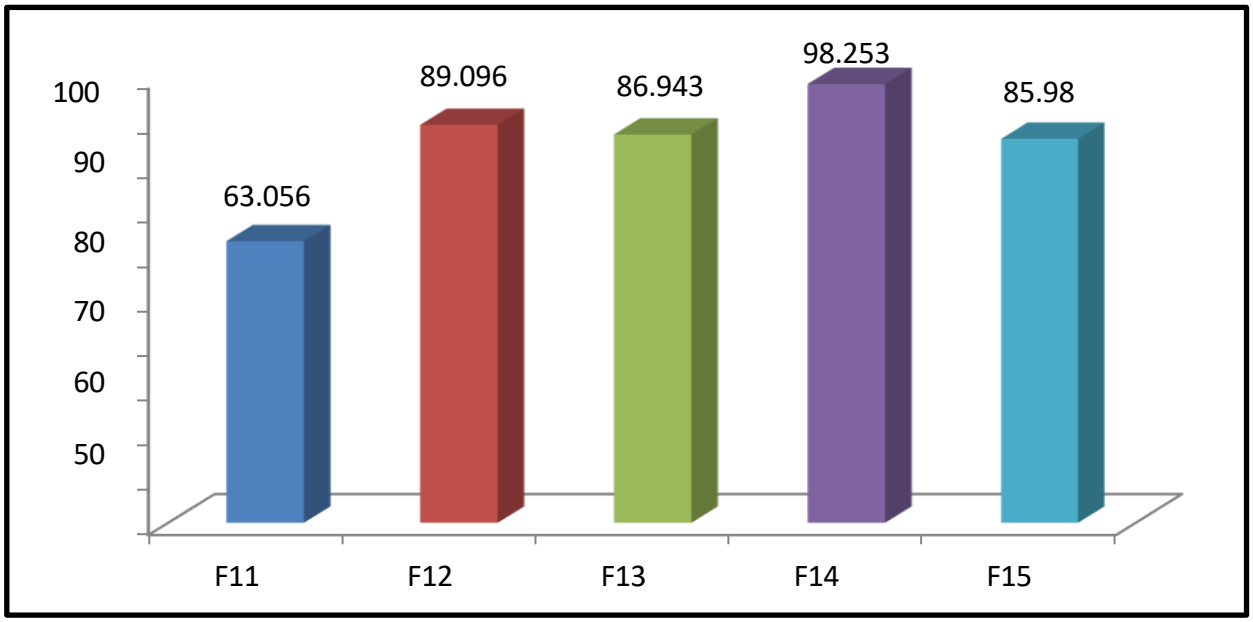


Fig 7. Comparative graph showing percentage yield distribution of various formulations of Eudragit S 100

Drug content: The drug content of different formulation is given table 6.4.7, 6.4.8, 6.4.9 and their graphical representation is shown in Fig. 6.17, 6.18, 6.19. The percentage drug content of MNZ microspheres in Batch 1 (different CAP formulations F1-F5) ranges 42.170±4.234 to 94.053±0.205 percentage. The highest percentage drug content was found in F5 formulation (1:5), it was 94.053±0.205percentage and the lowest was in F1 formulation i.e. 42.170±4.234 percentage. (Figures and Appendices II)

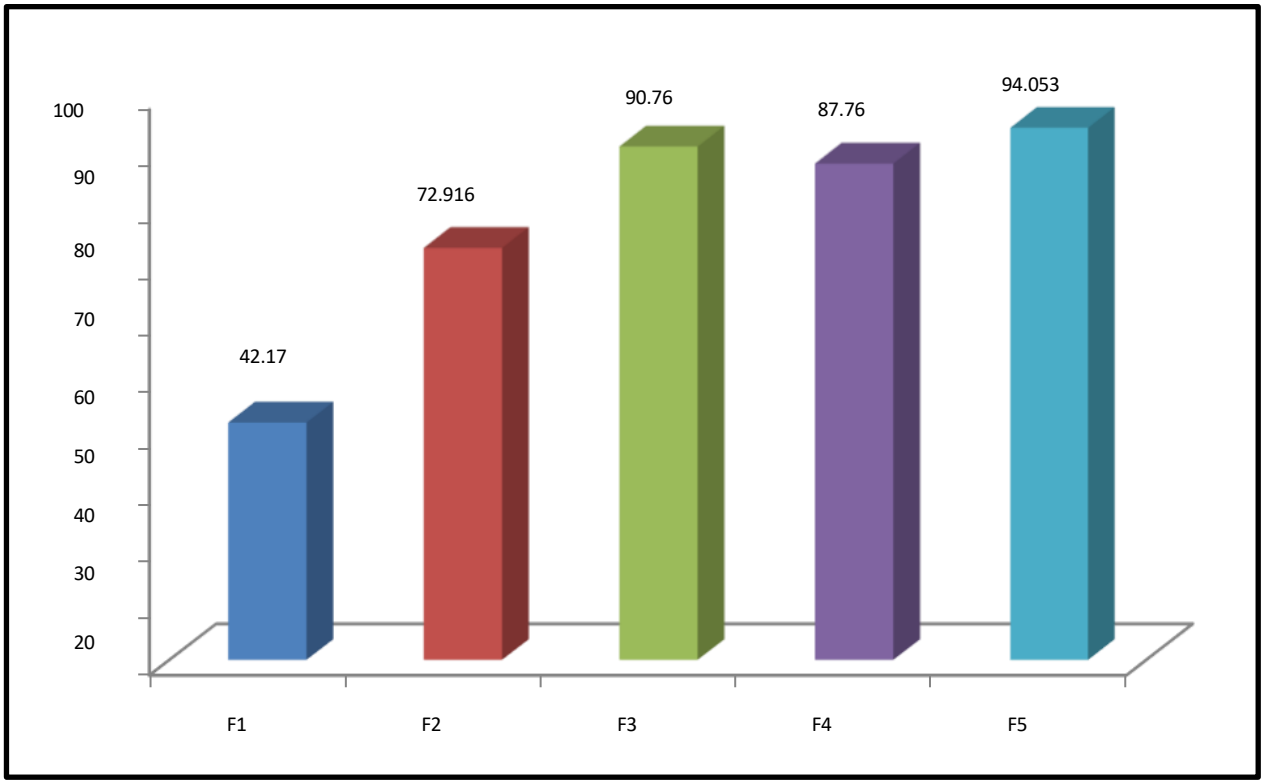


Fig 8. Comparative graph showing percentage drug content distribution of various formulations of CAP

The percentage drug content of MNZ microspheres in Batch 2 (different HPMCP formulations F6-F10) ranges 37.08±3.232 to 96.18±0.336 percentage. The highest yield was found in F8 formulation (1:3), it was 96.18±0.336 percentage and the lowest was in F6 formulation i.e. 37.08±3.232 percentage.

EVALUATIONOFTABLETS

The optimized microspheres were compressed into the tablet form and they were evaluated for various parameters like thickness, hardness, weight variation, friability, in vitro disintegrationtest and in vitro dissolution testing. The various evaluation parameters oftabletted microspheres and marketed preparation Metrogyl are given in Table

Table7. Evaluation parameter of tabletted microsphere and marketed tablets

Evaluation Parameters	TablettedMicrosphere			MarketedTablet (*Metrogyl*)
	T1	T2	T3	
Thickness(mm)	3.85±0.035	4.34±0.03	4.02±0.015	3.66±0.015
Hardness (kg/cm^2)	5.69±0.02	5.60±0.015	5.74±0.03	6.19±0.032
Weightvariation (mg)	252.33±1.15	248.33±0.57	252.66±0.57	199.6±1.15
Friability (percentage)	0.8	0.78	0.8	0.82
Disintegrationtime (min)	56±1	57.66±2.30	58.33±0.57	13.9±0.52
Drug content (percentage)	93.893±0.01	95.345±0.02	98.817±0.02	98.993±0.01

Invitrodrugrelease study

The drug release from the tabletted microsphere showed 96.59percentage drug release in 6.5 h and 44.473percentage in 3 h in phosphate buffer and 2.17percentage drug release in 0.1 N HCl whereas marketed tablet showed 90percentage drug release in 3 h and upto 75percentage drug release in 0.1 N HCl in 2 h. The comparative drug release data of tabletted microsphere and marketed formulation is shown in Table. Their graphical representation is shown in Figurebelow. (Appendices III)

Table 8.Cumulative data for drug release from various prepared tabletted microsphere and marketed tablet

S.No.	Time(h)	PercentageCDR			
		T1	**T2**	**T3**	***Metrogyl***
1.	0	0	0	0	20.45±0.07
2.	0.25	0.178±0.001	0	0	33.47±0.48
3.	0.5	573±0.03	0.029±0.001	0	40.04±0.02
4.	0.75	3.90±0.002	0.306±0.001	0.15	57.12±0.21
5.	1	6.98±0.001	2.13±0.0004	1.56±0.001	69.00±0.03
6.	1.5	9.13±0.001	3.22±0.002	1.87±0.0006	75.12±1.28
7.	2	10.98±0.001	5.56±0.03	2.17±0.001	87.07±0.07
8.	2.5	38.24±0.001	28.40±0.001	33.10±0.01	95.65±0.97
9.	3	59.08±0.001	35.22±0.002	44.47±0.005	
10.	3.5	71.15±0.001	49.40±0.003	64.45±0.005	
11.	4	76.08±0.003	57.783±0.006	73.56±0.001	
12.	4.5	80.52±0.004	79.48±0.007	77.22±0.001	
13.	5	85.57±0.001	88.21±0.005	81.14±0.001	
14.	5.5	91.39±0.002	93.39±0.004	86.50±0.001	

T1 ns (mean±S.D, n=3, p=0.5065two wayanova) T2 ns (mean±S.D, n=3, p=0.1208two way anova) T3 ns (mean±S.D, n=3, p=0.4363 two way anova)

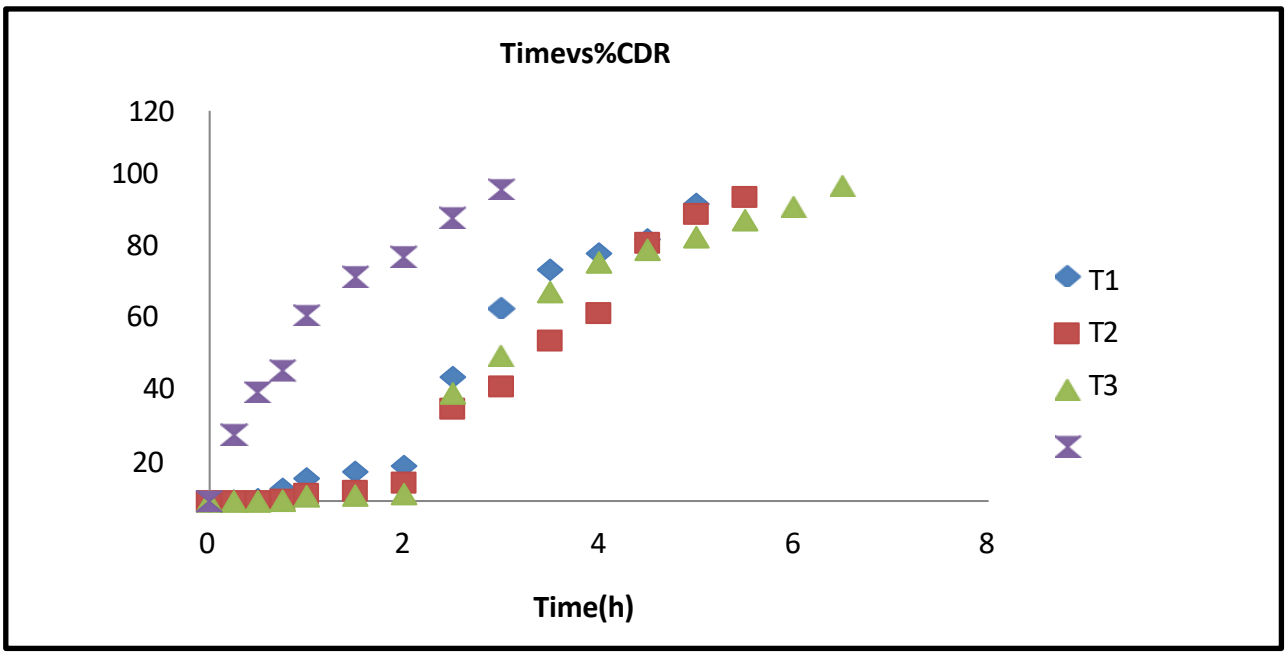


Fig9.Timev/spercentageCDRofpreparedtablettedmicrosphereandmarketedtablet

CONCLUSIONANDFUTURERESEARCH

After performing the above work it was found that the given drug was MNZ, basic, highly permeable drug and having high solubility in acidic media (0.1N HCl) as compare to the other media because of its basic nature. So coating of this drug by pH dependent polymer prevents its release in the gastric region and higher bioavailability will be achieved in the basic region. The drug was entrapped with CAP, HPMCP and Eudragit S 100 asthese polymers are known for pH dependent release. All the formulations were prepared by changing drug-polymer ratio from1:1 to 1:5. Then the formulations were optimized by applying statistical analysis in which F5, F8, F14 was found to best optimized inpercentage yield, percentage drug content, meanparticle size and in vitro drug release. Then tablet of these formulations were punched and in vitro drug release wasperformed. It wasfoundthat thetablet ofF14 formulationgave the goodrelease. It's 3percentagepartreleasedin0.1NHClin2h,restofdrugwasreleasedinphosphatebufferpH 7.4afterchangingthemedia.Therewas505releasein3hand96.414percentagereleasein5.5h. These formulations followed the zero order and peppas model concluded that release was diffusion controlled and accelerated stability testing of the formulations showed no significant difference. So it is concluded that MNZ successfully transferred to the colon and released completely.

It iswellknownthat Amoebiasis isa colonrelated disease caused bythe protozoaE.Histolytica. It is successfully cured by MNZ which kills the protozoa, but problem related with it, its solubility in the gastric region. So its delayed release is necessary to achieve the complete absorptionofdrug in colon. Inabove studydelayed release wasachieved bycoating ofdrug with pH dependent polymer, which also prevents the bittertasteofdrug. Thus this experimentalwork can be used in future to improve the patient compliance and absorption of drug in colon to successfully cure of the disease.

REFERENCES

1. Abdul, S. Chandewar, A.V. and Jaiswal, S.B. 2010. A flexible technology for modified release drugs: Multiple-unit pellet system (MUPS). Journal of Controlled Release, Vol. 147, pp. 2–16.
2. Agnihotri, N. Misha, R. Goda, C. and Arora, M. 2012. Microencapsulation – A Novel Approach in Drug Delivery: A Review. Indo Global Journal of Pharmaceutical Sciences.

Vol. 1, Issue 2, pp. 1-20.

3. Alagusundaram, M. Chetty, C. Umashankari, K. Badarinath, A.V. Lavanya, C. and Ramkanth, S. Jul Sep 2009. Microspheres as a Novel Drug Delivery System: A Review. International Journal of Chem. Tech. Research. Vol.1, Issue 3, pp. 526-534
4. Alli, A.S.M. Preparation and characterization of a coacervate extended-release microparticulate delivery system for Lactobacillus rhamnosus. Pubmed Beena,
5. Kumar, N. Rohilla, R.K. Roy, N. and Rawat, D.S. 2009. Synthesis and antibacterial activity evaluation of MNZ–triazole conjugates. Bioorganic & Medicinal Chemistry Letters, Vol. 19, pp. 1396–1398.
6. Bendas, E.R. and Ayres, J.W. 2008. Leaky enteric coating on ranitidine hydrochloride beads: Dissolutionand predictionofplasma data.EuropeanJournalofPharmaceuticsand Biopharmaceutics, Vol. 69, pp. 977–985.
7. Biju, S.S. Saisivam, S. Gerald, N.S.M. Misha, P.R. 2004. Dual coated erodible microcapsules for modified release of diclofenac sodium. European Journal of Pharmaceutics and Biopharmaceutics, Vol. 58, pp. 61–67.
8. Bogataj, M. Mrhar, A. Kristl, A. and Kozjek, F. 1991. Eudragit E Microspheres containing Bacampicilline: preparation by Solvent Removal Method. Journal of Microencapsulation. Vol. 8, Issue 3, pp. 401-406.
9. Brahmankar, D.M. and Jaiswal, S.B. 2012. Biopharmaceutics and Pharmacokinetics- A Treatise. Vallabh Prakashan. 2nd ed. pp. 432-433.
10. Chakraborti, C.K. and Beha, P.K. 2012. Development and Evaluation of gastroretentive controlledreleasepolymericsuspensioncontainingCiprofloxacineandcarbapolpolymer. Journal of Chemistry and Pharm Research. Vol. 4, Issue 4, pp. 2268-2284.
11. Chintagupta, P. Kavitha, K. and Kumar, S.N.A. 2010. Formulation and Evaluation of Trimetazidine Hcl Loaded chitosan Mtcrospheres. International Journal of Applied Pharmaceutics, Vol. 2, Issue 2, pp. 1-4.
12. Chourasia, M.K. and Jain, S.K. 2003. Pharmaceutical Approaches to Colon Targeted Drug Delivery System. J Pharm Pharmaceutical Sciences, Vol. 6, pp. 34.
13. Costa, P. and Sousa Lobo, J.M. 2001. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences.
14. Dey, S. Pramanik, S. and Malgope, A. 2011. Formulation and Optimization ofSustained Release Stavudine Microspheres Using Response SurfaceMethodology. International Scholarly Research Network ISRN Pharmaceutics, Vol. 11, pp. 1-8.
15. Fundueanu, G. Constantin, M. Bortolotti, F. Cortesi, R. Ascenzi, P. and Menegatti, E. 2007. Cellulose acetate butyrate pH/thermosensitive polymer microcapsules containing aminated poly(vinyl alcohol) microspheres for oral administration of DNA. European Journal of Pharmaceutics and Biopharmaceutics, Vol. 66, pp. 11–20. 66
16. Gavini, E. Alamanni, M.C. Cossu, M. and Giunchedi, P. 2005. Tabletted microspheres containing Cynara scolymus(var. Spinoso sardo)extract forthe preparationofcontrolled release nutraceutical matrices. Journal of Microencapsulation, Vol. 22, pp. 487–499.
17. Ghobashy, M. Abo-Talib, N. 2010. Spectrophotometric methods for the simultaneous determinationofbinarymixture ofMNZand diloxanide furoate without prior separation. Journal of Advanced Research. Vol. 1, pp. 323-329.
18. Giunchedi, P. E. Gavinie, E. Bonacucina, G. and Palmieri, G.F. 2000. Tabletted polylactide microspheres prepared by a w/o emulsion-spray drying method. Journal of Microencapsulation, Vol. 16, Issue 6, pp. 711-720.
19. Guersoy, A. and Cevik, S. 2000. Sustained release properties of alginate microspheres

and tabletted microspheresofdiclofenac sodium. JournalofMicroencapsulation, Vol. 16, Issue 5, pp. 565-575.

20. Guersoy, A. Karakuse, D. and Okar, I. 2009. Polymers for sustained release formulations of dipyridamole-alginate microspheres and tabletted microspheres. Journal of Microencapsulation, Vol. 16, Issue 4, pp. 39-452.

21. Jain, N. K. reprint 2009. Controlled and Novel Drug Delivery. CBS Publisher and Distributors. Kaurav, H. Kumar, S.L. and Kaur, A. 2012. Mucoadhesive Microspheres as carriers in Drug Delivery: a Review. International Journal of Drug Development and Research, Vol. 4, Issue 2, pp. 1-4.

22. Khan, S. Tiwari, T. Rao, N. Joshi, A. and Dubey, B.K. 2012. Microspheres: A Review. World Journal of Pharmacy and Pharmaceutical Sciences. Vol. 1, Issue 1, pp. 125-143.

23. Khidr, S.H. Niazy, E. M. and El-Sayed, Y. M. 1998. Development and In vitroEvaluation of sustained-release Meclofenamic Acid Microspheres. Journal of Microencapsulation. Vol. 15, Issue 2, pp. 153-160.

24. Kim, C. Kim, M. and Hee oh, K. 1994. Preparation and Evaluation ofSustained Release MicrospheresofTerbutalineSulfate.InternationalJournalofPharmaceutics.Vol.106, pp.213-219.

25. Korablev, M.V. Volynets, S.I. and Kurbatn, M. Effect of MNZ on The Action and Biological Transformation of ethyl Alcohol, UDC 615.283.612.1.015.4.015.2: 615.31: 547.262; 1-3.

26. Kurkuri, M.D. and Aminabhavi, T.M. 2004. Poly (vinyl alcohol) and poly(acrylic acid) sequential interpenetrating network pH-sensitive microspheres for the delivery of diclofenac sodium to the intestine. Journal of Controlled Release, Vol. 96, pp. 9– 20.

27. Lamprecht, A. Yamamoto, H. Takeuchi, H. and Kawashima, Y. 2003. Microsphere design for the colonic delivery of 5-fluorouracil. Journal of Controlled Release, Vol. 90, pp.313322.

28. Lamprecht, A. Yamamoto, H. Takeuchi, H. and Kawashima, Y. 2004. pH-sensitive microsphere delivery increases oral bioavailability of Calcitonin. Journal of Controlled Release, Vol. 98, pp. 1– 9.

29. Lamprecht, A. Yamamoto, H. Takeuchi, H. and Kawashima, Y., 2004. Design of pH sensitive microspheres for the colonic delivery of the immunosuppressive drug tacrolimus.European Journal of PharmaceuticsandBiopharmaceutics,Vol.58, pp.37–43.67