EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 3
Year: 2025

FORMULATION AND EVALUATION OF VANCOMYCIN MICROSPHERES

Naray Navya, M. Vijaya Laxmi* and Vijaya Kuchana

Department of Pharmaceutics, Teegala Krishna Reddy College of Pharmacy, Medbowli, Meerpet, Saroornagar, Hyderabad- 500097.

***Corresponding Author: M. Vijaya Laxmi**
Department of Pharmaceutics, Teegala Krishna Reddy College of Pharmacy, Medbowli, Meerpet, Saroornagar, Hyderabad-500097.

Article Received on 03/01/2025 **Article Revised on 23/01/2025** **Article Accepted on 13/02/2025**

ABSTRACT
The development of oral sustained or controlled release dosage form of Vancomycin has been an interested topic of research for a long period of time. Such drug is difficult to be delivered orally in a sustained or controlled release manner and, Due to its effectiveness and intensive use as a drug of choice in the treatment of colitis, numerous sustained and controlled release formulations of Vancomycin have been made and reported. The microspheres were evaluated with respect to the yield, particle size, incorporation efficiency, in vitro drug release and stability. Microspheres were characterized by FTIR studies. It was found that the particle size and incorporation efficiency of microspheres increases with increasing drug-to-polymer ratio.

KEYWORDS: Vancomycin, Ionic gelation method, Ethyl cellulose, Sodium alginate, FTIR studies, in vitro drug release studies.

INTRODUCTION
Novel drug delivery system delivers a therapeutic substance to the target site in a well-controlled and sustained model.[1] Microspheres are characterized as spherical microparticulate and free-flowing powders consisting of biodegradable polymers mostly. They ideally have a particle size ranging from 1 µm to 1000 µm. Microspheres can be loaded with drug and use for targeted drug delivering. As the drug is loaded in polymeric microspheres, it shows therapeutic action on targeted tissue only. Microspheres are designed to enhance the therapeutic effectiveness of the drug and achieve better bioavailability thereby minimizing the toxicity and minimal side effects.[2] Microspheres can be targeted to the desired location by active or passive targeting strategies. Passive targeting is based upon the size and general surface properties of the microspheres such as degree of hydrophobicity, surface charge, and non-specific adhesion, which directs them towards the particular organ.[3] Vancomycin, a tricyclic antibiotic, has been a key player in the antimicrobial war and is often used as the antibiotic of last resort in treating most resistant S. aureus infections. Vancomycin is highly water soluble and therefore lends itself to highly efficient encapsulation by albumin. Vancomycin has been combined with microspheres of tumor necrosis factor-α (TNF-α) to effectively protect rats from S. aureus induced peritonitis.[4] In this present study formulation and evaluation of vancomycin prepared by using ionotropic gelation technique.

MATERIALS
Vancomycin was obtained from Hetero lab, HYD. Sodium alginate and Ethyl cellulose were procured from Synpharma Research Labs, Hyderabad, and other chemicals, and the reagents used were of analytical grade.

METHODOLOGY
FT-IR study
FT-IR studies Chemical compatibility of Vancomycin with polymer were analyzed by FT-IR. Any changes in the chemical position or changes in functional groups characteristics peak of drug in IR spectra after combining with excipients were investigated.[5]

Formulation table

Table 1: Formulation development of Vancomycin microspheres.

F. No	Vancomycin (mg)	Sodium alginate(mg)	Ethyl cellulose(mg)	$CaCl_2$(%)
F1	500	1000	-	2
F2	500	900	-	2
F3	500	800	-	2
F4	500	700	-	2
F5	500	-	1000	2
F6	500	-	900	2
F7	500	-	800	2
F8	500	-	700	2

Ionic gelation method

Vancomycin microbeads were prepared by using blends of sodium alginate as the coat material by ionic gelation method. The sodium alginate mixture of different ratios were prepared. The drug Vancomycin [1gm] was added to this mixture and homogenized thoroughly with the help of magnetic stirrer to form a homogeneous dispersion. The homogenous dispersion was kept aside to remove the bubble. Now the bubble free dispersion was added drop wise manually with a 20ml syringe fitted with 22 gauge needle, 100 ml calcium chloride [$CaCl_2$] solution kept under the stirring in a 250 ml beaker. The gel forming [gelation] time of 15 min was allowed to complete the reaction and produce spherical microspheres. The prepared beads was collected by decantation, washed with alcohol and dried at room temperature, and finally dried at < 40°C for 2 hrs.[6]

Evaluation of microspheres

Particle size analysis

The volume average particle diameter (Mean size) and size distribution of all Vancomycin loaded microspheres were analyzed by a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, UK).[7]

Morphological characterization using SEM

The surface morphology of the microspheres was recorded with JEOL Scanning Electron Microscope (Model: JSM 5200). The samples were mounted on an Aluminium stub by using a double-sided adhesive tape. Then it was placed in an ion coater unit (Model: IB-2, Hitachi, Tokyo, Japan) for gold coating (200A). During gold coating process the sample were exposed to vacuum of 10-50 mm. After wards, an 50 accelerating voltage of 5 kV ws a applied and the image was photographed by Asia Pentex camera of 35 mm film.[8]

Drug Entrapment Efficiency

Taken 100 mg of microspheres and the amount of drug entrapped was estimated by crushing the microspheres and extracting drug into 100 ml methanol. After 24 hr, the extract was transferred to a 100 ml volumetric flask and the volume was made up using methanol. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 276 nm for Vancomycin respectively against methanol as a blank. Then practical drug content was calculated from respective calibration curves. Percent drug entrapment efficiency was calculated using the following equation.[9]

Drug entrapment efficiency (%) = Practical Drug content /Theoretical Drug content X 100

Percentage Yield

The relative yield was calculated based on the amount of microspheres of each formulation obtained relative to the amount of solid materials used in the dispersed phase. The percentage yield was calculated according to the following equation:[10]

Percentage Yield (%) = Actual weight of microspheres /Total weight of drug and polymer X 100

In-vitro drug release studies

100 mg of microsphere were taken and filled in capsules and subjected for dissolution test in dissolution test apparatus using basket method. Dissolution media was 900 ml of phosphate buffer pH 7.4 maintained at 37 ± 2C and rotated at 100 rpm. Sample were withdrawn at specified time intervals and replaced with the same volume of fresh medium, filtered and analyzed at 276 nm.[11]

Kinetics of drug release studies[12]

The quantitative elucidation of the values obtained in the dissolution study is facilitated by the usage of a generic equation that mathematically translates the dissolution curve in function of some parameters related to the microspheres. For understanding the mechanism of drug release and release rate kinetics of the drug 45 from dosage form, the Invitro drug dissolution data of optimized formulations obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix and Korsmeyer-Peppas models.

Zero order kinetics[13]

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly can be represented by the following equation:

$Q0 - Qt = K0t$

Arrangement of equation yields: $Qt = Q0 + K0t$

Where Qt is the amount of drug dissolved in time t,

Q0 is the initial amount of drug in the solution (most times, Q0 = 0)

and K0 is the zero-order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

First order Kinetics
The equation for first order release is given below
Log Qt = log Q0+ K1t/2.303
Where Qt is the amount of drug released in time t,
Q0 is the initial amount of drug in the solution
and K1 is the first order release constant.

A graph of the decimal logarithm of the released amount of drug versus time will be linear. Microspheres following this dissolution profile release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminishes.

Higuchi model
Higuchi described drug release as a diffusion process based on the Fick's law, square root time dependent. The simplified Higuchi equation is represented as
Qt=Kt ½
Where Qt = amount of drug released in time t,
K = Higuchi's constant
A linear relationship between amount of drug released (Q0 versus square root of time (t½) is observed if the drug release from the microspheres is diffusion controlled.

Korsmeyer-Peppas model
This mathematical model, also known as the Power Law, has been used, very frequently; to describe the drug release from several different pharmaceutical modified release dosage forms. The Korsmeyer-Peppas model relates drug release exponentially to time. It is described by the following equation
Mt/M∞ =atn

Where 'a' is a constant incorporating structural and geometric characteristics of microspheres, 'n' is the release exponent, indicative of the drug release mechanism, and the function of 't' is Mt/ M∞ (fractional release of drug).

Stability studies[14]
The external surface morphology and their internal cross section image of optimized batch of microspheres were obtained by scanning electron microscope (SEM, Philips XL20, Holland) under vacuum. The samples for SEM were prepared by mounting dried microspheres on a double adhesive tape stuck to an aluminium stub.

RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)
The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

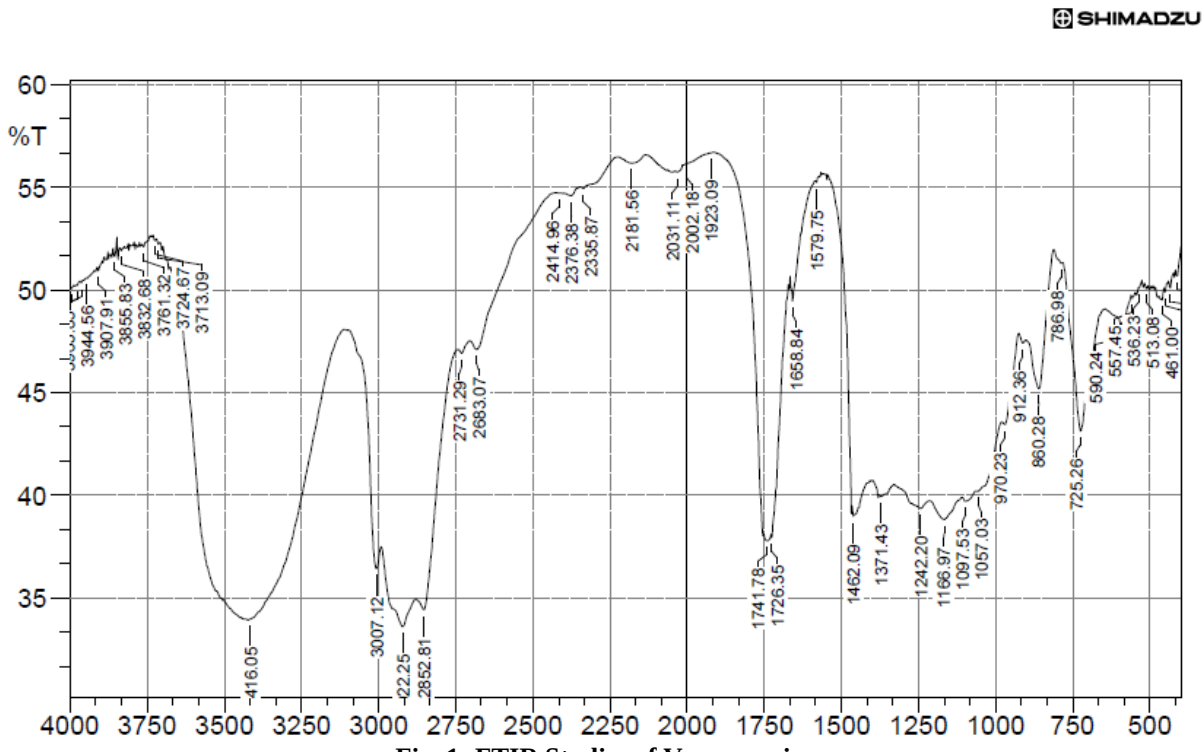

Fig. 1: FTIR Studies of Vancomycin.

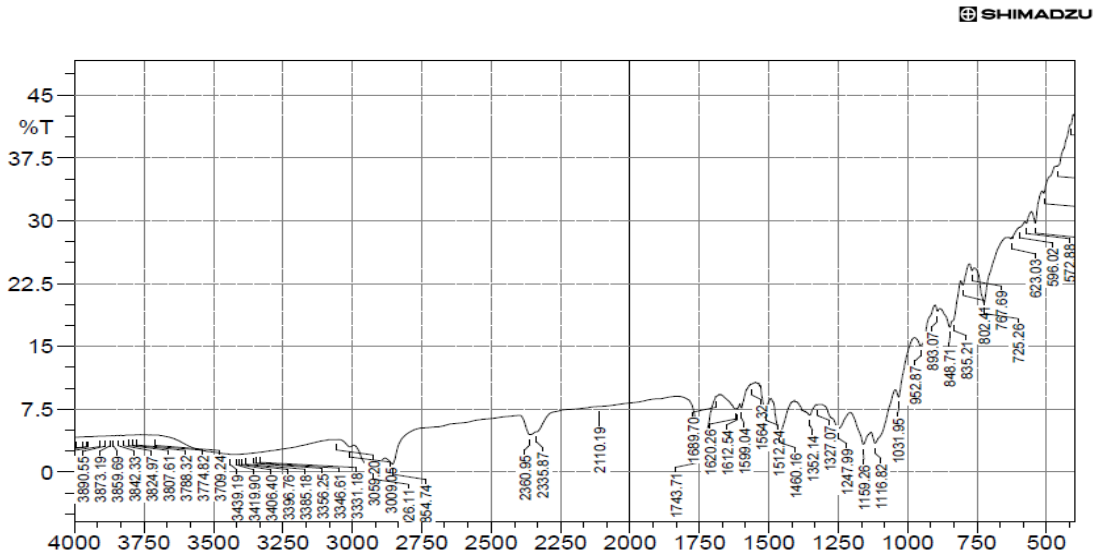

Fig. 2: FTIR Studies of optimized formulation.

Formulation and Evaluation of sustained release Microspheres of Vancomycin Optimization of formulation variables Therefore, the optimized conditions for the formulation of sustained release microspheres were: Results of the evaluation parameters of formulated sustained release microspheres.

The prepared sustained release microspheres were evaluated for various parameters such as yield, drug entrapment efficiency, particle size, and in vitro drug release. And effect of preparation and process variables such as drug polymer ratio, speed, type of polymer and combination of polymers on particle size, yield, entrapment efficiency, and in-vitro release of from Vancomycin sustained microspheres were also studied.

Characterization of microspheres by scanning electron microscopy (SEM) Figure A shows SEM photograph of optimized microspheres at 100□ magnification, at 1000□ magnification. SEM photographs showed discrete, spherical microspheres. SEM photographs also showed the presence of drug crystal on the surface of microspheres revealing that the microspheres were having some rough surface. The drug crystals on microspheres were may be due to the presence of un entrapped drug in dispersion medium.

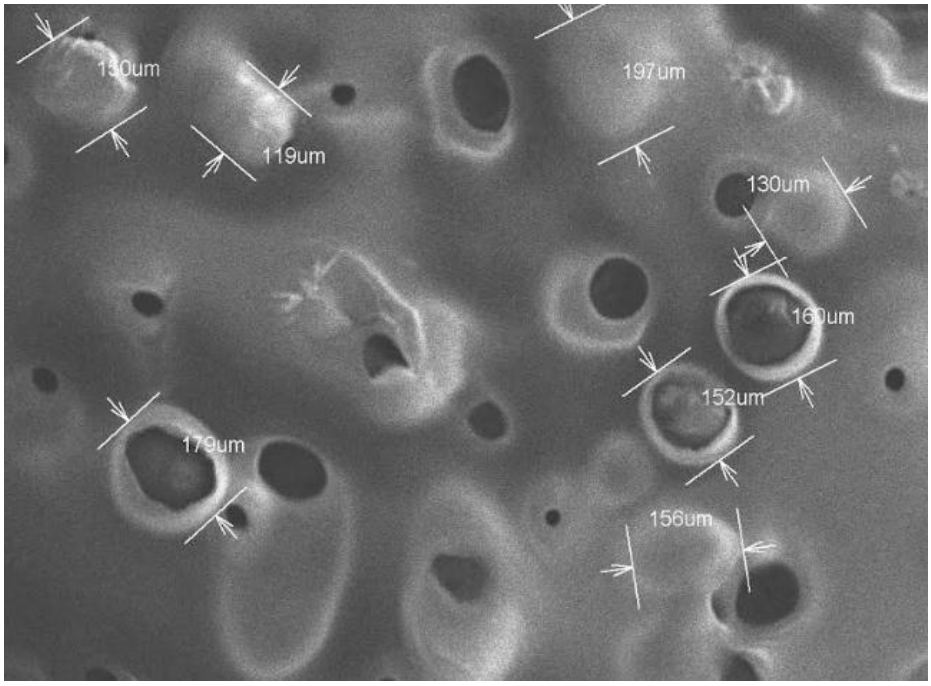

Fig-3: SEM photograph of optimized formulation.

Effect of formulation and process variables on Yield of sustained release microspheres, Particle size, Drug entrapment efficiency

Table 8: Effect of drug polymer ratio on Yield of microspheres, Particle size, Drug entrapment efficiency.

Formulation Code	% Yield	Particle size	Drug Entrapment Efficiency
F1	68.32±1.20	226±2.59	75.86±1.26
F2	65.92±1.56	176±2.31	77.18±2.10
F3	63.21±2.10	254±1.98	73.20±1.68
F4	72.18±1.69	179±2.30	78.93±1.96
F5	72.34±2.13	123±1.18	76.90±1.35
F6	70.23±2.16	201±2.34	75.88±1.50
F7	73.25±1.72	202±2.38	76.39±1.67
F8	75.18±1.98	158±1.37	79.38±1.83

Drug release studies

Table 9: *In vitro* release data of film F_1 to F_8.

Time (hrs.)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
0	0	0	0	0	0	0	0	0
1	15.23±1.70	15.15±2.10	14.96±2.13	16.10±2.36	17.14±2.12	15.20±2.16	18.30±2.16	19.21±2.13
2	23.65±2.10	24.68±2.14	25.32±1.34	24.96±2.21	26.93±1.53	25.89±2.14	27.98±1.98	28.75±2.18
3	33.95±1.54	35.79±1.60	35.38±1.14	36.39±1.39	36.38±1.60	36.87±2.13	38.36±1.36	39.52±2.56
4	45.84±1.34	43.52±1.63	42.67±1.20	44.67±1.62	46.94±2.15	45.23±1.98	45.14±2.34	48.66±1.85
5	69.75±1.69	67.45±2.02	66.98±1.25	68.21±2.13	61.35±2.16	68.35±1.67	66.33±1.58	69.85±1.75
6	76.89±2.10	73.69±1.28	73.65±1.60	76.98±2.05	77.15±1.59	70.34±1.38	75.10±1.04	76.35±1.38
7	86.41±1.35	82.36±1.26	83.52±1.17	81.25±2.18	83.90±1.43	86.77±1.47	87.82±2.05	88.95±1.60
8	92.32±2.01	93.62±1.14	92.68±1.60	93.35±2.03	94.45±1.20	93.50±1.39	94.99±1.98	96.55±2.13

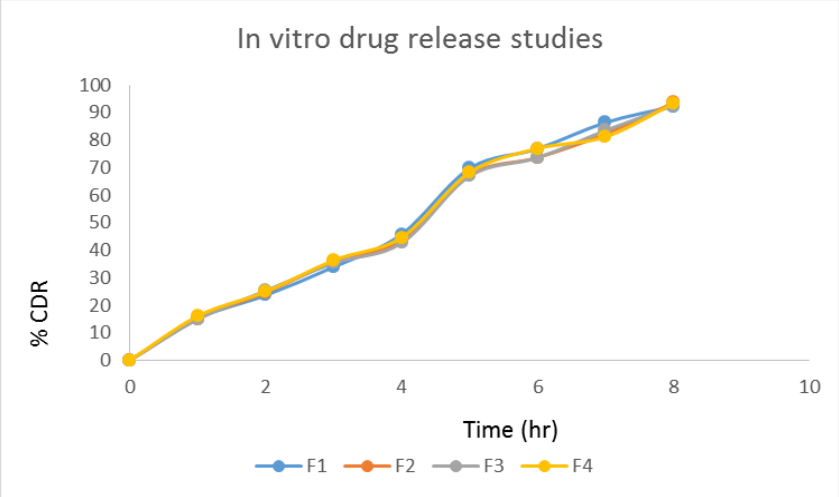

Fig. 4: In vitro drug release of (F1- F4) formulation.

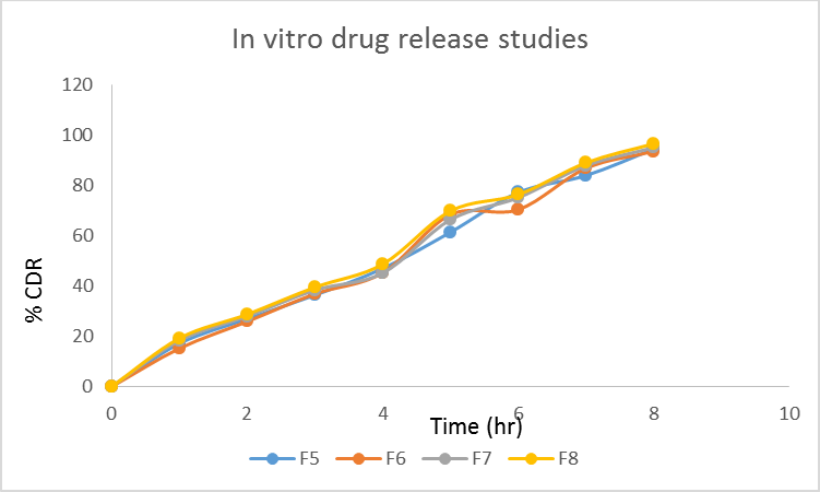

Fig. 5: In vitro drug release of (F5- F8) formulation.

Drug release kinetics

All the 8 formulation of Vancomycin microspheres prepared were subjected to in vitro release studies these studies were carried out using franz diffusion cell apparatus.

The dissolution medium consisted of 10 ml of Standard buffer pH 7.4 period of time.

The results obtaining in vitro release studies were plotted in different model of data treatment as follows

Cumulative percent drug released vs. time (zero order rate kinetics)

Log cumulative percent drug retained vs. time (First Order rate Kinetics)

Cumulative percent drug released vs. square root of time (Higuchi's

Classical Diffusion Equation)

Log of cumulative % release Vs log time (Peppas Exponential Equation)

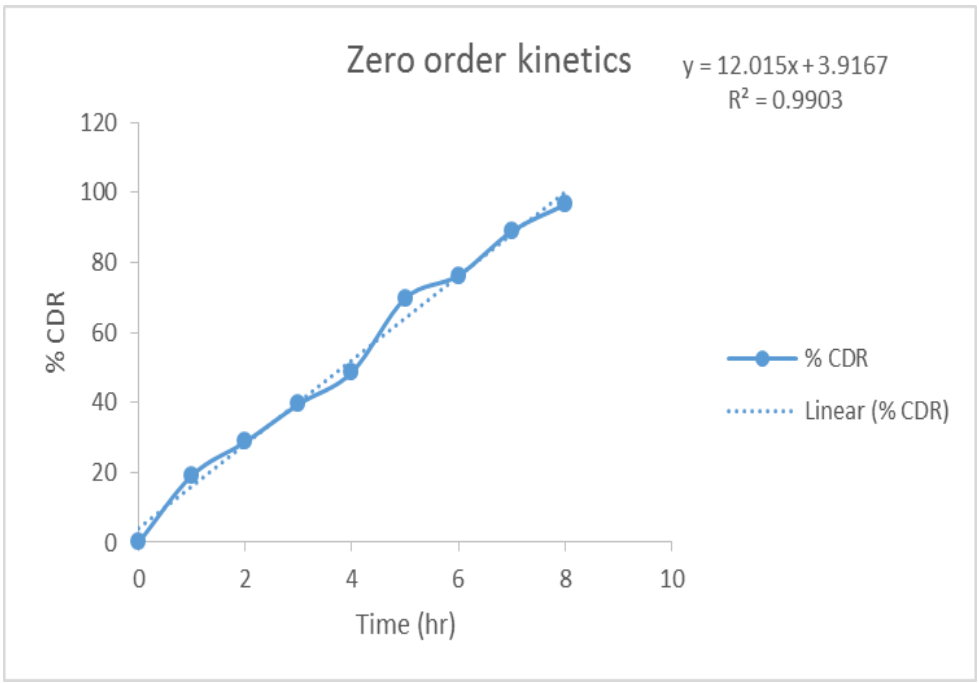

Fig. 6: Zero order kinetics of optimized formulation.

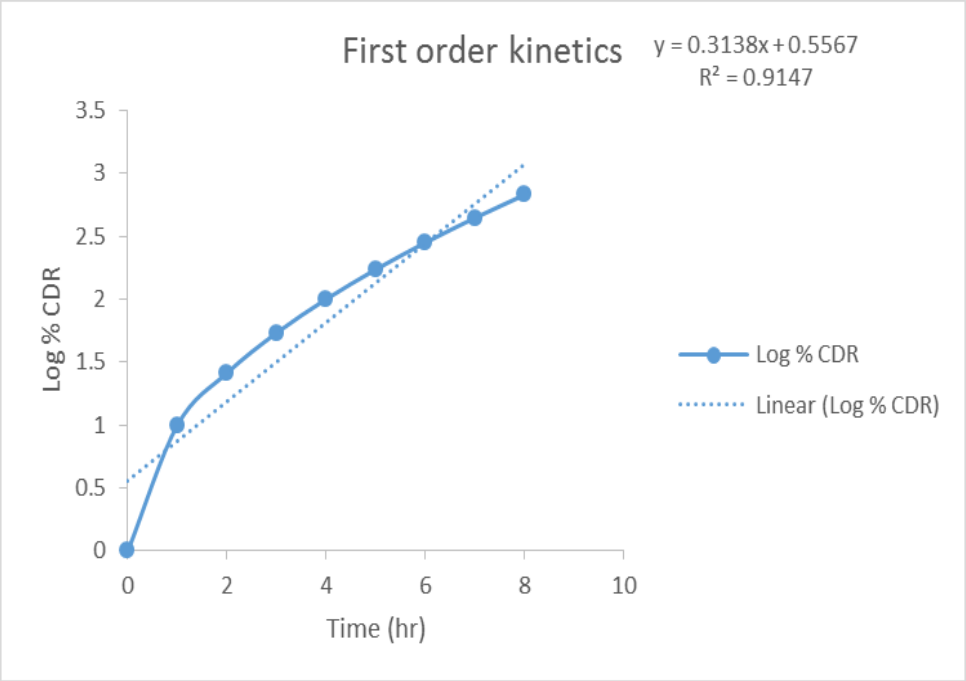

Fig. 7: First order kinetics of optimized formulation.

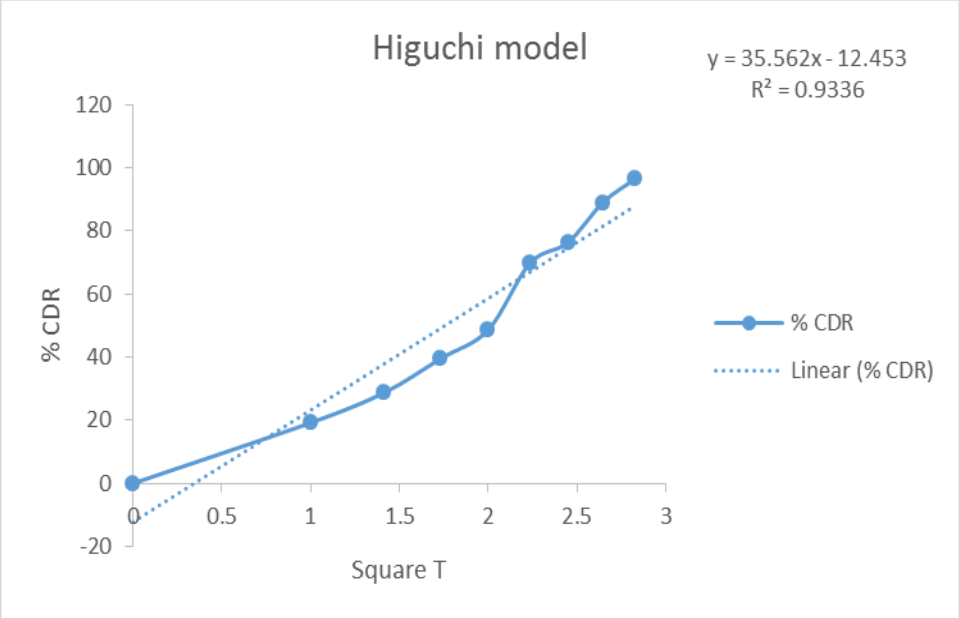

Fig. 8: Higuchi model of optimized formulation.

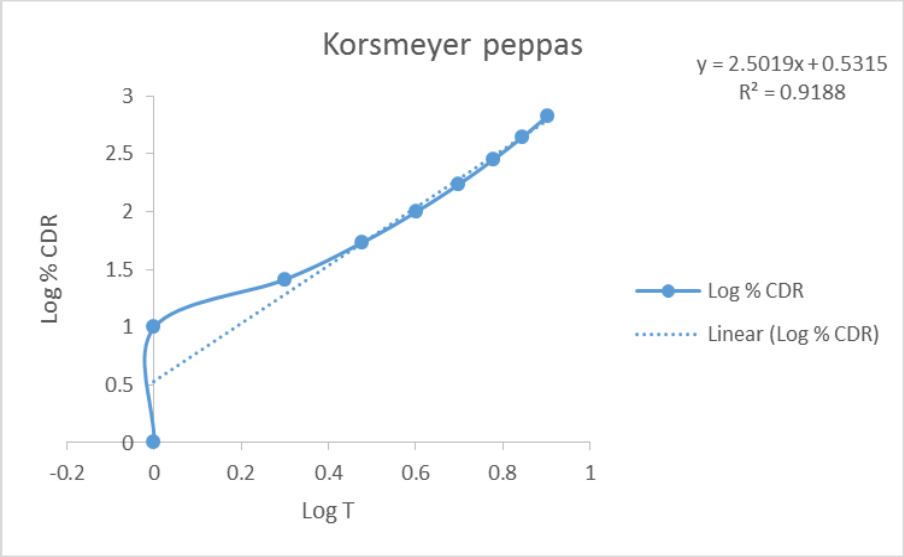

Fig. 9: Korsmayer peppas of optimized formulation.

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix, Peppas were respectively.

Regression values are higher with Zero order release kinetics. Therefore all the Vancomycin microspheres Zero order release kinetics.

The table indicates that r^2 values are higher for Higuchi's model compared for all the formulation. Hence Famotidine release from all the buccal films followed diffusion rate controlled mechanism.

Stability studies

There was no significant change in physical and chemical properties of the Microspheres optimized formulation after 90 days. Parameters quantified at various time intervals were shown;

Table 12: Results of stability studies of optimized formulation.

F. Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-8	25^0C/60%RH % Release	96.55±2.01	95.38±1.85	94.46±2.23	93.65±2.20	Not less than 85 %
F-8	30^0C/75% RH % Release	96.55±2.01	95.25±1.59	94.37±1.85	93.54±1.86	Not less than 85 %
F-8	40^0C/75% RH % Release	96.55±2.01	95.39±1.53	94.25±1.96	93.32±1.85	Not less than 85 %

CONCLUSION

Rationale of the present study was to prevent extensive metabolism of the drug and consequently to increase the oral bioavailability of the drug in the form of sustained release microspheres. Attempt has been made to prepare sustained release microspheres of Vancomycin, a highly water soluble drug. These microspheres are used to treatment of colitis. The microspheres were prepared by Ionotropic gelation technique method using natural and synthetic polymer as retarding polymers and evaluated for parameters like percentage yield, particle size, entrapment efficiency and the effect of preparation and process variables such as drug polymer ratio, speed, type of polymer and combination of polymers on evaluated parameters. Microspheres morphology was evaluated by SEM. The yield and entrapment efficiency was high for Sodium alginate microspheres were Particle size, entrapment efficiency and production yield were influenced by the type of polymer, polymer concentration, stirring speed and combination of polymers. *In vitro* diffusion of optimized formulations of various Polymer in pH 7.4 formulations are releasing the drug up to 8 hrs.

REFERENCES

1. Rajput S, Agrawal P, Pathak A, Shrivasatava N, Baghel SS, Baghel RS. A review on microspheres: methods of preparation and evaluation. World journal of pharmacy and pharmaceutical sciences, 2012; 1: 42238.
2. Sharma M, Dev SK, Kumar M, Shukla AK. Microspheres as a suitable drug carrier in sustained release drug delivery: An overview. Asian J Pharm Pharmacol., 2018; 4: 102-8.
3. M. D'Souza, C. W. Oettinger and G. V. Milton, "Microspheres Containing Neutralizing Antibodies to Tumor Necrosis Factor-α and Interleukin-1β Protect Rats from Staphylococcus aureus-Induced Peritonitis," Journal of Interferon & Cytokine Research, 2000; 20(10): 907-913.
4. Christina.e, preparation of microspheres of diclofenac sodium by ionotropic gelation technique, international journal of pharmacy and pharmaceutical sciences, int j pharm pharm sci., 5(1): 228-231.
5. S. Sahu formulation and evaluation of captropril microspheres by ionic gelation technique

international journal of pharmacy & life sciences int. J. Of pharm. & life sci. (ijpls), Jan.: 2012; 3(1): 1377-1379 1377.
6. Ravi kumar kota, formulation and evaluation of valsartan microspheres by ionotropic gelation technique. Iajps, 2018; 05(06): 5942-59.
7. Shilpa bhilegaonka, change in site of release offers advantages for candesartan cilexetil, int. J. Pharm. Sci. Rev. Res., jan – feb 2014; 24(2),04: 20-23.
8. Mohammad Khalid, Microsphere A Novel Drug Delivery System – A Review, International Journal of Scientific Research in Science and Technology, January-February-2023; 10 (1): 406-411.
9. Galande, P., Yadav, V., & Borkar, S. A Review on Microspheres: Preparation, Characterization and Applications. Asian Journal of Pharmaceutical Research and Development, 2022; 10(6): 128–133.
10. Dhadde Gurunath S., Mali Hanmant S., Raut Indrayani D., Nitalikar Manoj M., Bhutkar Mangesh A. A Review on Microspheres: Types, Method of Preparation, Characterization and Application. Asian Journal of Pharmacy and Technology, 2021; 11(2): 149-5.
11. Suhas Marutirao Kakade, Formulation and in vitro / in vivo Evaluation of Novel Biodegradable Microspheres for Treatment of Hormone Responsive Cancers, International Journal of Pharmaceutical Investigation, 2020; 10(2): 184-191.
12. Anshul Kumar, formulation and evaluation of naproxen and domperidone microbeads as controlled drug delivery system, Afr. J. Bio. Sc. 6.12(2024)
13. Pagar Madhumanjiri K, Formulation and Development of Sustained Release Oral Drug Delivery System Comprising Naproxen Sodium Microspheres, Biological Forum – An International Journal, 2023; 15(5a): 510-520.
14. SM Sarode, MK Kale, G Vidyasagar. Formulation and Evaluation of Ethyl Cellulose Coated Microspheres of Aceclofenac. Research J. Pharma. Dosage Forms and Tech., 2010; 2(1): 41-43.