Microspheres for Sustained Release of Cisplatin: A Comprehensive Formulation and Evaluation Study

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ABSTRACT :
The present work focused on the formulation and evaluation of Cisplatin loaded particulate systems with the aim of achieving high drug entrapment and controlled release. Eight formulations (F1–F8) were prepared by ionotropic gelation technique and characterized for percentage yield, particle size and drug entrapment efficiency (EE). The results showed that the percentage yield ranged from 75.98 to 83.36 %, indicating minimal processing loss and good reproducibility of the method. The particle size of the prepared particles was within the sub-micron range (555–692 nm), suitable for controlled drug delivery and providing a large surface area for dissolution. Drug entrapment efficiency was found to be 76.98–84.98 %, demonstrating efficient incorporation of venlafaxine within the polymeric matrix. Among all batches, F5 exhibited the most desirable characteristics with the highest yield (83.36 %), the smallest particle size (555 nm) and maximum drug entrapment (84.98 %). These findings confirm that the adopted preparation method can consistently produce venlafaxine-loaded particles with high yield and effective drug entrapment, and that formulation F5 is particularly promising for further optimization, in-vitro release studies and scale-up for extended-release drug delivery applications.
Keywords: Cisplatin, Ionotropic gelation method, Polymers, FTIR Studies, In vitro drug release studies.

I. INTRODUCTION

Microspheres are small, spherical, free-flowing particles (typically in the 1–1000 μm size range) composed of biodegradable polymers, natural biopolymers, or inorganic materials. They offer several attractive features such as controlled and sustained drug release, protection of the drug payload, improved drug stability, and better patient compliance by reducing the frequency of dosing.[1] Cisplatin (CDDP) is among the most effective and widely used chemotherapeutic drugs, indicated for a variety of cancers including testicular, ovarian, bladder, head-and-neck, and bone cancers.[2] Its cytotoxic effect stems from its ability to crosslink DNA, thereby inhibiting DNA replication and inducing apoptosis in rapidly dividing tumor cells.[3] However, despite its potent anticancer activity, the clinical use of cisplatin is severely limited by its dose-limiting toxicities and non-specific distribution, which often results in serious side-effects and reduced patient tolerance.[4] Therefore, the present study aims to develop a microsphere-based cisplatin delivery system that (1) achieves high drug entrapment efficiency (DEE), (2) maintains controlled and sustained drug release over an extended period, and (3) is biocompatible and suitable for potential clinical application.[5]

II. EXPERIMENTAL WORK

MATERIALS

Cisplatin procured from Hetero Labs, HYD. Sodium alginate and HPMCwere obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODLOGY

Fourier transform infrared spectroscopy studies

Drug and drug-polymer compatibility research identification procedure: The FTIR spectra of the pure drug, excipient and physical mixture of drug and excipient were recorded in between 400-4000 wave number (cm-1). No peaks are observed which interfere with the main drug peaks. The following spectrum and table show IR spectrum for drug and polymer and the wave number of characteristic bands for the same. [6]

Formulation table

Table-1: Formulation development of Cisplatin microspheres

F. no	Cisplatin	HPMC	Sodium alginate	$Cacl_2$
F1	10	100	-	1%
F2	10	200	-	1%
F3	10	300	-	1%
F4	10	400	-	1%
F5	10	-	100	1%
F6	10	-	200	1%
F7	10	-	300	1%
F8	10	-	400	1%

Ionotropic Gelation Technique

In this method, polymers in different concentrations was dispersed in suitable solvent solution and homogenized for 1hr. Drug polymer solution was prepared by dispersing the drug (10 mg) slowly into previously prepared polymers slurry in different ratios with continuous and uniform stirring for 3 hr. A gelation medium was prepared separately by dissolving different percentages of calcium chloride in distilled water. Bubble free dispersion medium was extruded through glass syringe (20 Guaze) into the gently agitated calcium chloride solution. The agitation was carried out by mechanical stirrer at different rpm. Microspheres were separated by filtration from the solution, washed with water and dried.[7]

Evaluation of microspheres

Percentage Yield

To prepared oral microsphere of all batches accurately weight. The measured weight of prepared microspheres was divided by total amount of all excipient and drug used in preparation of oral microspheres, which give the total percentage yield of total microspheres.[8]It was calculated by following equation;

$$\% \text{ yield} = \frac{\text{Actual weight of product}}{\text{Total weight of excipient and drug}} \times 100$$

Drug Entrapment Efficiency

Entrapment efficiency of oral microspores was evaluated by deriving percent drug entrapment. the drug content of drug loaded oral microsphere was determine by dispersing 10 mg of oral microspheres in 10 ml ethanol followed by agitation with of magnetic stirrer for about 30 min to extract the drug and dissolved completely. After filtration though paper the 1 ml of filtrate is pipette out and diluted up to 10 ml volumetric flask. Drug concentration in ethanol phase was recorded by taking absorbance of this solution. The drug concentration was calculated. Thus, the total drug entrapped in total yield of microspheres from the procedure was calculated. It is express in percentage it is called as % drug entrapment.[9] The amount of drug loaded and entrapped in oral microsphere was calculated by following formula.

% Drug Entrapment = Amount of drug actually present / Theoretical drug loaded expected × 100

Particle size analysis

Particle size was determined by using an optical microscope under regular polarized light and the mean particle size was calculated by measuring 50-100 particles with the help of a calibrated ocular micrometer.[10]

Morphological characterization using SEM

The prepared microspheres were coated with a thin layer of gold by sputtering (Emitech K450X, England) and then the microstructure were observed in a scanning electron microscope (SEM; AIS-2100 780, Seron, South Korea) that operated at an acceleration voltage of 20 kV.[11]

In-Vitro Dissolution Study

The release studies were carried out in 10 ml Franz diffusion cell containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10ml) was placed in a 10 ml beaker. The beaker was assembled on a magnetic stirrer

and the medium was equilibrated at 37±50C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Bisoprolol Microspheres was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.[12]

Kinetics of drug release studies[13]

Zero order release kinetics

It refers to the process of constant drug release from a drug delivery device independent of the concentration. In its simplest form, zero order release can be represented as

$Q = Q0 + K0\ t$

Where Q is the amount of drug released or dissolved,

Q0 is the initial amount of drug in solution (it is usually zero), and K0 is the zero order release constant. The plot made: cumulative drug release vs. time. Graphical representation of fraction of drug dissolved verses time will be linear. The slope of the curve gives the value of K in zero order release kinetics. This is ideal behaviour for a dosage form and leads to minimum fluctuations in drug plasma levels. This is expressed mainly by osmotic pump systems and also transdermal systems, matrix tablets with low soluble drugs and coated forms.

First order release kinetics

The first order Equation describes the release from system where release rate is concentration dependent, expressed by the equation:

$dC / dt = - Kt$

Where

K is first order rate constant expressed in units of time-1 .

This equation can be expressed as: $Log\ Ct = Log\ C0 – k\ t / 2.303$

Where,

C0 is the initial concentration of drug and

Ct is the concentration of drug in solution at time t.

The equation predicts a first order dependence on the concentration gradient (Co - Ct) between the static liquid layer next to the solid surface and the bulk liquid. The plot made: log cumulative of % drug remaining vs. time which would yield a straight line with a slope of –K/2.303.

Higuchi Model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1963 this model is applicable to study the release of water soluble and low soluble drugs incorporated in semisolid and solid matrices Model expression is given by the equation:

$Q = A\ [D\ (2C - Cs)\ Cs\ t]\ 1/2$

Where

Q is the amount of drug released in time t per unit area A,

C is the drug initial concentration,

Cs is the drug solubility in the media and D is the diffusivity of the drug molecules (diffusion coefficient) in the matrix.

Simplified Higuchi model describes the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Equation.

$Q = KH\ t1/ 2$

The data obtained were plotted as cumulative percentage drug release versus square root of time. The slope of the plot gives the Higuchi dissolution constant KH.

Korsmeyer - Peppas Model

To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer – Peppas model

$Mt / M\alpha = K\ tn$

Where

Mt/Mα is fraction of drug released at time t, k is the rate constant (having units of tn) incorporating structural and geometric characteristics of the delivery system. n is the release exponent indicative of the mechanism of transport of drug through the polymer. The n value is used to characterize different release mechanisms.

Stability studies[14]

Once the delivery system was developed, the practical utility of the formulation depends on the maintenance of the therapeutic efficacy throughout the shelf-life under different storage conditions. Various In vitro characterization parameters (physical appearance, entrapment efficacy, and drug release) of the microspheres were assessed after storage of the best formulations for 3 and 6 months at 40±2ºC/ 75±5% RH according to ICH guidelines, and results were compared with those obtained before storage.

III. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the API and the selected polymers and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-polymer mixture, which confirmed the absence of any chemical interaction between the drug, polymers and other chemicals.

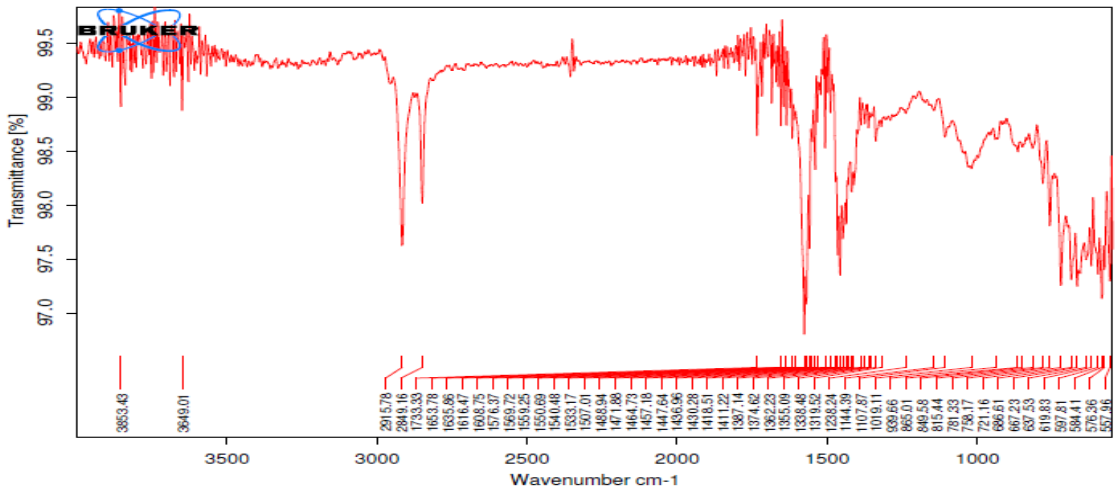

Fig-1: FT-IR Sample for Pure drug

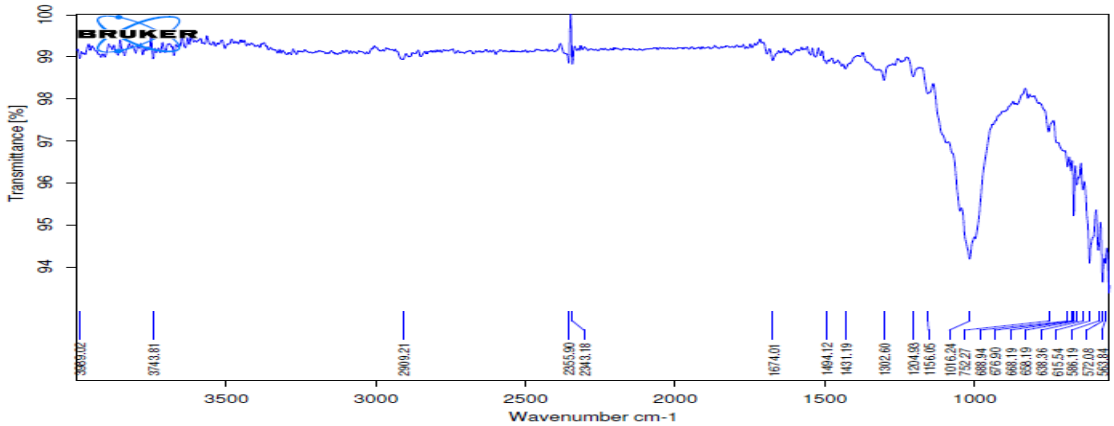

Fig-2: FT-IR Sample for Optimized formulation

Formulation and Evaluation of sustained release Microspheres of Cisplatin

Characterization of microspheres

Surface topography 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.

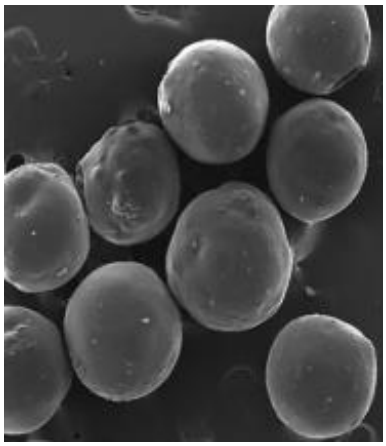

Fig-3: SEM photograph

The morphology of the prepared diverse types of microspheres was found to be virtually spherical in shape and have a rough surface, as illustrated in SEM photomicrographs of the microspheres.

Particle size:

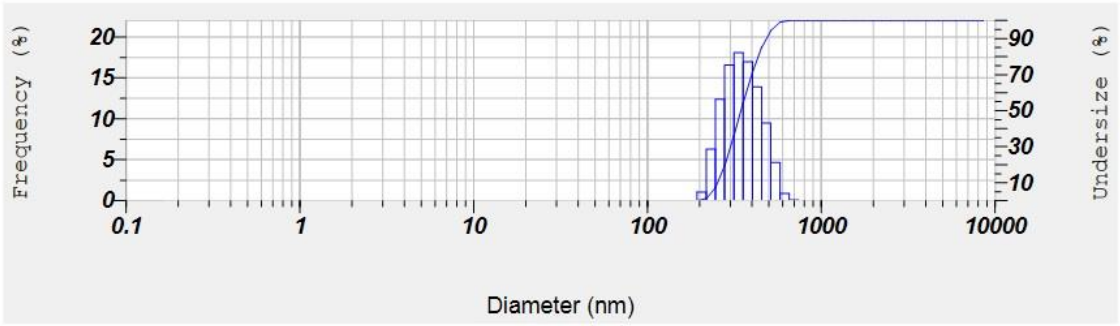

Fig-4: Particle size of optimized formulation

The mean particle size of optimized cisplatin microspheres was found to be 555 micrometers.

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

Table-2: Effect of drug polymer ratio on Yield of microspheres, Particle size, Drug entrapment efficiency

Formulation code	% Yield	Particle size	Drug Entrapment Efficiency
F1	75.98	692	76.98
F2	80.16	653	79.68
F3	79.89	658	80.25
F4	80.22	622	81.29
F5	83.36	555	84.98
F6	80.19	589	83.22
F7	78.47	593	80.98
F8	81.22	585	79.69

Percentage Yield

The % yield ranged from 75.98 % (F1) to 83.36 % (F5). All batches exhibited satisfactory recovery,

indicating minimal processing loss during preparation. The slightly higher yield observed in F5 (83.36 %) could be due to better precipitation/solidification efficiency or minimal loss during washing and collection. The lowest yield for F1 (75.98 %) might be related to polymer loss during filtration or transfer steps.

Particle Size

Particle sizes varied between 555 nm (F5) and 692 nm (F1). All formulations fall within the typical submicron range desirable for controlled drug delivery systems. The relatively smaller particle size of F5 (555 nm) and F8 (585 nm) may enhance surface area and consequently improve drug release rate. Larger particles in F1 (692 nm) might be due to higher polymer concentration or insufficient stirring/sonication during preparation.

Drug Entrapment Efficiency (EE)

Entrapment efficiency ranged from 76.98 % (F1) to 84.98 % (F5). A higher EE indicates effective incorporation of venlafaxine into the polymer matrix. F5 (84.98 %) achieved the highest EE, which may be attributed to optimal polymer–drug ratio and efficient encapsulation conditions. The relatively lower EE in F1 (76.98 %) may reflect inadequate interaction between drug and polymer or higher loss of drug during washing.

Drug release studies

Table-3: *In vitro* release data of Microspheres 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	14.90	15.15	15.80	15.56	16.13	15.58	14.89	15.10
2	26.70	25.89	26.50	25.55	26.45	25.55	25.60	24.65
3	37.89	36.87	37.70	38.25	37.89	38.55	33.59	35.65
4	48.18	45.23	44.50	47.59	48.89	48.66	49.89	48.24
5	69.75	68.35	67.65	66.55	68.98	67.55	69.12	69.32
6	76.89	79.34	71.98	78.32	79.21	80.55	81.25	82.65
7	88.86	86.77	85.32	84.28	85.90	86.99	88.96	89.23
8	94.45	92.50	93.12	95.22	98.10	96.89	96.92	95.78

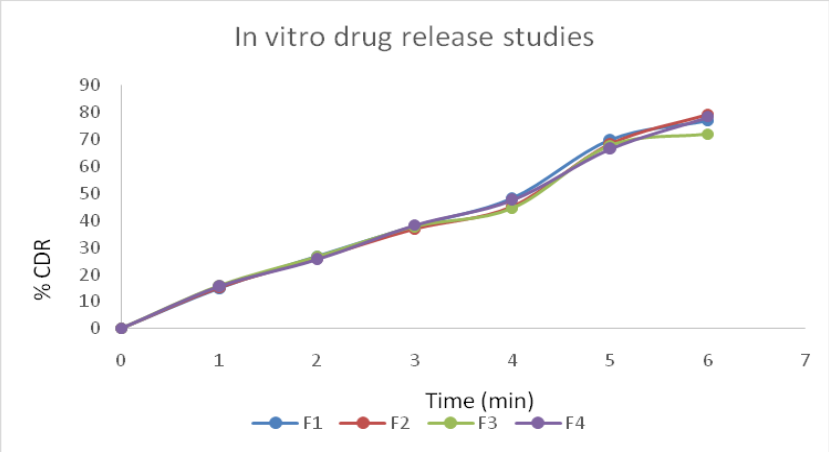

Fig-5: In vitro drug release of (F1- F4) formulation

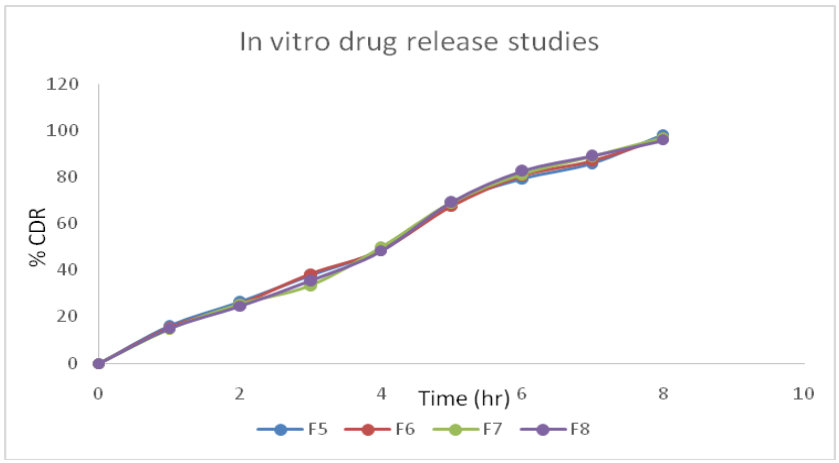

Fig-6: In vitro drug release of (F5- F8) formulation

Drug release kinetics:

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

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

Zero order kinetics

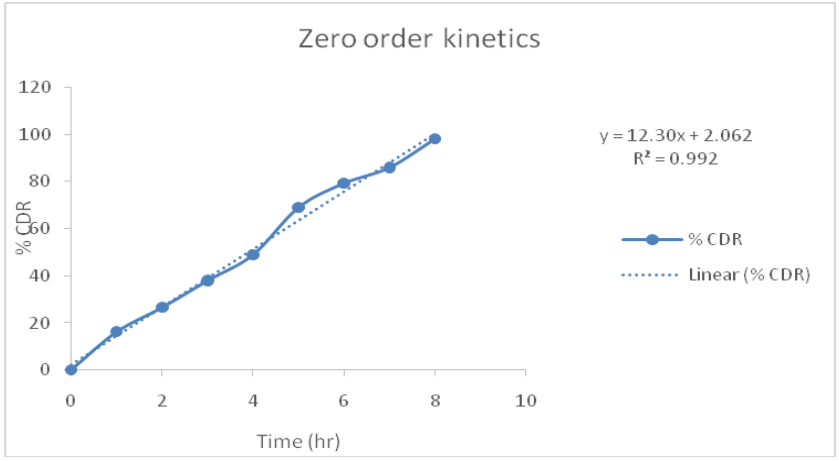

Fig-7: Zero order kinetics of optimized formulation

First order kinetics

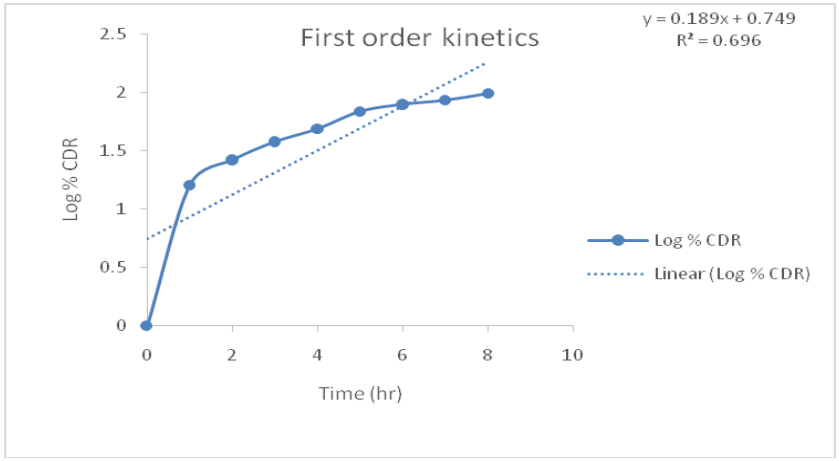

Fig-8: First order kinetics of optimized formulation

Higuchi model

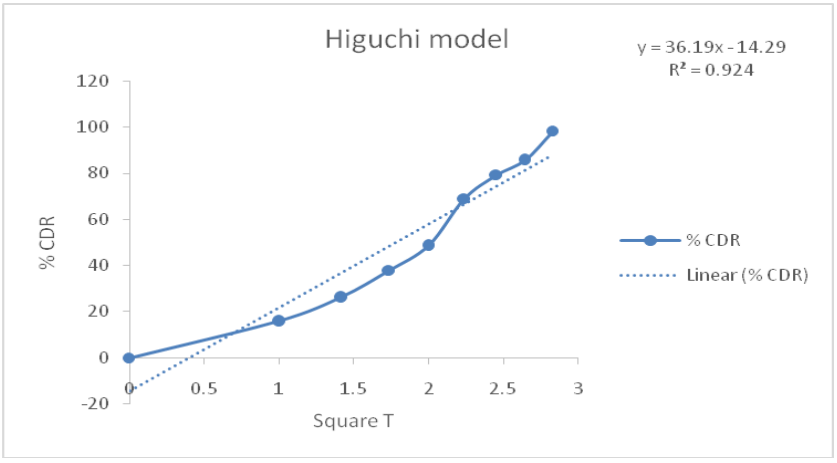

Fig-9: Higuchi model of optimized formulation

korsmeyer peppas

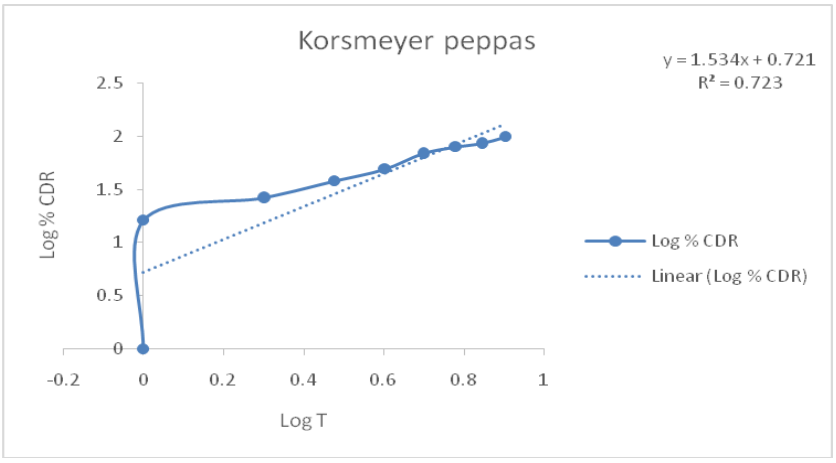

Fig-10: korsmeyer peppas of optimized formulation

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 Cisplatin microspheres Zero order release kinetics.

The table indicates that r^2 values are higher for Higuchi's model compared for all the formulation. Hence cisplatin release from all the microspheres followed dissolution 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-4: Results of stability studies of optimized formulation

F. Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-5	25^{0}C/60%RH % Release	98.10	97.86	96.24	95.98	Not less than 85 %
F-5	30^{0}C/75% RH % Release	98.10	97.58	96.22	95.65	Not less than 85 %
F-5	40^{0}C/75% RH % Release	98.10	97.36	96.01	95.32	Not less than 85 %

CONCLUSION

- Sustained-release microspheres of Cisplatin were successfully developed using HPMC and Sodium Alginate via ionotropic gelation.
- F5 was identified as the optimized formulation, showing the highest yield, particle size uniformity, and maximum drug entrapment efficiency.
- In-vitro drug release studies confirmed sustained and controlled release following zero-order kinetics, suitable for reducing dosing frequency and improving patient compliance.
- FTIR studies confirmed compatibility between drug and polymers, while SEM analysis demonstrated spherical morphology of microspheres.
- The formulation remained physically and chemically stable under various storage conditions for up to 3 months.

Overall, the study demonstrates that polymeric microspheres of Cisplatin can provide a controlled and sustained drug release profile, making them a promising approach for improved chemotherapy administration with potentially reduced side effects.

REFERENCES

1. Nair R, Reddy B. Application of chitosan microspheres as drug carriers: a review. J Pharm Sci Res 2009; 1:1-12.
2. Kadam NR, Suvarna V. Microsphere a brief review. Asian J Biomed Pharm Sci 2015;5:13-9.
3. Virmani T, Gupta J. Pharmaceutical application of microspheres: an approach for the treatment of various diseases. Int J Pharm Sci Res. 2017; 8(8):3253-60.
4. .Li SP, Kowarski CR, Feld KM, Grim WM. Recent advances in microencapsulation technology and equipment. Drug Development and Industrial Pharmacy. 1988 Jan 1; 14(2-3):353-76.
5. Kreuter J, Nefzger M, Liehl E, CzokR VR. Microspheres–A Novel Approach in Drug Delivery System. J Pharm sci. 1983; 72:1146.7.
6. Margel S, Wiesel E. Acrolein polymerization: monodisperse, homo, and hybrido microspheres, synthesis, mechanism, and reactions. Journal of Polymer Science: Polymer Chemistry Edition. 1984 Jan; 22(1):145-58.
7. Wakiyama N, Juni K, Nakano M. Preparation and evaluation in vitro of polylactic acid microspheres containing local anesthetics. Chemical and Pharmaceutical Bulletin. 1981 Nov 25; 29(11):3363-8
8. Patel NR, Patel DA, Bharadia PD, Pandya V, Modi D. Microsphere as a novel drug delivery. International Journal of Pharmacy & Life Sciences. 2011 Aug 1; 2(8).
9. Toshio Y, Mitsuru H, Shozo M, Hitoshi S. Specific delivery of mitomycin c to the liver, spleen and lung: Nano-and m1crospherical carriers of gelatin. International Journal of Pharmaceutics. 1981 Apr 1;8(2):131-41.
10. Patel NR, Patel DA, Bharadia PD, Pandya V, Modi D. Microsphere as a novel drug delivery. International Journal of Pharmacy & Life Sciences.2011 Aug 1;2(8).
11. Herfarth H, Obermeier F, Andus T, Rogler G, Nikolaus S, Kuehbacher T, Schreiber S. Improvement of arthritis and arthralgia after treatment with infliximab (Remicade) in a German prospective, open-label, multicenter trial in refractory Crohn's disease. The American journal of gastroenterology. 2002 Oct 1;97(10):2688.
12. Kavita K, Ashvini VR, Ganesh NS. Albumin microspheres. Unique system as drug delivery carriers for non steroidal anti-inflammatory drugs. Int J Pharm Sci Rev Res. 2010; 5(2):10.
13. .Vishal Yadav, A Review on Microspheres: Preparation, Characterization and Applications, Asian Journal ofPharmaceutical Research and Development. 2022; 10(6): 128-133.
14. Patel KS, Patel MB. Preparation and evaluation of chitosan microspheres containing nicorandil. Int J Pharm Investig. 2014 Jan;4(1):32-7.