DEVELOPMENT AND CHARACTERIZATION OF CHLORAMBUCIL LOADED POLYMERIC NANOPARTICLES

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***ABSTRACT :** Nanoparticles are formulated to target the drug to the specific organ site and to control the rate of delivery of drug. By encapsulating a drug into nanostructures, the being of the drug in the systemic circulation can be prolonged and thus improve perforation into target tissue and decrease the toxicity. The main aim of this study is to achieve prolonged release of Chlorambucil such that the dosing frequency of the drug can be reduced by which we may decrease the side effects and improve the patient compliance. By formulating Chlorambucil as nanoparticles we can directly deliver the drug to the cancer cell and prevent the normal cells from the adverse effects of Chlorambucil. Investigation of the preparation, characterization and in-vitro delivery of the nanoparticles was carried out. The different formulations of with different concentration of drug-polymer and surfactant were examined and finalized. which can accomplish belongings in drug encapsulation and drug delivery kinetics of the nanoparticles.*

***Key words:** Chlorambucil, polymer, solvent evaporation method, in vitro drug release studies, Zero order kinetics*.

I. INTRODUCTION

In the last 30 years, particle size reduction technologies turned from an exploratory approach into a mature commercial drug delivery platform. Nanonization technologies have gained a special importance due to a steadily increasing number of development compounds showing poor aqueous solubility[1]. Many drug delivery companies and academic research groups have contributed to the currently existing large variety of different technologies to produce drug nanoparticles[2]. The prefix "nano" has found in last decade an ever-increasing application to different fields of the knowledge. Nanoscience, nanotechnology, nanomaterials or Nano chemistry are only a few of the new nano-containing terms that occur frequently in scientific reports, in popular books as well as in newspapers and that have become familiar to a wide public, even of non- experts[3]. Pharmaceutical nanoparticles are defined as solid, submicron-sized (less than100 nm in diameter) drug carrier that may or may not be biodegradable. The term nanoparticle is a combined name for both nanospheres and nano capsules.[4] The main aim of this study is to achieve prolonged release of Chlorumbucil such that the dosing frequency of the drug can be reduced by which we may reduce the side effects and increase the patient compliance[5]. By formulating Chlorumbucil as nanoparticles we can directly deliver the drug to the cancer cell and prevent the normal cells from the adverse effects of Chlorumbucil. Chlorambucil is an antineoplastic in the class of alkylating agents that is used to treat various forms of cancer[6]. Alkylating agents are so named because of their ability to add alkyl groups to many electronegative groups under conditions present in cells. They stop tumor growth by cross-linking guanine bases in DNA double-helix strands - directly attacking DNA[7].

II.MATERIALS AND METHODS

2.1MATERIALS

Chlorumbucil was collected as a gift sample from Hetero Drugs Ltd, Hyd, Tragacanth, Sodium alginate and various excipients were purchased from AR Chemicals, HYD.

2.2 METHODOLOGY

Compatibility studies Fourier transform infrared (FTIR) analysis

The FTIR analysis of the Chlorambucil was carried out for qualitative compound identification. To check the compatibility of the drug with various polymers, IR spectra of drug, polymers, and combination of the drug and polymers were taken on an FTIR spectrophotometer in the wave number region of 4000-400/cm[8].

Method of preparation of Chlorambucil loaded nanoparticles:

Nanoparticles formulations were prepared by solvent evaporation method. The various different amount of

polymers was dissolved in solvent mixture of methanol (2 ml) and dichloromethane (8 ml) very slowly on a magnetic stirrer and Chlorambucil (5mg) was added to it and the contents were allowed to stand at room temperature for 30 to 45 minutes with occasional vortexing to allow complete solubilisation of drug and polymer. This solution was poured into 5 ml of each different concentration aqueous polyvinyl alcohol solution. The resulting solution was homogenized by using high pressure homogenizer for 3 minutes to form o/w emulsion. This emulsion was immediately added drop wise to 125 ml of aqueous PVA solution. The contents were stirred for 6 hours at room temperature with a magnetic stirrer to evaporate organic volatile solvent, allowing the formation of a turbid nanoparticulate suspension. The suspension was filtered through membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and supernatant was collected. Further the ultracentrifugation (3200 rpm for 1 hour) was carried for supernatants. Following ultracentrifugation, the pellet was washed and collected two times with deionized water to remove adsorbed drug and was suspended in deionized water to prevent clumping on storage[9].

Table-: 1 Composition of the Nanoparticles

Ingredients	Batch no							
	F1	F2	F3	F4	F5	F6	F7	F8
Chlorambucil(mg)	5	5	5	5	5	5	5	5
Tragacanth (mg)	50	100	150	200	-	-	-	-
Sodium alginate(mg)	-	-	-	-	50	100	150	200
Methanol (ml)	5	8	8	8	8	8	8	8
Polyvinyl Alcohol(ml)	10	10	10	10	10	10	10	10
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation of Chlorambucil loaded nanoparticles:

Particlesize:

All the prepared batches of nanoparticles were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of nanoparticle dispersion on it and average size of nanoparticles were determined[10].

SEM analysis

The morphology of NPs was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the NPs was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 Ma[11].

Drug encapsulation efficiency:

Lyophilized nanoparticles 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Chlorambucil in nanoparticles to the theoretical amount of the drug used in the preparation .The entrapment of the Chlorambucil nanoparticles was expressed as loading capacity[12].

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies:

The release studies were carried out by franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non entrapped Chlorambucil dispersion 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[13].

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{Da}{Dt} \times 100$$

Where, Dt = Total amount of the drug in the patch
Da = The amount of drug released

Release kinetics

Drug release mechanisms and kinetics are the two important characteristics of a drug delivery system in describing drug dissolution profile. Mathematical models are used to evaluate the kinetics and mechanism of drug release from the tablets. The model that best fits the release data was selected based on the correlation coefficient(R) value in various models. The models that have show high 'R' value was considered as the best fit on the release data[14].

% drug release = concentration × no.of dilutions × volume of dissolution fluid/1000

1. Zero Order Release Equation:

The equation for zero order release is

$$Q_t = Q_o + K_o t$$

Where ,

Q_o = Initial amount of drug
Q_t = Cumulative amount of drug release at time "t"
K_o= Zero order release constant
T= Time in hours

The zero-order kinetics describes the systems in which the drug release rate is independent of its concentration of the dissolved substance. A graph was plotted between the time taken on x-axis and the cumulative percentage of drug release on y-axis.

2. First Order Release Equation:

The first order release equation is

$$\text{Log } Q_t = \text{Log } Q_o + K_t /2.303$$

Q_t = Cumulative amount of drug release at time "t"
K= First order release constant
T= Time in hours

Here, the drug release rate depends on its concentration .The first order kinetics describes the systems in which the drug release rate is concentration dependent.

3. Higuchi Release Equation

The Higuchi release equation is

$$\mathbf{Q_t = K_H \sqrt{t}}$$

Where ,

Q = Cumulative amount of drug release at time "t"
K_H = Higuchi constant
T = Time in hrs

Higuchi described the release of drug from an insoluble matrix as square root of time dependent process.The Higuchi square root model also gives the drug release from a planar surface of an insoluble heterogeneous matrix by diffusion through the intra granular openings created by porosity of the formulation. A graph is plotted between the square root of time taken on x-axis and the cumulative percentage of drug release on y-axis.

4. Korsmeyer -Peppas Release Equation:

Korsmeyer –Peppas equation is

$$F=M_t / M = K_m t^n$$

Where,

F = fraction of drug released at time 't'

M_t = amount of drug released at time 't'

M = total amount of drug in dosage form

K_m= kinetic constant

n = diffusion or release exponent

t = time in hrs

'n' = Linear regression of log (M_t / M) versus log t

In case of Korsmeyer-Peppas model, the drug release from such devices having constant geometry will be observed till the polymer chains rearrange to equilibrium state. A graph is plotted between the log time taken on x-axis and the log percentage of drug release on y-axis.

Stability studies:

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^{0}C/60% RH analyzed every month for period of three months.
2. 30^{0}C/75% RH analyzed every month for period of three months.
3. 40^{0}C/75% RH analyzed every month for period of three months[15].

III.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.

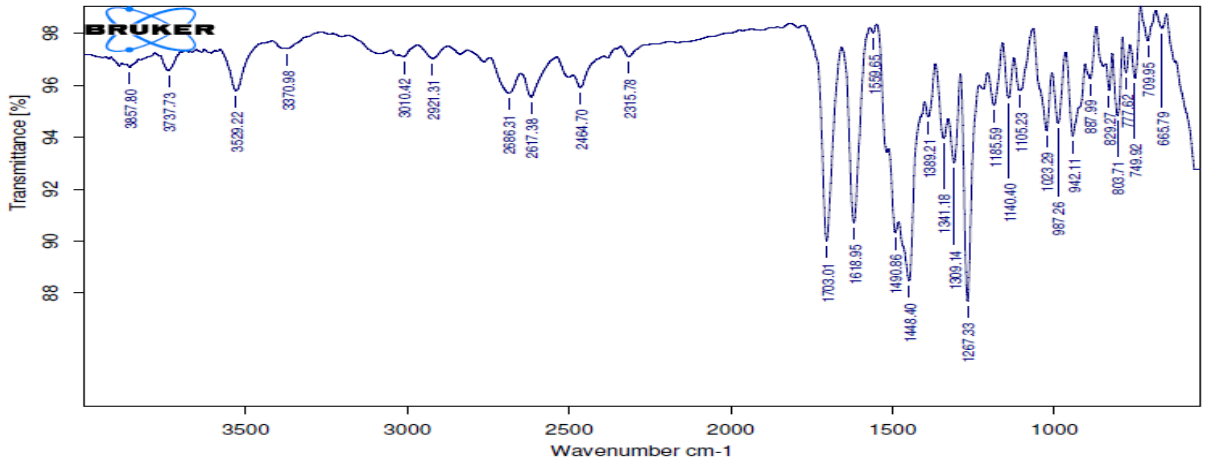

Fig.1. FT-IR Sample for Chlorambucil

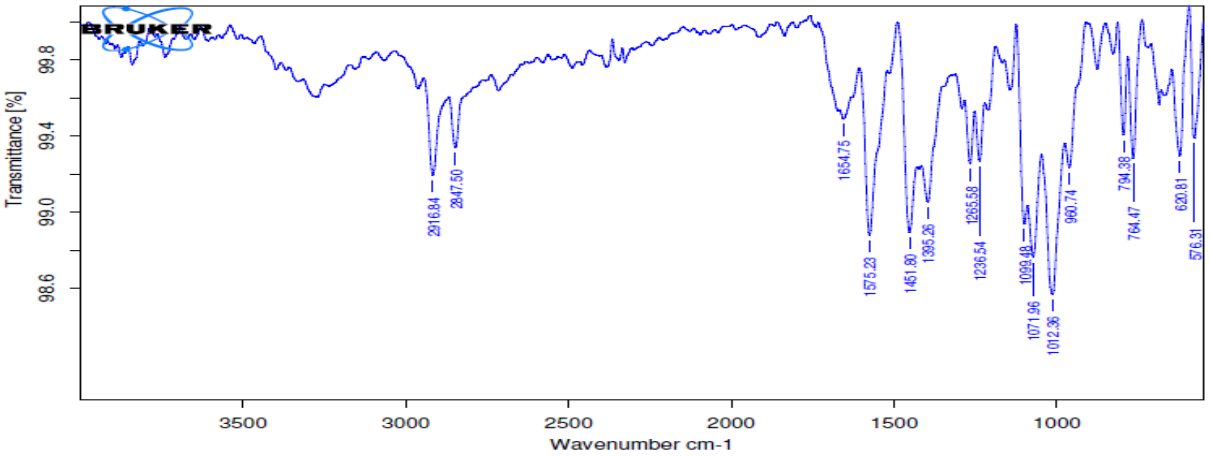

Fig.2. FT-IR Sample for Optimised Formulation

EVALUATION PARAMETERS

The nanoparticles prepared were evaluated as per the following parameters-

- Entrapment efficiency
- Particle size and SEM analysis
- In vitro release study
- Drug release kinetics
- Stability studies

Table: 2 Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency, Particle size

Batch No	Particle size (nm)	Entrapment Efficiency (%)
F1	186.8	44.6
F2	152.5	52.2
F3	176.9	69.6
F4	192.1	75.1
F5	116.8	64.9
F6	132.3	71.6
F7	121.5	73.6
F8	122.4	70.8

Surface morphology

Scanning electron microscopy (SEM) SEM revealed that the Chlorambucil nanoparticles

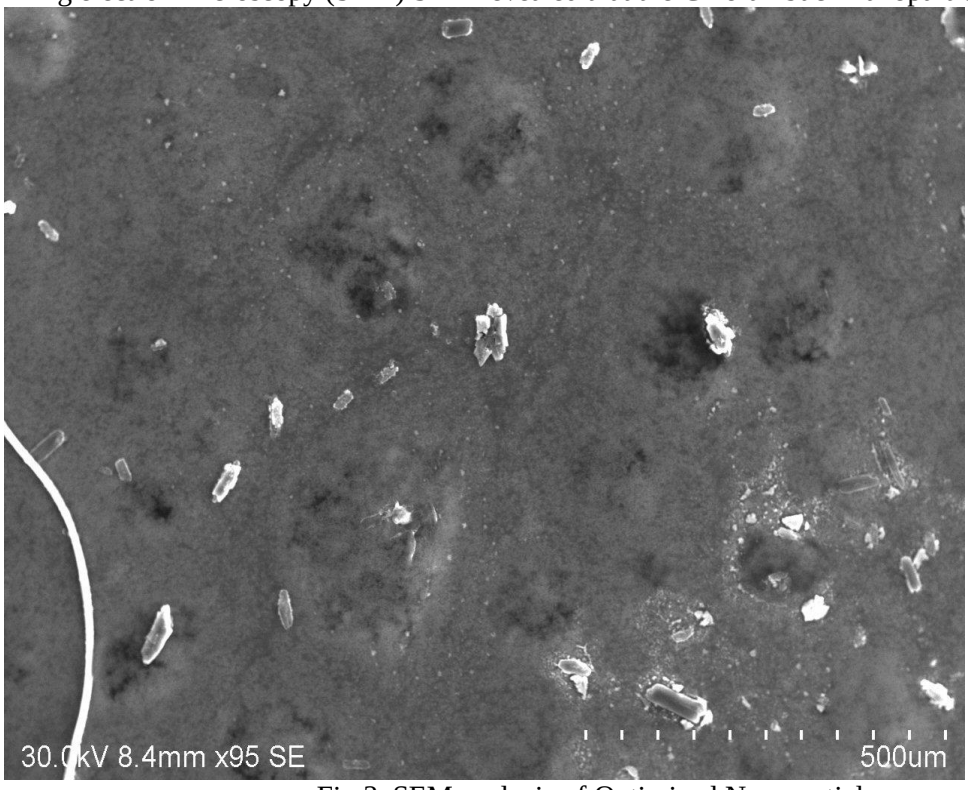

Fig.3. SEM analysis of Optimized Nanoparticles

Drug release studies

Table-: 3 Diffusion study profiles for all formulations

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	33.62	32.10	31.50	32.10	29.43	28.82	24.29	29.94
2	40.19	39.50	40.19	39.50	32.51	31.28	30.78	31.52
3	49.28	52.69	47.30	52.69	41.78	39.61	40.76	45.21
4	62.62	61.19	61.50	61.19	50.7	49.20	52.32	51.87
5	70.74	71.40	69.50	71.40	59.2	55.81	66.49	61.71
6	78.56	82.91	75.47	82.91	65.3	71.76	78.77	70.86
7	92.68	92.9	89.71	92.9	79.2	83.63	85.84	88.82
8	94.82	98.62	91.80	97.26	80.3	84.32	87.24	90.12

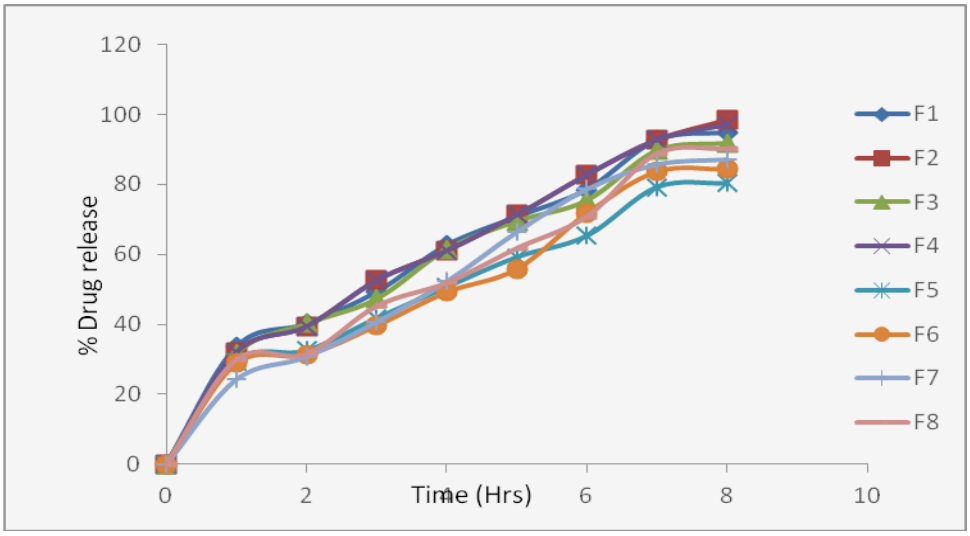

Fig.4. In vitro drug release studies for all formulations

The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 8 hours. Initially the release of drug from all the batches was found to be about 25-35% in 8 hours. This was due to the release of adsorbed drug from the surface of Nanoparticles. Later on a constant and slow drug release was observed for 8hrs. F4 formulation which had drug polymer tragacanth was decided to be the optimized formulation.

Kinetic modeling of drug release

All the eight formulation of prepared of Chlorambucil nanoparticles were subjected to in vitro release studies these studies were carried out using dissolution apparatus.

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

1. Cumulative percent drug released vs. time (zero order rate kinetics)
2. Log cumulative percent drug retained vs. time (First Order rate Kinetics)
3. Cumulative percent drug released vs. square root of time (Higuchi's Classical Diffusion Equation)
4. Log of cumulative % release Vs log time (Peppas Exponential Equation)

Dissolution data of above two methods was fitted in Zero order, First order and Higuchi equations. The mechanism of drug release was determined by using Higuchi equation.

Fig.5. zero order plot for optimized formula

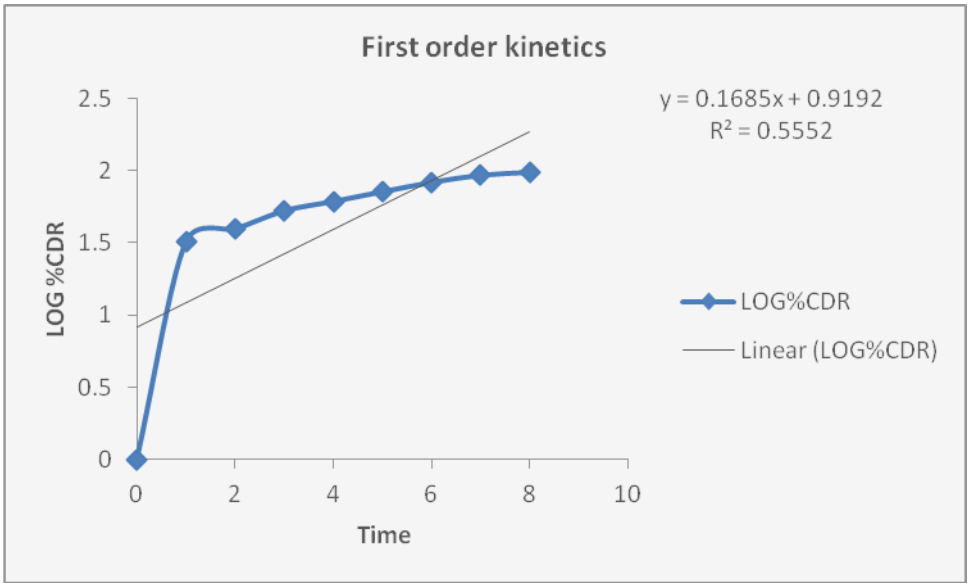

Fig.6. First order for optimized formula

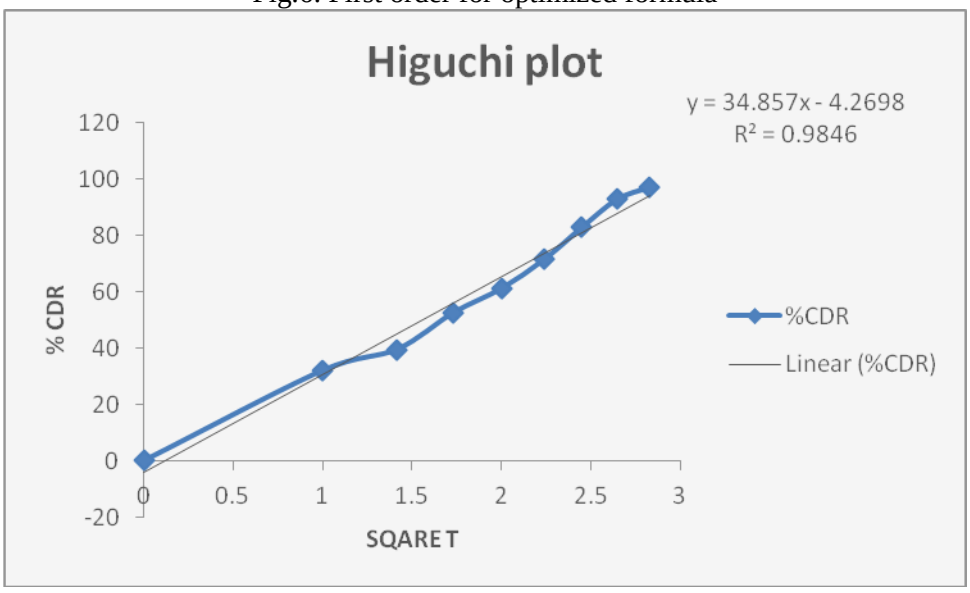

Fig.7. Higuchi plot for optimized formula

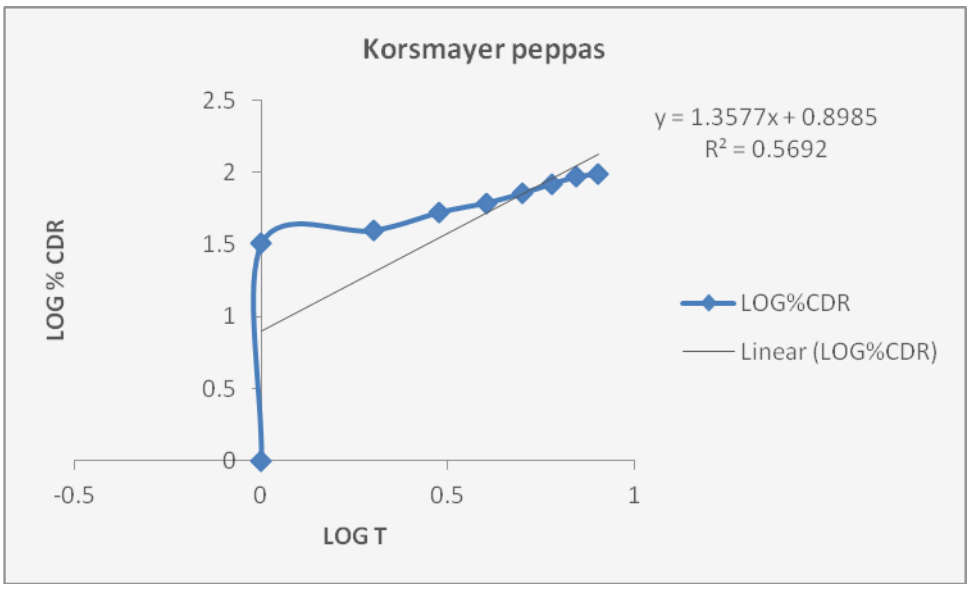

Fig.8. Korsmayer peppas plot for optimized formula

The drug release from the Nanoparticles was found to follow Zero order release based on the "r" value obtained for Zero order (0.958) and first order (0.555) for F4 formulation. Also, the drug release mechanism was found to be "Diffusion" based on the "r" value of 0.984 obtained for Higuchi's plot. Similarly, the drug release mechanism was found to be of Anomalous diffusion mechanism based on the "n" value of 0.569 obtained for Peppa's equation.

Stability studies

There was no significant change in physical and chemical properties of the tablets of formulation F-4 after 3 months. Parameters quantified at various time intervals were shown;

Table- : 5 Results of stability studies of optimized formulation F-4

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	25^0C/60%RH % Release	97.26	96.54	96.49	96.39	Not less than 85 %
F-4	30^0C/75% RH % Release	97.26	96.54	96.49	96.39	Not less than 85 %
F-4	40^0C/75% RH % Release	97.26	96.54	96.49	96.39	Not less than 85 %

IV. CONCLUSION

The present research proposed a novel formulation Chlorambucil Nanoparticles for controlled release. Investigation of the preparation, characterization and in-vitro release of the Nanoparticles was carried out. The different formulations of with various ratios of drug-polymer and surfactant were evaluated and optimised. In this research, in vitro drug release as high as 97.26 has been achieved. The method used for the formulation of Chlorambucil containing Tragacanth and sodium alginate nanoparticles was solvent evaporation method followed by sonication to reduce the particle size.

Nanoparticle's formulations showed good results in terms of the assayed drug content and encapsulation efficiency. This indicates that the method used for the formulation produced good yield and it was suitable and reproducible in nature. Formulation (F-4) showed the highest encapsulation efficiency. Permeation studies with dialysis membrane were carried out as per the method reported. The formulations showed good drug release from the polymer, the *in vitro* drug release profiles of all the formulations are within limits.

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