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Development and characterization of chlorambucil polymeric nanoparticles

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Abstract
The goal of this study was to assess the efficacy of a method based on the creation of polymeric nanoparticles as an innovative formulation of Chlorambucil with enhanced therapeutic efficacy. Chlorambucil has low solubility and permeability, which result in limited and variable bioavailability; its low stability makes it difficult to develop stable aqueous liquid formulations. The Chlorambucil Polymeric nanoparticles were created using the solvent evaporation process. The numerous formulations with varied drug-polymer and surfactant ratios were analyzed and improved. Chlorambucil Polymeric nanoparticles containing PLGA were created using the solvent evaporation method, then the particle size was decreased by sonication. Particle size, surface morphology by SEM, drug excipient compatibility by FTIR, and in-vitro drug release experiments were used to characterize the produced nanoparticles. The formulation with the best encapsulation efficiency was (F-4) A drug encapsulation effectiveness of up to 92.85% has been attained in this study. It was discovered that the efficiency of encapsulation improved along with the polymer content. According to the results of the current investigation, the manufacture of Chlorambucil Polymeric nanoparticles can be done using a solvent evaporation process followed by sonication.

Keywords: Chlorambucil drug, polymeric nano particles, solvent evaporation, lipid, FTIR, *in vitro* drug release

Introduction
Polymeric nanoparticles (NPs) are particles within the size range from 1 to 1000 nm and can be loaded with active compounds entrapped within or surface-adsorbed onto the polymeric core. The term "nanoparticle" stands for both nano capsules and nanospheres, which are distinguished by their morphological structure [1]. Polymeric NPs have shown great potential for targeted delivery of drugs for the treatment of several diseases. Polymeric nanoparticles (NPs) have attracted considerable interest over recent years due to their properties resulting from their small size [2]. Advantages of polymeric NPs as drug carriers include their potential use for controlled release, the ability to protect drug and other molecules with biological activity against the environment, improve their bioavailability and therapeutic index [3]. 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 reduce the side effects and increase the patient compliance [4]. 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. Chlorambucil is an antineoplastic in the class of alkylating agents that is used to treat various forms of cancer [5].

Materials
Chlorambucil was obtained from Micro lab. PLGA, SLS, and Poloxamer 407 were procured from Synpharma Research Labs, Hyderabad, and other chemicals the reagents used were of analytical grade.

Methodology
Compatibility study (IR spectroscopy) [6]
The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of the drug and polymer to an Infrared spectrophotometric study.

Method of preparation of Chlorambucil loaded nanoparticles

Formulation development

Table 1: Composition of the Nanoparticles

Ingredients	Batch no			
	F1	F2	F3	F4
Chlorambucil	2	2	2	2
PLGA	5	10	15	20
Poloxamer 407	0.5	1	1.5	0.5
SLS	5	5	5	5

Drug-loaded polymeric nanoparticles were prepared using PLGA as a poloxamer 407, and sodium lauryl sulphate (SLS) as a stabilizer utilizing the solvent evaporation method. PLGA concentration was kept constant (10 mg), while poloxamer 407, SLS, and drug were used in varying concentrations. The developed Nano formulations were characterized for their physicochemical properties [size, polydispersity index (PDI), and zeta potential], drug loading, % entrapment efficiency, and stability. The optimized Nano formulations were then decorated with Paclitaxel.

Evaluation of Chlorambucil loaded polymeric nanoparticles

Particle size [7]

All of the generated batches of nanoparticles were observed under a microscope to establish their sizes. The average size of the nanoparticles was determined by measuring the size of each batch's nanoparticles in a small drop of nanoparticle dispersion on a slide.

SEM analysis [8]

The morphology of nanoparticles was examined using the scanning electron microscope (SEM, Hitachi, Tokyo, Japan). After being properly diluted (1:100) in double-distilled water, Chlorambucil-freeze-dried SLNs were added to a drop of the nanoparticle formulation and left to air dry. The sample was then observed under various magnifications and a 15,000 volt accelerating voltage. The imaging was performed in a high vacuum.

Drug encapsulation efficiency [9]

A set volume of the SLNs dispersion (10 ml) was poured into a centrifuge tube at room temperature, and it was spun at 18,000 rpm for 20 minutes (Remi Instruments Pvt. Ltd, India). The drug's absorbance in the supernatant was measured spectrophotometrically at a maximum wavelength of 270 nm after the lipid component was removed (Shimadzu 1800, Japan).

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

In-vitro drug release studies [10]

Utilizing the dialysis bag approach, in vitro release tests were carried out. Before the release trials, the dialysis membrane (molecular weight cut off between 12,000 and 14,000) was immersed in double distilled water for an overnight period. As releasing media, phosphate buffer pH 6.8 and hydrochloric acid (0.1 N) were also employed. A donor compartment and a receptor compartment make up the experimental unit. A boiling tube that was cut open at one end and tied with a dialysis membrane at the other end serves as the donor compartment, into which 3 ml of SLN dispersion was injected for the release research. The receptor compartment is made up of a 250 ml beaker that contains 100 ml of release media and was kept at a temperature of 37 0.5 °C. Every 3 ml sample was taken out of the receiver compartment and replaced with the same amount of release medium at the 1, 2, 3, 4, 5, 6, 7, and 8h periods. The collected samples were appropriately diluted before being examined at 240 nm with a UV-visible spectrophotometer.

The percentage of drug release was determined using the following formula.

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

Where

Dt = Total amount of the drug

Da = The amount of drug released

Stability studies [11]

Over the course of 90 days, the stability of Chlorambucil nanoparticle dispersion in screw-capped glass vials was assessed. Six samples were split into two groups and kept at 4°C and 25°C, respectively. At the end of the 90 days, the amount of drug leaking from nanoparticles and the average particle size of the samples were calculated.

Results and Discussion

Drug - excipient compatibility studies (FT-IR)

Using the FTIR peak matching approach, the compatibility of the medicine with the chosen lipid and other excipients was assessed. The drug-lipid mixture showed no peaks that appeared or vanished, indicating that there was no chemical interaction between the medication, lipid, and other molecules.

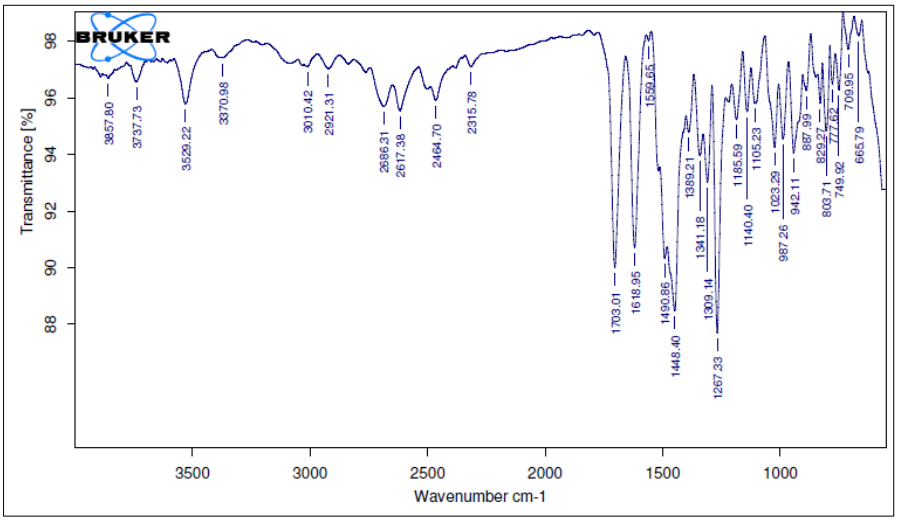

Fig 1: FT-IR Sample for Chlorambucil

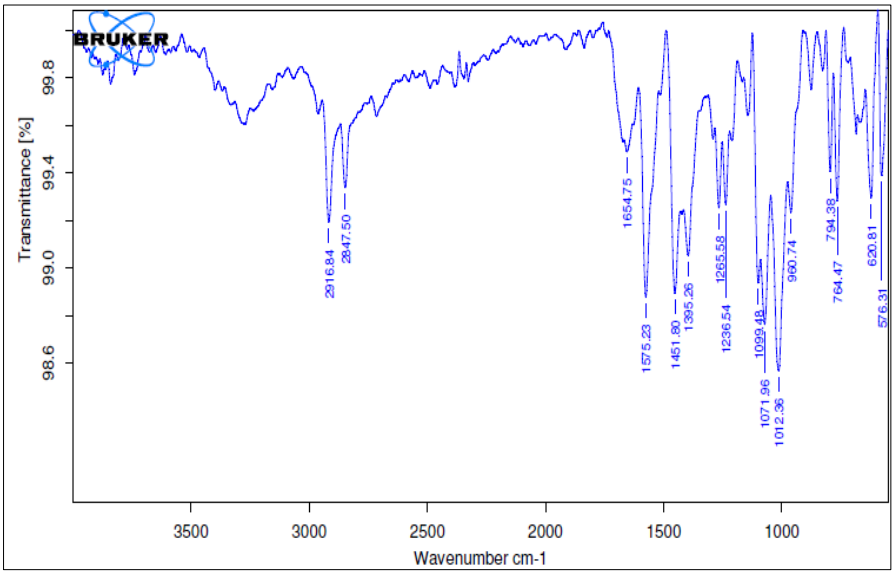

Fig 2: FT-IR Sample for Optimized Formulation

Evaluation Parameters

Particle size

With an increase in lipid concentration, the particle size increased based on entrapment effectiveness and particle size distribution.

Surface morphology

According to scanning electron microscopy (SEM), the polymeric nanoparticles were round, smooth.

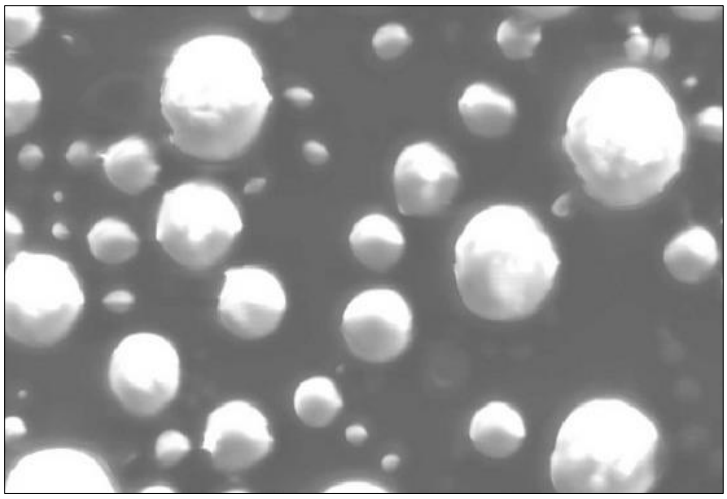

Fig 3: SEM analysis of Optimized polymeric nanoparticle

Drug entrapment efficiency

Optimizing the polymer concentration to be used in the creation of polymeric nanoparticles was the first step of the work plan. Based on the particle size and entrapment effectiveness of the discovered polymeric nanoparticles, the polymer content was optimized.

Table 2: Evaluation Studies of Prepared polymeric nanoparticles: Entrapment Efficiency and Particle size

Batch No	Particle size (nm)	Entrapment Efficiency (%)
F1	232	85.20
F2	226	88.63
F3	255	90.18
F4	228	92.85

In vitro drug release studies

Using a dialysis membrane and a pH 7.4 buffer, the in vitro diffusion investigations were carried out for eight hours. The initial release of the medication from all three batches was discovered to be between 25 and 30 percent in 8 hours. This resulted from the drug's release from the surface of the solid lipid nanoparticles. Later, for 8 hours, a consistent and gradual medication release was seen. The lipid and surfactant ratio in the F4 formulation was shown to be the most effective one.

Table3: *In vitro* drug release profiles of Chlorambucil polymeric nanoparticles (F1-F4)

Time	F1	F2	F3	F4
0	0	0	0	0
1	12.80	14.46	13.63	12.89
2	26.98	25.35	26.52	24.67
3	37.12	38.92	35.86	36.627
4	45.36	50.30	48.12	40.18
5	53.82	63.52	56.38	56.71
6	68.34	77.52	64.89	69.2
7	75.82	85.20	78.85	81.76
8	90.18	92.48	94.24	95.63

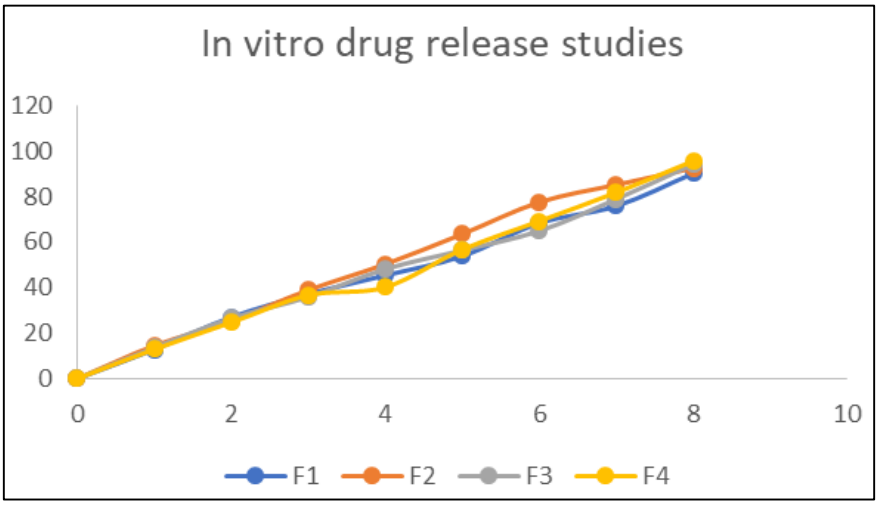

Fig 4: Drug release for all formulations

Stability studies:

After three months, the physical and chemical characteristics of the nanoparticles of formulation F-4 had not significantly changed. The parameters quantified at various times were displayed.

Table 4: 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	95.63	95.45	94.86	93.15	Not less than 85 %
F-4	30^0C/75% RH % Release	95.63	95.42	94.75	93.18	Not less than 85 %
F-4	40^0C/75% RH % Release	95.63	95.23	94.63	93.02	Not less than 85 %

Conclusion

The current study suggested a unique Chlorambucil polymeric nanoparticle formulation for regulated release. Investigation into the polymeric nanoparticles' production, characterization, and in-vitro release was done. The numerous formulations with varied drug-polymer and surfactant ratios were analysed and improved. A drug encapsulation effectiveness of up to 92.85% has been attained in this study. Chlorambucil polymeric nanoparticles containing polymers were created using the solvent evaporation method, then the particle size was decreased by sonication. formulation using polymeric nanoparticles performed well in terms of medication content and encapsulation effectiveness. This shows that the formulation procedure was suitable and reproducible in nature, and it provided a good yield. The formulation with the best encapsulation efficiency was (F-4) It was discovered that the percentage of encapsulation efficiency along with the polymer concentration. According to the method described, permeation studies with dialysis membrane were conducted. The in vitro drug release profiles of all the formulations indicated an initial burst effect, followed by a gradual drug release. The formulations demonstrated good drug release from the polymer. These polymeric nanoparticles contained more Chlorambucil and released it more quickly.

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