Formulation And Evaluation of Implantable Drug Delivery System for Temozolamide

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Abstract:

The central nervous system (CNS) comprises both the brain and the spinal cord. CNS is the foundation of our being, intelligence, personality, memories, ideas, speech, and comprehension, as well as our fundamental bodily processes and how we interact with the world around us, are all under its control. The brain stem, cerebellum, and cerebrum are the three primary areas of the human brain that can be distinguished despite its enormous interconnections Hydrophilic polymer was used in the preparation of temozolomide implants. Utilizing PLA, PGA, and PLGA, nine formulations were created. The powder blend underwent the pre-formulation parameters. Results from each formulation were all within acceptable ranges. Extrusion technology was used to prepare the implants. All of the formulations' physical parameters were found to be within the limit. All nine formulations were subjected to an in vitro dissolving test, with formulation F3 demonstrating the highest drug release (98.78% in 12 hours) compared to the other formulations. Temozolomide and the polymers utilized in the study have no chemical interactions, according to FTIR tests. Implants' appearance and drug content did not significantly alter over the short term, according to research using stable formulations.

Keywords: Implants, Temozolomide, PLGA, Central nervous system, Pre-compression.

INTRODUCTION

The human brain ranks among the preeminent and intricate organs in the human body, comprising an impressive array of over 100 billion neurons, intricately interconnected via an extensive network of trillions of synapses.[1] The central nervous system (CNS) comprises both the brain and the spinal cord. CNS is the foundation of our being, intelligence, personality, memories, ideas, speech, and comprehension, as well as our fundamental bodily processes and how we interact with the world around us, are all under its control.[2] The brain stem, cerebellum, and cerebrum are the three primary areas of the human brain that can be distinguished despite its enormous interconnections. In addition to linking the brain with the spinal cord and therest of the body, the brainstem, which is made up of the, midbrain, pons, and medulla, regulates respiration, heart rate, digestion, and other autonomic functions.

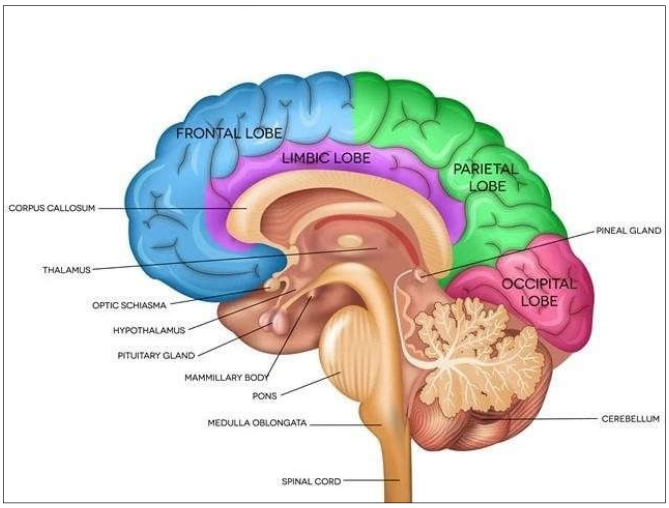

Figure no. 1 Brain Anatomy

MATERIAL AND METHOD

Temozolomide implants were prepared using a method that involved the incorporation of different grades of Carbopol, following a specific formulation. The process began by dissolving the drug in a 5% acetic acid solution. The medication solution was gradually mixed with Carbopol powder, and the mixture was let to soak for 10 to 15 minutes. During this period, the Carbopol swelled, forming a cohesive mass. The swelling mixture was fully blended into a sticky, malleable dough lumpsin a glass mortar for homogeneity. The cylinder of an extruder was then fed with this doughy material. The dough was extruded through the nozzle of the extruder in the shape of long rods, giving the implants the appropriate shape. The freshly created rods were then carefully placed onto a glass plate after the extrusion and allowed to dry for the night.[3] For the implants to acquire the requisite solid-state and

dimensional stability, this drying process was essential. The lengthy rods were painstakingly chopped into implants that each measured 27 mm in length after drying. After that, the implants were dried at 40 °C.

CROSS LINKING OF IMPLANTS

Implant preparation required a particular cross-linking technique with glutaraldehyde as a crucial step. First, a 100 ml beaker was filled with an exact volume of 25 ml of a 25% glutaraldehyde solution that had been metered out with the utmost care.[4] The beaker was then put into an empty desiccator to create a confined environment that was suitable for the cross- linking procedure. The desiccator was filled with a wire mesh carrying the implants to aid in the cross-linking process, and it was quickly shut. For varied periods of 6, 12, and 24 hours, respectively, the implants were subjected to glutaraldehyde vapours. In order to ensure that the reaction that occurred between Carbopol and glutaraldehyde was fully completed, the implants were cautiously removed after this exposure and left to air dry for 72 hours.[5] The implants were kept in an open environment for a week to encourage the evaporation of any leftover glutaraldehyde in order to completely remove it.[4] To achieve the best outcomes, the entire cross-linking procedure was methodically carried out at low temperatures. Additionally, the residual glutaraldehyde was effectively removed by employing an 2% aqueous solution of sodium metabisulfite. Subsequently, the implants were promptly transferred to an absolute alcohol bath.[6] This meticulous cross-linking process, utilizing glutaraldehyde, not only significantly enhanced the mechanical strength and stability of the implants but also played a crucial role in controlling their drug release properties. The successful implementation of this cross-linking methodology is anticipated to have valuable implications for the development of advanced implant materials with enhanced performance and therapeutic efficacy.[7]

Table No. 1: Drug and excipient details used in formulations

Formulation	**FA**	**FB**	**FC**	**F1**	**F2**	**F3**	**FX**	**FY**	**FZ**
Temozolomide mg	20	20	20	20	20	20	20	20	20
PLA	200	400	600	-	-	-	-	-	-
PGA	-	-	-	200	400	600	-	-	-
PLGA	-	-	-	-	-	-	200	400	600
5% acetic acid(ml)	5	5	5	5	5	5	5	5	5
25%Glutaraldehyde solution	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

PRE-COMPRESSION PARAMETERS

The assessment of pre-compression parameters for powder blend is a necessary step. The prepared powder formulation undergoes a series of tests to determine key characteristics that directly influence its suitability for compression processes. These assessments include measuring the tapped density, bulk density, Carr's compressibility index, angle of repose, and Hausner's ratio, all of which are conducted following standardized procedures.

Angle of repose

It is a crucial parameter that assesses the flowability and cohesiveness of the powder blend. A steep angle indicates poor flow, which can lead to issues during the manufacturing process, such as segregation anduneven tablet content.

Bulk density and **tapped density** measurements provide valuable insights into the packing properties of the powder. These measurements allow manufacturers to optimize the filling of powder into the die cavity during compression, ensuring consistent tablet weight and content uniformity.

Carr's compressibility index, also known as the Carr index, It helps in determining the required compression force and the potential for powder capping and sticking during tablet formation.[7]

Hausner's ratio, determined as the ratio of tapped density to apparentdensity, which helps to indicates the powder's ability to undergo compression without significant volume reduction.

EVALUATION PARAMETERS FOR IMPLANTS

Uniformity of weight

The uniformity of weight is an essential parameter to assess the consistency in the mass of individual implants within a batch. Implants with consistent weight ensure that each unit delivers the same amount of drug, providing reliable therapeutic efficacy. This test is carried out to keep the weight of individual implant consistent. This is accomplished by randomly weighing 20 implants and determining the average weight. Only two of the individual weights depart from the average weight by a percentage greater than that, and none do so by a margin greater than twice that. Determined and reported were the mean and standard deviation.[8]

Diameter of Implants.

Implants diameter is a critical dimension to evaluate, as it directly impacts theimplant's

release rate and biocompatibility. With the aid of Vernier callipers, the diameter and length of implants from each batch were measured. From each batch, three samples were selected for the study, and the mean value was given.

Procedure for drug content uniformity test

Every batch's implant's drug content was estimated. The implants were broken up into little pieces and placed in a 50 ml volumetric flask. 45 ml of glacial acetic acid was then added, and the volume was increased to 50 ml byadding more glacial acetic acid. By appropriately diluting this solution with glacial acetic acid and assessing the absorbance at 330 nm, the concentrationof temozolomide was determined.[9]

Swelling index

The implant formulations were submerged in a swelling solution with a phosphate buffer pH 7 while the swelling index was being examined.[10] The weight of the implants was measured after one hour of being submerged in aswelling solution, and any excess solution was carefully removed by tapping the outer layer with a piece of dry filter paper. Using the following equation, the level of swelling for individual implant formulation at a particular periodwas determined:

$$H = Wt - \frac{wo}{Wo} * 100$$

where W_t and W_0, respectively, represent the sample's weight at any given moment and when it is in dry state.

***In-vitro* dissolution studies**

In-vitro dissolution studies are a critical component, these studies involve the evaluation of the release of active pharmaceutical ingredients (APIs) from the dosage form in a simulated physiological environment. Assessment of the drug release kinetics, which is essential for establishing the medication's bioavailability and therapeutic effectiveness in the body, is the main goal of in vitro dissolution studies.[11] The dissolution medium (900 ml) was 0.1N hydrochloric acid, and the temperature was maintained at 37°C + 0.5°C while stirring at 50 rpm. At regular intervals, 5 ml samples were taken out, filtered, and replaced with 5 ml of fresh dissolving medium. When necessary, the obtained samples were appropriately diluted with dissolving fluid before being examined using a double-beam ultraviolet spectrophotometer.

Stability study

As per the ICH guidelines the primary objective of stability testing is to offer empirical evidence regarding the alterations in the quality attributes of a drug substance or drugproduct over time, taking into account the impact of diverse environmental factors such as temperature, humidity, and light. This essential assessment facilitates the establishment of appropriate storage conditions, retest intervals, and shelf lives, ensuring the maintenance of the product's quality and efficacy throughout its intended period of use. The chosen formulation's ICH guidelines stability studies were run for three months at 25°C/75% RH. The chosen formulations were then sealed in butter paper, kept at 37°C and 75% RH for three months, and tested for physical quality and drug content at certain intervals.[12]

Interactions Between Drugs and Polymers

The FTIR series model 1615 spectrometer from Perkin Elmer was used to measure the major peaks in the IR spectra of temozolamide with a resolution of 4 cm-1 spanning the range 4000-400 cm-1. Place 1 to 2 mg of the sample and 200–300 rng of KBr in a mortar. Prepare the pellets for sampling by compressing them under vacuum at a pressure of 800 MPa after thoroughly triturating the sample. FT-IR spectrophotometer was used to record the spectrum. The spectrum of an optimized formulation was compared to the spectrum of a pure medication. Fig. displayed spectra. In order to choose optimal chemically compatible excipients, FTIR analysis can be utilized as the easiest way to estimate any physiochemical interactions among within the components in a formulation.

pH solubility Profile

Water and several buffers, including 0.1N HCl, 4.5 pH acetic acid, 6.8 pH phosphate buffer, and 7.4 pH phosphate buffer, were used to test the solubility of temozolomide. Drug was added to 1 ml of the aforementioned buffers in excess up until no more drug was soluble in it. Following that, solutions were maintained for 24 hours at 37° C in an orbital shaker for shaking. After 24 hours, the solution was centrifuged for 20 minutes at 10,000 RPM in an ultracentrifuge. Then super latent solution was obtained for UV spectrophotometric absorption. To assist in the selection of acceptable dissolution media for development purposes and to guarantee that "sink conditions" are maintained therein, the solubility of Temozolomide IP in various pH ranges of the gastrointestinal system was evaluated. In Table 8.7, the solubility profile is displayed.

RESULTS

Pre-Compression Evaluation Parameters of TemozolomideFormulation Blend

The powder mixtures were carefully prepared by precisely combining the specified elements. These mixtures were employed to evaluate and characterize diverse powder flow properties. Through systematic analysis, it was established that all formulations exhibited bulk densities ranging from 0.46 ± 0.04 to 0.59 ± 0.04 (g/cm3), indicating highly favourable flow characteristics of the powders. The various formulations, (Table No. 1 and Table No. 2). Notably, all formulations demonstrated remarkably consistent tapped densities, lying within a narrow range of 0.56±0.02 to 0.69±0.03. Similarly, the compressibility indices exhibited a tightly clustereddistribution, ranging from 15.98±0.04 to 17.89±0.02. Hausner ratio ranges of 0.63±0.04 to 1.16±0.04, depicting good to excellent flow properties as the result of investigation for the temozolamide formulation blend. Table No.3:Findings of Pre-compression evaluation parameters of temozolomide formulation blend of bulk and tapped density.

Formulation code	Bulk density	Tapped density
IMPFA	0.48±0.08	0.56±0.02
IMPFB	0.55±0.07	0.61±0.04
IMPFC	0.51±0.04	0.67±0.06
IMPF1	0.53±0.03	0.65±0.07
IMPF2	0.55±0.07	0.68±0.04
IMPF3	0.54±0.04	0.65±0.06
IMPFX	0.59±0.04	0.69±0.03
IMPFY	0.46±0.04	0.56±0.04
IMPFZ	0.53±0.07	0.61±0.05

TableNo.3. Findings of Pre-compression evaluation parameters carr's index and hausner's ratio.

Formulation code	Carr's index	Hausner's ratio
IMPFA	15.98±0.04	0.76±0.04
IMPFB	16.57±0.04	0.99±0.06
IMPFC	17.18±0.06	0.63±0.04
IMPF1	17.45±0.07	1.14±0.05
IMPF2	16.72±0.02	1.12±0.09
IMPF3	17.25±0.07	1.05±0.08

IMPFX	16.33±0.03	0.76±0.02
IMPFY	17.89±0.02	1.14±0.07
IMPFZ	17.74±0.06	1.16±0.05

RESULT OF EVALUATED PARAMETERS OF TEMOZOLAMIDE IMPLANTS

Physical characteristics

The quantification analysis of variance in weight and drug content variability in Temozolomide Implant Formulations FA, FB, FC, F1, F2, F3, FX. FY & FZ, were measured, and the results of all formulations have been determined to be within the ranges prescribed in official texts.

Drug content

The medication concentrations of individual implant formulations were carefully determined and found to exhibit a high degree of uniformity, with values ranging from 96.9% to 102.3% of temozolomide content. The observed close alignment of the concentration levels within this allowable range attests to the consistency and accuracy of the medication content in the implant formulations**.**

Uniformity of weight

The quantification analysis of variance in weight for all formulations. Since the percentage of weight variation was within the pharmacopoeial limitations, all implants passed the weight variation test. All implant formulation weights were discovered to be between 50±5 mg.

Table No. 4: Report for evaluation parameters of temozolomide implants forweight variation and drug content

Formulation code	**Mean Weight (mg) (±SD)**	**Drug content (%) (±SD)**
IMPFA	54±0.03	100.6±0.03
IMPFB	51±0.02	96.9±0.03
IMPFC	48±0.09	99.9±0.04
IMPF1	47±0.02	102.3±0.03
IMPF2	50±0.08	100.4±0.06
IMPF3	52±0.09	95.3±0.07
IMPFX	45±0.09	98.5±0.06

IMPFY	55±0.05	100.1±0.03
IMPFZ	50±0.09	99.4±0.07

Diameters of implants

TableNo.4 provides a comprehensive record of the measured diameters for the fabricated implants. Across all batches of implant formulations, the mean implant diameters exhibited a remarkably consistent range, spanning from 1.01 mm to 1.74 mm.

% swelling index

The % swelling indices of the prepared implants were carefully assessed and reported, presenting a range spanning from 87% to 176%, as indicated in TableNo.5.

Table No. 5: Evaluation parameters of temozolomide implants for swelling index and diameter of implants

Formulation code	% Swelling index	Diameter of implants
IMPFA	88	1.01
IMPFB	111	1.53
IMPFC	136	1.31
IMPF1	105	1.25
IMPF2	117	1.07
IMPF3	143	1.66
IMPFX	87	1.74
IMPFY	133	1.44
IMPFZ	176	1.58

***IN- VITRO* DRUG RELEASE**

Dissolution tests were conducted using a rotating paddle method with apparatus 2, adhering to the guidelines of USP XXIV.

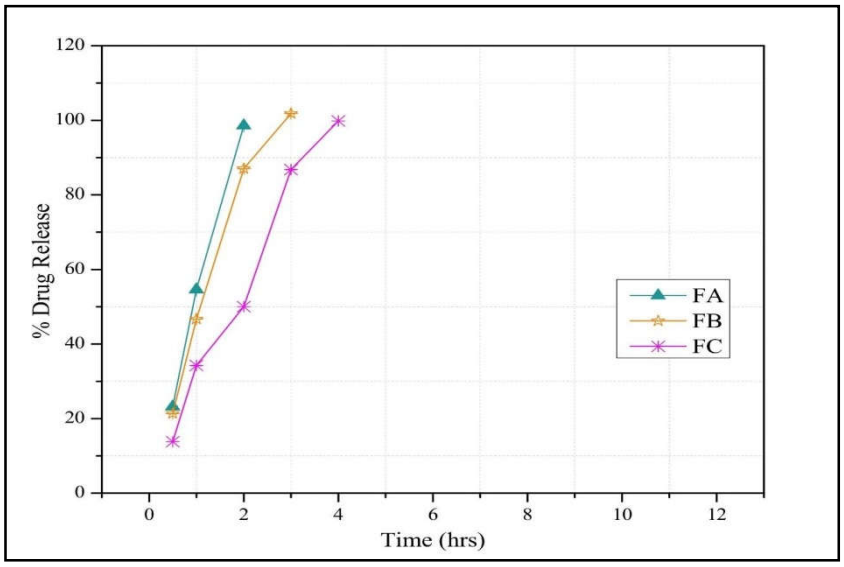

Fig. 2: *In vitro* drug release of implants with PLA (FA, FB and FC)

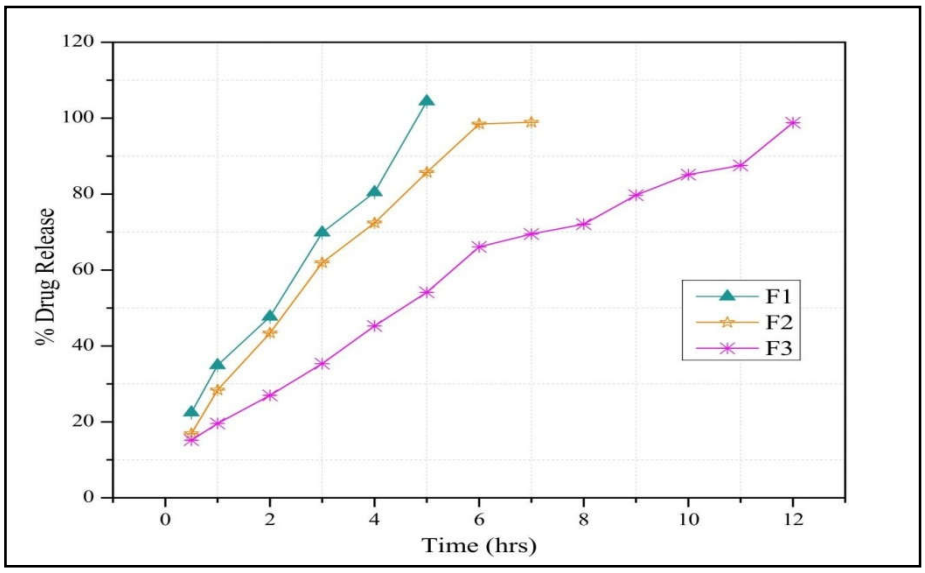

Fig. 3: *In vitro* drug release of implants with Carbopol 971P NF (F1, F2 and F3)

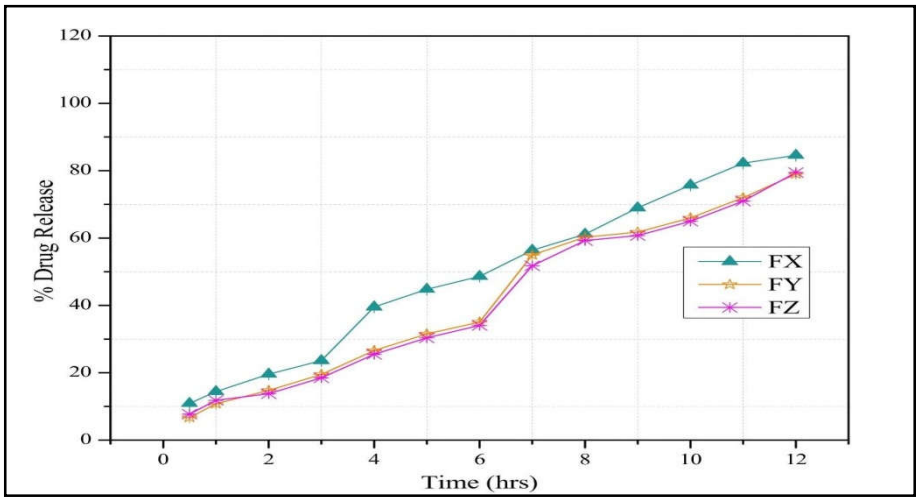

Fig.4. *In vitro* drug release of implants with PLGA (FX, FY and FZ)

Formulations as the optimal candidate among all formulations. Therefore, based on the results of the dissolution study, it can be concluded that formulation F3 exhibits superior characteristics compared to other.

Time (hrs.)	% Cumulative drug release								
	FA	**FB**	**FC**	**F1**	**F2**	**F3**	**FX**	**FY**	**FZ**
0.5	22.98±0.04	21.49±0.07	14.01±0.03	23.04±0.06	16.88±0.06	15.19±0.07	10.85±0.02	6.67±0.08	7.69±0.07
1	54.02±0.03	45.57±0.02	35.34±0.06	34.95±0.07	28.77±0.04	19.55±0.05	14.33±0.03	10.74±0.04	11.80±0.01
2	98.38±0.06	86.92±0.04	51.01±0.04	48.50±0.06	43.46±0.08	26.92±0.04	19.54±0.04	14.71±0.05	13.76±0.06
3		102.01±0.08	87.09±0.08	70.01±0.09	61.97±0.04	35.37±0.03	23.66±0.06	19.56±0.04	18.50±0.03
4			98.92±0.03	80.56±0.08	72.62±0.01	45.29±0.07	39.51±0.07	26.57±0.07	25.48±0.03
5				104.04±0.04	85.78±0.07	54.13±0.04	44.75±0.06	31.52±0.03	30.34±0.01
6					98.57±0.08	66.05±0.03	48.63±0.04	35.06±0.04	34.04±0.03
7					98.81±0.04	69.44±0.06	56.37±0.03	54.91±0.05	51.76±0.07
8						72.04±0.08	61.15±0.07	60.28±0.07	59.26±0.09
9						79.77±0.04	68.91±0.06	61.77±0.08	60.75±0.02
10						85.15±0.03	75.96±0.04	65.91±0.04	64.97±0.03
11						87.56±0.04	82.31±0.08	71.94±0.06	70.98±0.07
12						98.72±0.09	84.44±0.03	78.96±0.04	79.45±0.06

Table 6. Drug release profile of temozolomide implants

STABILITY STUDIES

Stability studies were conducted under controlled conditions of 25°C/75% RH and 37°C/75% RH over a duration of 3 months for the chosen formulation. To ensure protection, the chosen formulas were painstakingly wrapped in butter paper. At predefined intervals, their physical characteristics and medication content wereroutinely assessed.

Table No.7: Stability studies for optimized formulation (F3)

S.no	Optimized formulation (F3) duration (months)	25º C (75% RH)	37º C (75% RH)
1	1	96.45	97.82
2	2	96.85	97.70
3	3	96.80	97.65

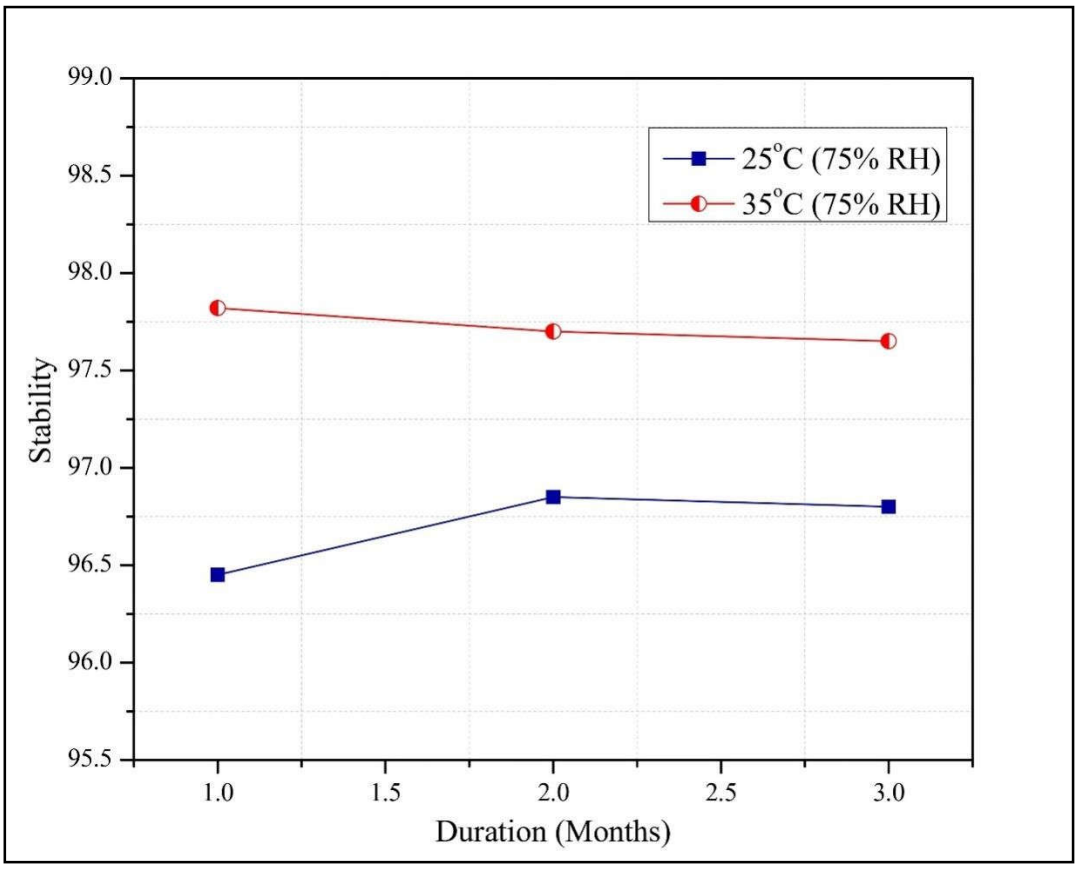

Fig 5: Stability studies for optimized formulation

COMPATIBILITY STUDIES BY FTIR

FTIR was employed to study the drug and excipient compatibility. Using potassium bromide (KBr) pellets, the IR spectra of TMZ was captured throughout the range of 4000-400 cm-1 at a resolution of 4 cm-1. Peaks for the respective functional groups in TMZ were visible in the study. There were no significant changes in the peaks when the trial was conducted with TMZ and polymers. The FTIR spectra shown above show no difference between internal structures and molecular confirmation of these substances. Fig. 6 and 7 demonstrate that there is no interaction between the medicine and the polymers in use.

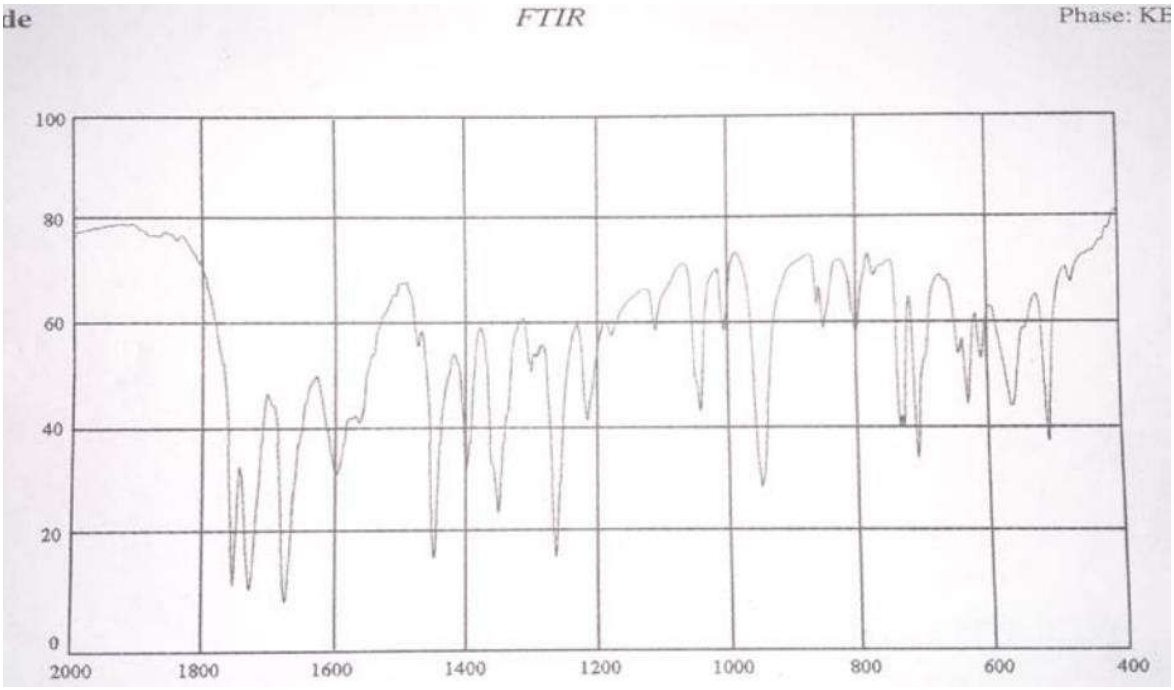

Fig.6: Reference spectra of Temozolamide from IP 2014

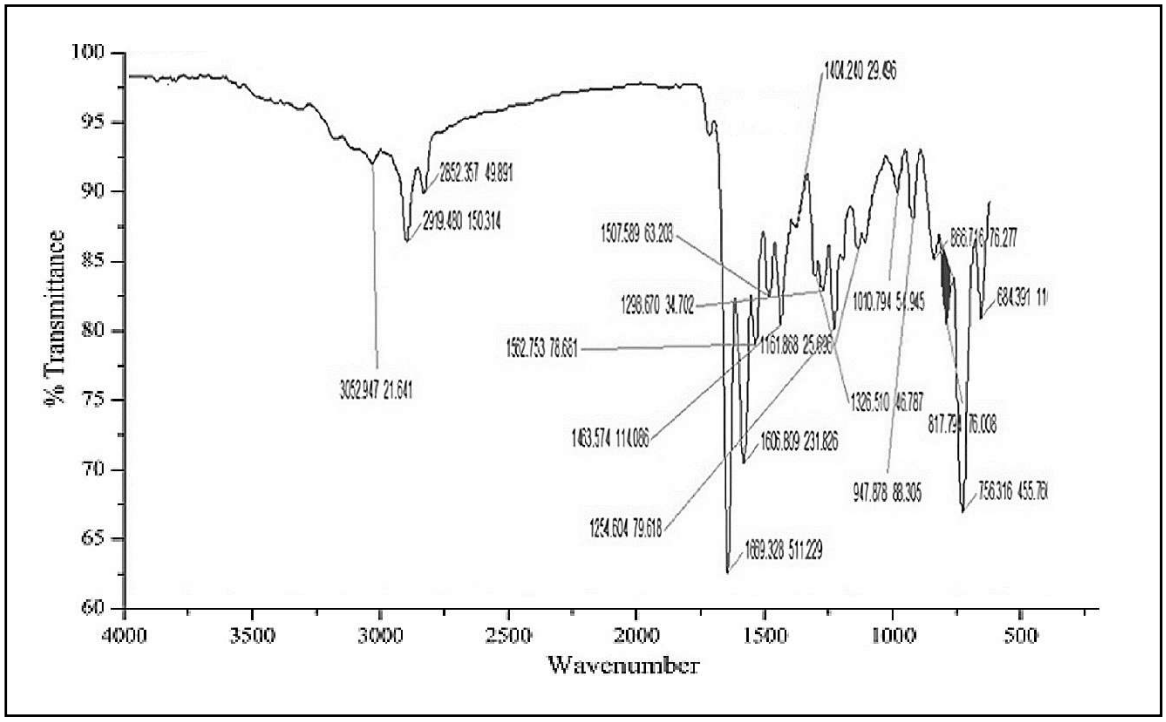

Fig. 7. Fourier transform infrared spectra of pure drug

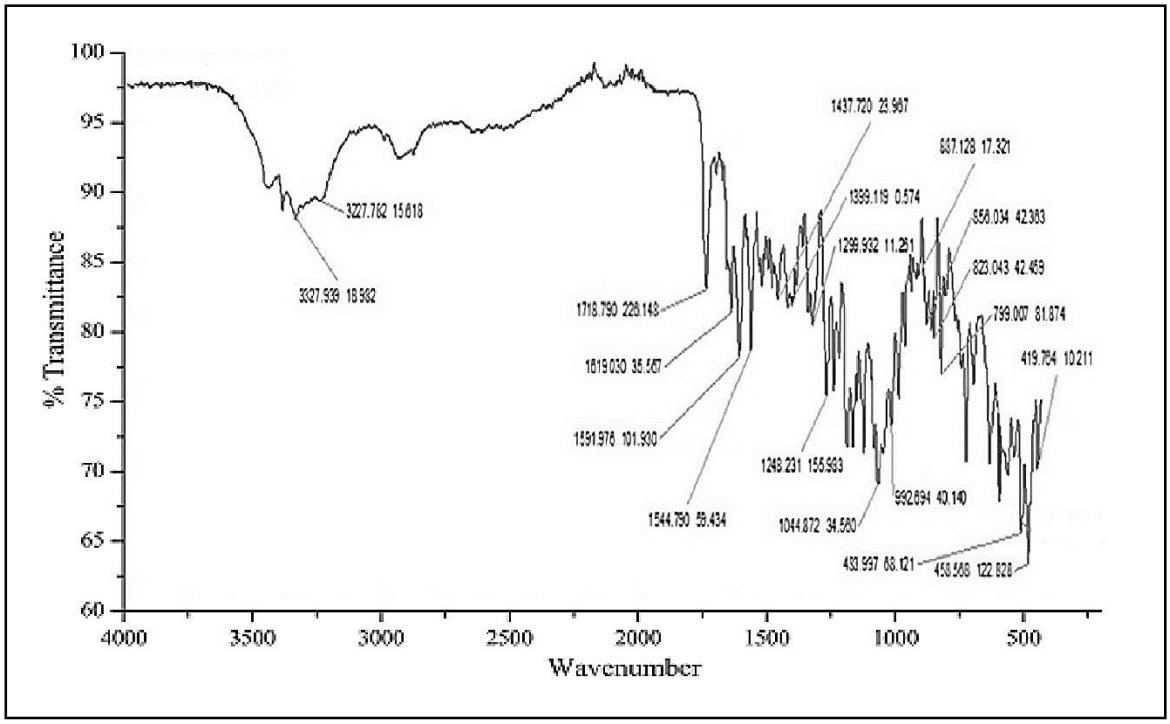

Fig. 8. Fourier transform infrared spectra of optimized drug

pH solubility Profile

The ability to dissolve of Temozolamide IP in various pH ranges of the gastrointestinal

system was assessed to aid in the selection of suitable dissolving media for the purpose of development and to ensure that "sink conditions" are maintained therein. Table No.7provides the information for the solubility profile.

DISCUSSION

1. Temozolamide implantable Drug Delivery systems can be prepared by using extrusion method using different grades of Carbopol.
2. All the prepared implant formulations were found to be good percentage of weight variation, which was within the pharmacopoeial limitations.
3. The flow properties of all formulation were evaluated and found within the acceptable range.
4. FTIR Spectra of physical mixture showed no interaction betweendrug and excipient in the prepared formulation.
5. The *In-vitro* dissolution profiles of all the prepared temozolamide implantable drug delivery system formulations demonstrated a notable retarding effect on drug release, effectively prolonging the releaseduration beyond 12 hours. Specifically, Formulation F3 exhibited aremarkable drug release of 98.78% within the 12-hour timeframe.
6. Comparing the all formulations, implantable drug delivery system formulation of F3 was considered as an ideal formulation which exhibited98.78%ofdrugreleasein12- hours timeframe.
7. The given implants of temozolamide were made to prepare with different polymer combination such as PLA, PGA and PLGA.

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