TEMOZOLOMIDE AFLOAT MICROSPHERES FOR CARCINOMA OF THE BRAIN: FABRICATION AND CHARACTERIZATION

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ABSTRACT

Floating microspheres (FM) of Temozolomide (TZM) were made to build its scope to achieve good blood concentration and to show lengthened medication discharge. FM of TZM was set up by Ion gelation strategy utilizing ethyl cellulose, Carrageenan gum, and sodium alginate. The medication stacked FM were structured and evaluated for its physicochemical attributes including drug-excipient inviting conduct by Differential Scanning Calorimetry (DSC) and Fourier Transform Infra-red (FTIR). The DSC and FTIR study uncovered the similarity of TZM with the excipients utilized. The rate yield of FM from all details was acceptable and demonstrated palatable lightness and coasting time. % TZM discharge for the FM was found up to 98.25% (F-8) till tenth h. The investigation infers that FM with TZM can be figured utilizing sodium alginate, ethyl cellulose, and carrageenan gum by exfoliation technique.

Keywords: Temozolomide, carrageenan, microspheres, buyout
INTRODUCTION
The floating drug delivery systems (FDDS) is intended for gastrointestinal maintenance for a long length (Ahad et al., 2011). Gastro holding of solid dosage forms can be accomplished by the creations of adjusted density, bodily fluid attachment, development, unfurling, and so on (Meka et al., 2008). Floating Microspheres (FM) with low-density polymers are getting intriguing for many researchers (Harsha et al., 2020).

Temozolomide (TZM) is an alkylating agent given orally to tackle carcinoma of the brain (Barvaux et al., 2004). TZM is of imidazotetrazine derivative, contains imidazole ring fused to tetrazine ring. TZN is a prodrug and converts into an active form in the body. It mainly eliminates by renal and traces by feces (Ashley et al., 2009).

MATERIALS AND METHODS
Material
The materials required in this work are delineated in Table 1.

| Materials              | Suppliers/Manufacturer                        |
|------------------------|-----------------------------------------------|
| Temozolomide           | A gift from Cipla, Hyderabad, Telangana        |
| Sodium alginate        | Fischer Chemic Ltd, Hyderabad                 |
| Ethyl Cellulose        | Fischer Chemic Ltd, Hyderabad                 |
| Carrageenan            | Fischer Chemic Ltd, Hyderabad                 |
| Calcium chloride       | Fischer Chemic Ltd, Hyderabad                 |
| Double distilled water | Own distillation unit                         |

Table 1: List of materials

The equipment utilized was (table 2) listed.

| Devices                  | Manufacturers                                  |
|--------------------------|-----------------------------------------------|
| Digital balance          | Vibra technologies, Bangalore                 |
| Magnetic stirrer         | Remi, Secunderabad                            |
| Melting point apparatus  | Sisco Ltd. Hyderabad, India                   |
| DSC scanner              | Perkin Elmer                                  |
| FTIR                     | Bruker alfa                                   |
| UV visible spectroscopy  | Shimadzu-S1210                                |
| Membrane filter          | Millipore                                     |

Table 2: List of equipment used in the study
Drug excipient compatibility studies

Differential scanning calorimetry (DSC)

The DSC examinations of TZM and definition mix were performed with Perkin Elmer, FTIR spectrophotometer to check any medication excipient collaboration. Each sample was situated in an aluminum skillet discreetly with warming paces of 10°C/min from 50-300°C under nitrogen (50 ml/min).

FTIR study

FTIR spectra of TZM and its combination with excipient blend were made by Bruker IR spectrophotometer.

Preparation of FM

The FM of the TZM was set up by the exfoliation method. The sodium alginate cross-connecting polymers were inundated in water for 24 h. The TZM taken in 10 ml of water broke up and afterward blended in with the above polymer blend. The above arrangement was poured gradually utilizing a 24-measure carrageenan needle containing calcium chloride (3%). The shaped FM was left for 30 min in the above arrangement with mixing to finish the response and to frame round MS. The readied MS were separated, sprinkled with refined water lastly dried at 45°C (Hennink and Franssen, 2002). The dried MS safeguarded in a water/air proof holder (Table 3) (Abdul et al., 2011).

| Ingredients (mg) | Formulations |
|-----------------|--------------|
|                 | F-1         | F-2 | F-3 | F-4 | F-5 | F-6 | F-7 | F-8 | F-9 |
| Temozolomide    | 100         | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Sodium alginate | 10          | 20  | 30  | 10  | 20  | 30  | 10  | 20  | 30  |
| Ethyl Cellulose | -           | -   | -   | 10  | 20  | 30  | 10  | 20  | 30  |
| Carrageenan     | 5           | 5   | 5   | 10  | 10  | 10  | 15  | 15  | 15  |
| Calcium chloride| 3           | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 3   |

Characterization of FM

Determination of Entrapment Efficiency

A 100 mg of FM were taken, cautiously ground, and suspended in HCl (0.1 N). In this way, the substance suspended in the water was kept up by test sonication (Power sonic 505) for 20 min and mixed with a magnetic instigator (Remi) for the entire extraction of the TZM from the FM. The ensuing arrangement was explained through a 0.45μ film channel (Millipore). The TZM was
bounded by a UV-visible spectrophotometer (Shimadzu-S1210) at 329 nm. The entrapping was determined by utilizing the accompanying formula (Srivastava et al., 2005).

\[
\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100
\]

**Particle size distribution**

Sieve analysis with the help of sieves #16, #20, #30, #40, #60, and #80 was used to determine by passing the MS from coarse size to finer sieves (Muneer et al., 2017; Kumae et al., 2010).

**Buoyancy percentage**

The FM (0.3g) was put in USP XXIV dissolution assembly (type II) loaded up with 900 ml of 0.1M HCl with Tween 80 (0.01%). The medium was inspired at 100 rpm for 12 h. The drifting and balanced out part of the FM was recouped independently. The FM were emaciated and contemplated. The buoyancy rate was planned as the extent of the majority of the FM that persevered through fluctuating and the complete mass of the FM (Ma et al., 2008; Hindustan et al., 2010).

\[
\% \text{ Buoyancy} = \frac{\text{Weight of the FM settled}}{\text{Weight of the settled FM}} \times 100
\]

**In Vitro Release studies**

TZM release from FM was performed by the USP basket dissolution apparatus. FM of the required amount was suspended in 900 ml of HCl (0.1 M of pH 1.2). The medium was enthused (100 rpm) and maintained at 37±0.5°C by keeping up sink conditions. After sufficient solution, the samples were investigated for measurement of the TZM at 329 nm utilizing the Shimadzu UV-VIS double beam spectrophotometer (model: S1210) (Ishaq et al., 2013; Rani et al., 2011). The TZM was measured from the calibration curve as describes by Ishaq et al., 2014.

**RESULTS AND DISCUSSIONS**

The TZM thermogram was described by a solitary intense endothermic at 211.78°C and the TZM blend demonstrated an endothermic top at 201.84°C. These thermograms showed that a little change to one side when joined with excipients, this could be attributable to the TZM liquefying or to its transformation into a nebulous structure. These thermograms show that there are no indications of contrariness among TZM and excipients (Fig.1).
The FTIR spectra of TZM demonstrated stretches and peaks were likewise in FTIR of TZM-excipient mix represents no remarkable contra issues of TZM with the excipients of the study.

**Drug entrapment efficiency (DEE)**

It was discovered that the DEE was acceptable in all the FM at the awakening of 500 rpm (Table 4). The DEE was from 55.95±0.35 to 78.07±0.32% for F-1 to F-9. The DEE was expanded by climb the polymer focus in FM s. This might be attributable to the low dissolvability of TZM in water, which encourages the dissemination of a piece of the TZM caught in the encompassing during the FM preparation.

**Particle size analysis**

The size of the FM differed based on composition (Table 4). The F-1 demonstrated a moderately enormous level of huge size (299.54±1.48μ) and the F-8 detailing indicated fluctuating FM of generally little size (278.84±2.69μ) as the viscidity of the medium raised to a higher polymer fixation, bringing about the improved interfacial strain. The FM s were globular with no noteworthy obvious inconsistency on a superficial level (Table 4).

**The Buoyancy Percentage**

The buoyant rate for all FM was >75% (for 10 h), and ranged from 75.09±0.02% to 91.36±2.16%. The uppermost% was gained with the plan F-8.

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**Table 4: Efficiency of FM s of Temozolomide FM**

| Formulation | Yield   | Particle | Drug     | Buoyancy | Floating |
|-------------|---------|----------|----------|----------|----------|
|             |         |          |          |          |          |

Fig. 1. DSC thermograms of drug and excipient
| (%) | Size (μ) | Entrapment (%) | Percentage (%) | time (h) |
|-----|----------|----------------|----------------|----------|
| F-1 | 65.48±0.58 | 299.48±1.45 | 56.48±1.20 | 75.09±0.02 | 5.0±0.07 |
| F-2 | 64.58±1.49 | 298.54±1.48 | 55.95±0.35 | 76.26±1.38 | 4.8±0.07 |
| F-3 | 69.54±1.05 | 297.49±2.25 | 72.19±0.68 | 80.28±1.65 | 3.8±0.05 |
| F-4 | 71.35±2.15 | 287.03±7.84 | 68.25±2.25 | 81.41±0.26 | 5.1±0.01 |
| F-5 | 68.57±1.15 | 295.98±2.36 | 74.49±0.49 | 85.22±0.38 | 7.3±0.05 |
| F-6 | 81.10±0.65 | 294.91±1.20 | 69.55±0.31 | 86.84±0.13 | 8.5±0.11 |
| F-7 | 69.05±0.48 | 299.30±1.26 | 67.22±0.95 | 78.58±1.48 | 7.0±0.05 |
| F-8 | 75.84±0.29 | 278.84±2.69 | 76.04±0.25 | 91.36±2.16 | 8.9±0.09 |
| F-9 | 80.11±1.49 | 285.65±0.67 | 78.07±0.32 | 87.65±1.49 | 8.5±0.05 |

Values are in mean± SD; Trials made=3

**Calibration curve**

The TZM concentrations were estimated by the regression equation \( y = 0.017x - 0.0007, \quad R^2 = 0.9976 \) of the calibration curve of TZM in 0.1 N HCl (pH 1.2). The calibration curve of TZM was tabulated in table 5 and shown in fig. 2.

**In-vitro dissolution study**

The FM s showed a prolonged emission of the TZM in an acidic medium and the release of the TZM was approximately linear. About 40% of TZM was initially released. Furthermore, the release of the TZM from the FM s matrix was regulated by the polymer. This can reduce the total drug release (DR) from the polymer matrix. Furthermore, the smaller FM are designed at a minor polymer level and has a wider surface wide-open to the dissolution medium, which results in a faster DR (fig.3).

**Table 5: Calibration data of drug**

| Concentration (µg/ml) | Absorbance (nm) |
|-----------------------|-----------------|
| 0                     | 0.000           |
| 10                    | 0.156           |
| 20                    | 0.356           |
| 30                    | 0.526           |
| 40                    | 0.658           |
| 50                    | 0.852           |
CONCLUSION

In-vitro data obtained for floating microspheres (FM) of Temozolomide (TZM) displayed good incorporation effectiveness, good buoyancy, and lengthy drug release. The microspheres of various sizes and TZM content could be attained by fluctuating the ingredients. The study concludes that TZM can be formulated as FM using sodium alginate, Ethyl cellulose and carrageenan gum by ionotropic gelation method. The FM disclosed better buoyancy, and TZM discharge.

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