IJBPAS, January, 2026, 15(1): 377-395

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

IN VITRO CHARACTERIZATION AND CYTOTOXICITY STUDY OF TRIFLURIDINE-TIPIRACIL HCL LOADED FLOATING MICROSPHERE FOR TREATMENT OF GASTRIC CANCER

RAHEVAR KH[1*] AND PATEL M[2]

1: Department of Pharmaceutics, Pioneer Pharmacy College, Vadodara, Gujarat, India., India

2: Department of Pharmaceutics, Faculty of Pharmacy Dharmsinh Desai University, Nadiad, Gujarat, India

***Corresponding Author: Dr. Komal H Rahevar: E Mail: komalrahevar.kr@gmail.com**

Received 13th Sept. 2024; Revised 25th Nov. 2024; Accepted 25th Jan. 2025; Available online 1st Jan. 2026

https://doi.org/10.31032/IJBPAS/2026/15.1.9790

ABSTRACT

Gastric cancer is a leading disease for considerable death across the globe and the research aimed to treat it with improved patient compliance and bioavailability by targeting the stomach as a site of action. Trifluridine-Tipiracil (TFD–TPI) is a fixed-dose medication combined with two active pharmaceutical ingredients. TPI increases the bioavailability of TFD by preventing the rapid metabolism of TFD. The drawback of shorter biological half-life and low bioavailability of TFD can be resolved by combining it with TPI in floating microspheres which releases the drug for an extended period in the stomach with better patient compliance.

TPI-TFD floating microspheres are prepared by water-in-oil-in-oil (W/O1/O2) double emulsion solvent diffusion method using ethyl cellulose, and hydroxy propyl methyl cellulose. The polymer ratio, rotational speed and solvent volume are optimised by Box Behnken design. The percentage entrapment efficiency (Y1), Time required for 50% drug release (Y2) and particle size (Y3) were taken as dependent variables. The % EE of the optimised batch was 76.5 and 77%, T50% was 5.39 and 5.06 hours, particle size was 300 µm and the % drug release was 100.30 %. A kinetic study of in vitro drug released by anomalous diffusion. The FT-IR and DSC analysis confirmed compatibility between drug and excipients in spherical-shaped microspheres. Gas chromatography reveals that the trace amount of residual solvents was within accepted limits. The average sizes of spherical-shaped microspheres were found to have a thickness of 303 nm. The optimised batch (B-13) was subjected to a short-term stability study per ICH guidelines indicating no significant changes in evaluation criteria at 40°C/75% RH after 6 months. The MTT assay using AGS gastric cancer cell lines shows TFD-TP-

loaded microspheres inhibited AGS cell growth. The findings demonstrate that the developed floating microspheres would provide stomach-specific controlled drug release for 12 hours with improved bioavailability to treat gastric cancer.

Keywords: Gastric cancer, Box Behnken Design, floating microsphere, In vitro cytotoxicity-study, Trifluridine, Tipiracil HCl

INTRODUCTION :

The most efficient and widely used way to administer drugs is orally. Solid oral dosage forms have historically been created to release their drug content in the upper part of the gastrointestinal tract, which has better drug absorption and breakdown conditions. Controlling the site of medication release from oral formulations has received more attention recently to increase patient compliance and treatment effectiveness. Nevertheless, this approach contains several physiological medications in a controlled and repeatable manner **[1, 2]**.

GIT, which is comprised of the stomach and the small intestine, is the region of the GIT that is the most dilated. The pyloric sphincter is in charge of controlling how it opens into the duodenum. Physiologically, it is divided into four anatomical regions: the fundus, the body, the antrum, and the pylorus. GRDDS are helpful for such medications by further developing their bioavailability, therapeutic proficiency, and possible portion reduction. Supporting constant therapeutic levels over a long period and reducing changes in the helpful levels reduces drug waste and makes it easier for less soluble drugs to dissolve in an environment with a high pH **[3]**.

Low-density systems with sufficient buoyancy to float over the contents of the stomach and remain buoyant there for a long time without reducing the rate at which the stomach empties are known as hydrodynamically controlled systems, also known as floating drug delivery systems (FDDS). The drug is slowly released from the system at the desired rate while the system is floating on the contents of the stomach.

Microspheres are small, spherical particles that typically have dimensions between one and one thousand micrometers. Numerous organic and synthetic materials can be used to make microspheres. Solid, hollow microspheres come in a wide range of densities and are employed in a variety of ways as a result. Typically, hollow microspheres are added to materials to reduce their density **[4, 5]**.

The third-highest cause of cancer death, causing 783,000 deaths globally in 2020, is gastric cancer, which is the fifth-most common disease diagnosed worldwide. Although the incidence of the accumulation

of multiple genes such as oncogenic, tumour suppressor, and mismatch repair genes in gastric cancer is declining, it remains a major health problem and a common cause of cancer mortality worldwide. The term "gastric cancer carcinogenesis" refers to the dynamic balance between cell proliferation and death, which is crucial to maintaining homeostasis in the human body. The key etiological factors for stomach cancer are Helicobacter pylori infection and exposure to chemical carcinogens, and its development is thought to be a slow process **[6, 7]**.

(TFD), a potent cytotoxic agent was originally synthesized in the 1960s. When administered alone, Trifluridine is rapidly metabolized by thymidine phosphorylase (TPase) in the liver and GI tract to inactive forms with decreased cytotoxicity, which is responsible for its poor bioavailability and toxicity **[8]**. TPI, a thymidine phosphorylase (TPase) inhibitor, prevents the metabolic degradation of TFD **[9]**. The combination of TFD with TPI thus increases the systemic exposure of TFD with the required cytotoxicity. TPI is used in combination with TFD, in a ratio of 1:0.5, to increase TFD bioavailability by inhibiting its catabolism. Based on its efficacy and tolerability, the current National Comprehensive Cancer Network (NCCN) guidelines recommend TFD or TP as a preferred option for third- or subsequent-line treatment of gastric cancer **[10, 11]**. The goal of this research was to find a way to treat gastric cancer with TFD-TPI stomach-targeted sustained-release microspheres. These microspheres release the drug steadily for 12 hours, improving its bioavailability and making it less harmful.

MATERIALS AND METHODS:

Materials

Trifluridine and Tipiracil HCl were received as samples gratis from Emcure Pharma Ltd. (Ahmedabad), and Methocel K15M and Ethyl cellulose 45 were procured from Colorcon Asia Pvt. Ltd. (Goa). Dichloromethane, Acetone, HCl, and Tween 80 were purchased from S.D. Fine Chemicals (Mumbai, India). Liquid paraffin (Acme Chemicals Ltd., Mumbai).

Methods:

Cell culture

Adenocarcinoma gastric cell line (AGS) (stomach cancer) cell culture was procured from the National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Ham's F-12K (Kaighn's) Medium (F-12 K media) supplemented with 10% inactivated fetal bovine serum (FBS), Antimycotic solution (100X), (10,000 unit /ml penicillin, streptomycin (10,000 µg/ml), and Gibco amphotericin B (25 µg/ml) and HEPES buffer were added to a final concentration of 1.25mM, to

complete the growth medium. Make volume up to 50 ml by adding the appropriate media and mix by inverting the tube and filter using 0.22 µ filter and Store at 2-8°C (up to 4-6 weeks) **[12, 13]**.

Preparation of floating microsphere

The finding of the literature survey shows that various hydrophilic and lipophilic polymers were explored and optimised in the design and formulation of floating microspheres. The present preliminary screening was aimed to establish the range of Drug: Polymer ratio with a suitable proportion of hydrophilic: Lipophilic polymer. The water-oil-oil emulsion (w/o/o) technique was employed to produce floating microspheres. The aqueous phase was formulated by dissolving TPI and TFD in distilled water. Conversely, the organic phase was prepared by dissolving specific polymers in a 30 ml organic solvent blend comprising dichloromethane and Acetone in a 1:1 ratio. Drugs containing an aqueous solution were then combined with the organic phase containing the polymer to generate the primary emulsion (w/o). The primary emulsion was gradually introduced into 50 ml of light liquid paraffin which contained 0.01% Tween 80 as a surfactant while maintaining constant stirring at 1500 rpm for 2 hours. The resultant microspheres were isolated by filtering through Whatman filter paper made free from liquid paraffin and washed with pet ether. The washed microspheres were air-dried overnight for 12 hours. The prepared preliminary batches were assessed for various evaluation parameters for further optimization study **[14[**.

Optimization of variables using Box Behnken Design

The result of preliminary screening revealed that the total polymer load and the type of polymer have the greatest influence on various qualitative and quantitative parameters of prepared floating microspheres, hence it was decided to optimize the hydrophilic: lipophilic polymer ratio and various processing parameters using Box Behnken design. The polynomial equation generated by this experiment using Statistica and Microsoft Excel is shown in Equation 1:

Yi = b0 + b1X1, b2X2, b3X3, b12X1X2, b13X1X3, b23X2X3, b11X1 2, b22X2, and b33X3 (2) **(Table 1)**.

Table I: Optimization using Box Behnken Design (Independent Variables)

Batch	Coded value			% Ratio	Polymer weight (X1)		DCM: Acetone	RPM
	X1	X2	X3	X1 (HPMC15:EC45)	HPMC15(mg)	EC (mg)	X2	X3
~~1~~	~~-1~~	~~-1~~	~~0~~	~~25:75~~	~~34~~	~~101~~	~~20~~	~~1500~~
~~2~~	~~1~~	~~-1~~	~~0~~	~~75:25~~	~~101~~	~~34~~	~~20~~	~~1500~~
~~3~~	~~-1~~	~~1~~	~~0~~	~~25:75~~	~~34~~	~~101~~	~~40~~	~~1500~~
~~4~~	~~1~~	~~1~~	~~0~~	~~75:25~~	~~101~~	~~34~~	~~40~~	~~1500~~
~~5~~	~~-1~~	~~0~~	~~-1~~	~~25:75~~	~~34~~	~~101~~	~~30~~	~~1200~~
~~6~~	~~1~~	~~0~~	~~-1~~	~~75:25~~	~~101~~	~~34~~	~~30~~	~~1200~~

7	-1	0	1	25:75	34	101	30	1800
8	1	0	1	75:25	101	34	30	1800
9	0	-1	-1	50:50	68	67	20	1200
10	0	1	-1	50:50	68	67	40	1200
11	0	-1	1	50:50	68	67	20	1800
12	0	1	1	50:50	68	67	40	1800
13	0	0	0	50:50	68	67	30	1500
14	0	0	0	50:50	68	67	30	1500
15	0	0	0	50:50	68	67	30	1500

Evaluation of floating microspheres:

Determination of dissolution parameters

"Dissolution efficiency (DE) is a parameter used to quantitatively assess the extent and rate of drug dissolution from a dosage form over a specified period". It is often expressed as a percentage and is calculated using the area under the dissolution curve.

The formula for calculating dissolution efficiency (DE) is as follows:

$$DE = \frac{\int_0^t y \cdot dt}{y_{100} \cdot t} \cdot 100\%$$

DE is determined as the percentage of the area under the dissolution curve (y) up to a particular time point (t) compared to the area of the rectangle representing 100% dissolution at the same time **[15]**.

Mean dissolution time

MDT is a parameter applied to represent the average time taken for a drug substance to dissolve in a dissolution test. It provides valuable information about the rate of dissolution of a drug from its Dosage regimen.

Where (i) represents the number assigned to each sample.

(n)denotes the total number of samples undergoing dissolution.

(t*i) refers to the midpoint time between t and t(i-1), where t is the current time and t(i- 1) is the previous time point.

(ΔMi) represents the additional amount of drug dissolved between the current time (t) and the previous time point (t(i-1)).

Model-independent approaches were employed to analysed dissolution profiles of various formulations. These analyses were performed using the DD-Solver program, an add-in for Microsoft Excel, facilitating the comparison of dissolution profiles **[16]**.

Characterization of optimized floating microspheres

a) Differential Scanning Colorimetric Studies (DSC)

Differential Scanning Calorimetry (DSC) stands as one of the most extensively utilized calorimetric methods for investigating drug compatibility and ingredient interactions. In this study, samples of Microsphere, TPI, and TFD were placed in flat, round-bottomed aluminium pans and subjected to heating from 45°C to 300°C at a rate of 10°C/min under nitrogen purging (at a flow rate of 50 mL/min). Alumina served as the reference

standard in the differential scanning calorimeter. The objective was to assess any potential interactions between the drug and the excipients. DSC analysis is effective because it can reveal changes in appearance, shifts in melting endotherms and exotherms, and variations in accompanying enthalpies of reactions, allowing for rapid examination of potential incompatibilities. Both a batch of Microspheres that had been optimized and the DSC thermogram of the pure drug was recorded, providing valuable insights into the thermal behaviour and compatibility of the drug and its excipients **[17]**.

b) Transmission electron microscope (TEM)

The high-resolution transmission electron microscope was used to investigate the microstructures. Transmission electron microscopy (TEM) images were taken with a FEG - Transmission Electron Microscope (HR-TEM), Thermo Fisher Scientific, Talos F200i S/TEM using an accelerating voltage of 200 kV was further investigated by TEM. TEM images of the as-prepared floating microspheres with different magnifications **[18]**.

c) Scanning Electron Microscopy (SEM)

Scanning Electron Microscope XL 30 ESEM with EDAX: Resolution: up to 2; Acc. voltage: 30 kV; Magnification: up to 2,50,000x used to examine the morphology of microspheres. They were sprinkled over a double-sided adhesive tape mounted on a metal specimen stub. Before examination with SEM **[19]**.

d) Residual Solvent Analysis

A gas chromatograph with headspace (GC-HS) was used to analyse microsphere samples for detecting volatile organic content in drugs. Precipitates were removed using a Spin-X centrifuge filter, and GC analysis was performed on the filtrate. An aliquot of the internal standard solution was injected into the Shimadzu GC 2010. The concentration of residual solvents was determined using a calibration plot **[20, 21]**.

e) ***In vitro*** **Drug Release Kinetics Study:**

To study the mechanism for the release and release rate kinetics of the dosage form, the obtained data were fitted into the Higuchi matrix, Peppas, and Hixson Crowell models. In this case, the best-fit model was selected by comparing the R-values that were produced **[22]**.

f) Stability studies:

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. microspheres formulation was filled in tightly closed glass vials and subjected to stability testing according to the International Conference on Harmonization (ICH) guidelines for

zone III. The packed containers of microspheres were kept at under accelerated condition (40±2°C/75±5% RH) in a stability chamber for a period of six months. The samples (n=3) were analysed at intervals of 3 and 6 months and evaluated for physical appearance, particle size, drug content, entrapment efficiency and drug release studies **[23]**.

g) *In vitro* cytotoxicity study

MTT Assay [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]

A culture flask with 80-90% confluent cells was prepared. The media was removed by washing the cells with 1ml Phosphate buffered saline (PBS) twice. PBS was removed and the cells were Trypsinized by adding 1ml Trypsin-EDTA solution. The flask was incubated at 37°C in a CO2 incubator for 7-8 minutes at 5% CO2. The rounding of cells was observed and the flask was taped by hand for the complete detachment of cells. One ml of the Cell suspension is transferred to each of the three 1.5 ml microcentrifuge tubes. The cells were centrifuged at 500g for 10 minutes at 25°C. Media was removed carefully without disturbing the pellet. One ml 1X PBS was added to each vial and mixed gently to remove cell clumps. cell suspension (10µl) was taken and proceeded with cell counting. The cells were Centrifuged at 200g for 10 minutes at room temperature. The supernatant was removed without disturbing the pellet. One ml of media was added and mixed gently. 5000 to 10000 cells were added in each well of a 96-well plate according to cell count (kept inverting the tube while adding the cells). The volume of each well is made up to 100µl by adding complete media. Incubated at 37°C for 24 hours. The media was removed from each well after 24 hours. Drugs were added at different concentrations (10, 20, 40, 60, 120 & 160 µg/ml) in triplicate and the volume of each well is adjusted up to 300 µl with media. One set of the three wells was kept untreated as control and incubated at 37°C in a CO2 incubator for 24 hours **[24]**.

RESULTS:

Effect of % Entrapment Efficiency Y1 on independent variables:

Following the polynomial equation by the statistical analysis.

(% EE-TP) = 76.85+ 0.29X1 -0.90X2 – 3.00X3 + 0.32X1X2+ 0.23X1X3 - 0.18X2X3- 0.07X12 +0.40X22 – 0.96X32

(% EE-TFD) = 76.69+ 0.01X1 -0.96X2 – 3.10X3 + 0.09X1X2- 0.17X1X3 - 0.37X2X3 + 0.20X12 +0.34X22 – 0.83X32

The P-values determined were found less than 0.05 indicating a significant level of the model. The difference between adjusted R2 (0.9601) and Predicted R^2 (0.8439). The Model F-value of 14.20 indicates that the model is statistically significant. All of the observed values show that the model is

significant and can be applied to design. The coefficient of X1 bears a positive sign with a p-value more than 0.05, whereas the coefficient values for X2 and X3 carry a negative sign with a p-value less than 0.05, indicating that the effect of X2 and X3 is significant on % EE. The maximum coefficient value of X3 indicates that the variation in X3 (RPM) affects more of the EE than X1 and X2. Response surface plot and contour plot show that the negative effect of X2 and X3 on % EE is greater as compared to the positive effect of X1. The significant coefficient values of X2 and X3 and insignificant values for X1 found in the polynomial equation were reflected in the plots **(Figure 1) [25]**.

Effect of Time to release 50%(T50%) of drug (Y2) on independent variables

T50% = 5.23 -2.74 X1 -0.08 X2 -0.26 X3 + 0.109X1X2 + 0.046 X1X3 + 0.047X2X3 +1.77 X12- 0.318 X22 + 0.308 X32

T50% = 5.05 -2.74 X1 -0.12 X2 -0.261X3 + 0.111X1X2 + 0.13 X1X3 + 0.047X2X3 +1.80 X12- 0.21X22 + 0.311 X32

The P-value for the overall applied model remains less than 0.005 indicating the significance level of the model. Furthermore, the difference obtained between the predicted R^2 of 0.9632 and the adjusted R^2 of 0.9935, remaining less than 0.2, also indicates that the model is reliable. The Model F-value of 240.31 demonstrates that the model is significant. The coefficients of X1, X2 and X3 bear negative signs indicating the negative effect of all three independent variables on T50% furthermore the higher value of b1 than b2 and b3 indicates a prominent effect of X1 on T50% than X2 & X3. The significance level of coefficients b1, b3, b11, and b33 being less than p=0.05 indicates that they have a significant effect on T50%. The results of the statistical analysis are presented in the table. The coefficient of X1 that is b1, b2 and b3 bear a negative sign indicating the response decreases (antagonist effect), thus, X1 X2 and X3 increase the T50% decrease. The significance level of coefficients b1 and b3 was found to be less than p=0.05, thus they had a significant effect on T50%. The plot shows that T50% microspheres was greatly dependent and influenced by changes in the levels of X1 and X3 from 1 to 1. There is a significant reduction in T50% was revealed with an increase in the levels of X1 and X3. The effect of X2 is not as prominent as the outcome of X1 and X3. The results of the statistical analysis are presented in **Figure 2**.

Effect of Particle size Y3 on independent variables:

The selected model's quadratic equation, which shows the impact on the aforementioned variables, is shown below.

(P. size) = 300 + 2.59X1 – 20.77X2 – 49.72X3 + 1.19X1X2+ 0.53X1X3 – 2.75X2X3 +2.62X12+ 0.22X22 - 2.47X32

The applied model remains significant with overall P- value less than 0.005. The Model F-value of 150.01 suggests that the model is significant. Additionally, the negligible difference between the predicted R^2 (0.9410) and the adjusted R^2 (0.9897) remains less than 0.2, indicating acceptable agreement between them. The coefficient of X1, which is b1, bears a positive sign with P value higher than 0.05 shows insignificant positive effect of selected X1 variable (ratio of HPMC K 15 M to EC45) on particle size of microspheres. The coefficients of X2 and X3, which are b2 and b3, bear a negative sign with P value less than 0.005 demonstrate a significant negative effect of both the independent variables on selected response (particle size). Higher value for b2(coefficient of X2) and b3(coefficient of X3) with negative sign indicates stronger negative dependency of response (Particle size) on X2 (solvent volume) X3(Rotational speed) as smaller sized microspheres were obtained with higher level of solvent volume rotational speed. The plot shows that the particle size of microspheres was greatly dependent on and influenced by changes in levels of X3 and X2 from -1 to 1. There is a significant decrease in particle size revealed with an increase in levels of X2 and X3. The results of statistical analysis are shown in **Figure 3**.

Validation of Model by Checkpoint batch

The overlay plots give the yellow-coloured region of all the possible batches with a previously set range of all dependent variables. The batches selected from this yellow region were evaluated for all three dependent responses and comparison of result was described in **Figure 4**. 17 Prediction error was calculated by comparing observed and predicted values of % EE, T50% and PS. The value of prediction error was remained less than 5% which suggest good correlation between predicted values and the values obtained from prepared checkpoint batches. Furthermore, the strong agreements between predicted and observed values confirm the validation of applied model with conclusion that the applied statistical model will be used to predict theoretical response values within design.

Differential Scanning Calorimetry (DSC)

DSC thermograms of pure TFD and TPI were compared with TFD-TPI loaded microspheres exhibited a sharp endothermic peak of TFD at about 186.28˚C corresponding to its melting point (186-189˚C), and TPI exhibited a sharp endothermic peak at 253.45˚C corresponding to its melting point (251-2530C) representing its crystalline nature

and compatibility of the polymer with the drug in the microspheres **(Figure 5)**.

Transmission electron microscope (TEM)

The high-resolution transmission electron microscope (HRTEM) was utilized to examine the microstructures. The TEM (Figure) was utilized to estimate both the average sizes of the microparticles and the particle morphology. TEM images were captured using a FEG - TRANSMISSION ELECTRON MICROSCOPE (HR-TEM), specifically the Thermo Fisher Scientific, Talos F200i S/TEM, with an accelerating voltage of 200 kV. Further investigation was conducted via TEM. **Figures 6A and 6B** illustrate TEM images of the as-prepared floating microspheres at varying magnifications. The low-magnification TEM image, depicted in **Figure 6a**, showcases the uniform diameter of approximately 200 nm for the as-prepared microspheres. This observation aligns with the findings observed in SEM images. In **Figure 6b**, a high-magnification image unveils a hollow structure within the sample, with a spherical shell thickness measuring approximately 303 nm. This hollow configuration formed within the microspheres, and the structure was disrupted. Additionally, the high-magnification TEM image depicted in the figure showcases microspheres characterized by a porous structure **[26]**.

SEM study

SEM pictures of a batch of B13 microspheres that had been improved were taken to get topographical data on the formulation. The findings are shown in **Figure 7** and indicate that the formulated B13 microspheres are spherical, smooth-surfaced, and highly porous. Additionally, there were no drug crystals observed on the surface.

Residual Solvent Analysis

Acetone and dichloromethane were utilized as organic solvents in the formation of the floating microsphere. The ICH recommendations "Q3C" for residual solvents classify acetone as a class 3 residual solvent, which is thought to be less harmful since it poses no risk to human health at concentrations typically permitted in medicines. However, DCM is a class 1 residual solvent, and its concentration in the final formulation must not exceed the limit (600 ppm). The graphic displays the typical DCM solution's gas chromatogram. DCM reached its peak at 5.39 minutes. As shown in the normal preparation, the acetone retention period was 4.72 min. The peak sample preparation time was 10.08. Acetone and dichloromethane residue were within the limits of the floating microsphere. As a result, the developed formulation is thought to be safe for use by people **(Figure 8)**.

Accelerated short-term stability study for Optimized Formulation as per ICH Guidelines

A stability study for the optimized floating microsphere revealed that the formulation was physically and chemically stable for six months under the specified test circumstances. For six months, samples were set aside in a stability chamber at accelerated storage conditions of 40 ± 2 ºC and 75 ± 5% relative humidity with humidity and temperature control. At three and six months of the stability investigation, the samples were examined for physical variations, buoyancy, drug content, and % CDR for 12 hr. After six months of storage, there was no notable alteration. in the colour, shape, drug content, or drug release pattern of the drug. The dissolution study of the preparation was conducted after three and six months. The result derived from the t-test and the value of similarity-dissimilarity factors shows nearly identical drug release between batch no 13 stored as per ICH guideline and Theoretical drug release profile **(Table 2) (Figure 9) [27]**.

In-vitro cytotoxic effect using MTT assay

The obtained results, which measured the viability of AGS cells following exposure to floating microspheres at concentrations ranging from 10 to 160 µg/ml, were compared against controls including a placebo, TFD, and TPI for a duration of 24 hours in an AGS cell culture. They obtained 60.05, 56.46, 55.46, and 47.57% cell survival for floating microsphere, placebo, TFD, and TPI in the AGS cell line. determined the percentage survival of cells after 24 hours of treatment with free TFD-TPI and TFD-TPI-loaded floating microspheres at a concentration of 160 µg/mL. Additionally, the median inhibitory concentration (IC50) was estimated using the GraphPad Prism program[185]. The IC50 results are given in **Figure 10**. TFD-TPI inhibited AGS cell growth with IC50 values of 113.3, 31.27, 23.24, and 32.76 µg/mL, respectively.

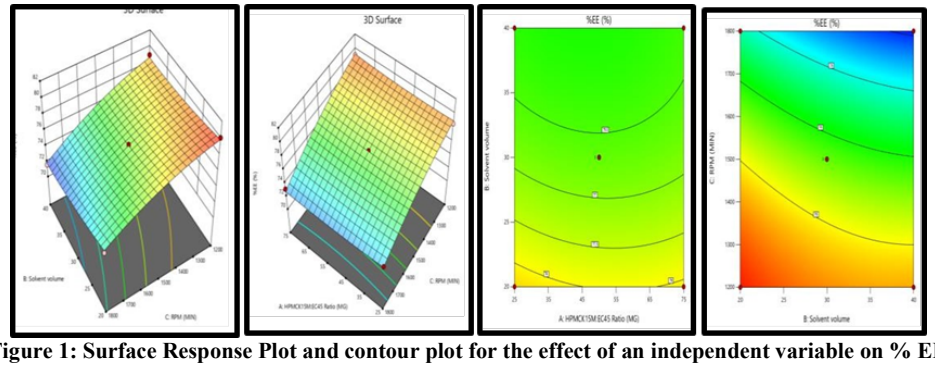

Figure 1: Surface Response Plot and contour plot for the effect of an independent variable on % EE

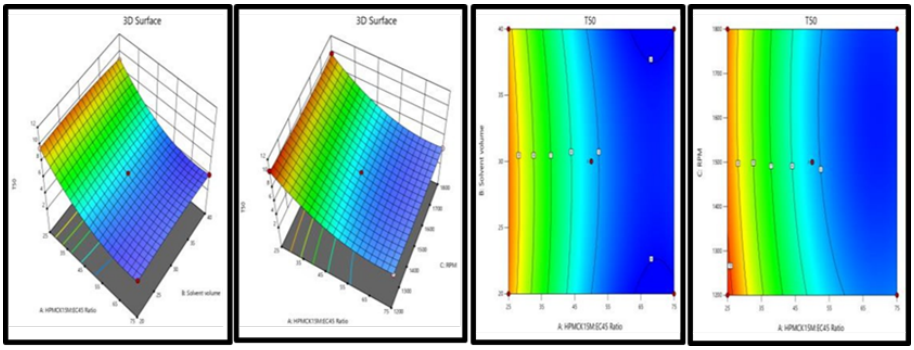

Figure 2: Surface Response and contour Plot for effect of an independent variable on T50%

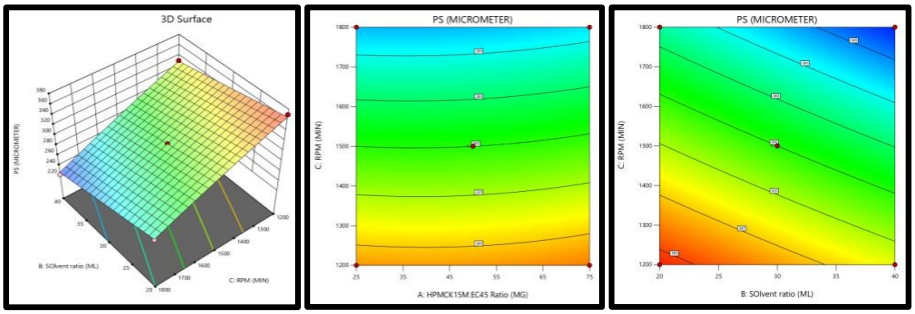

Figure 3: Surface Response & contour plot showing effect of X2 and X3 and X1, X3 on Particle size

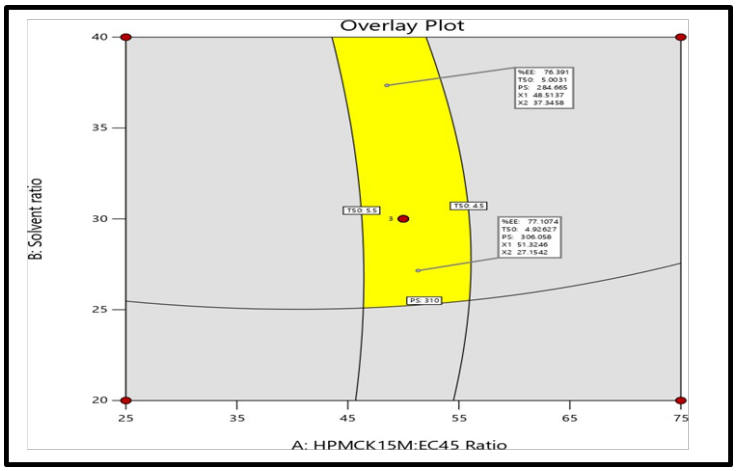

Figure 4: Overlay plot of responses for all dependent variables

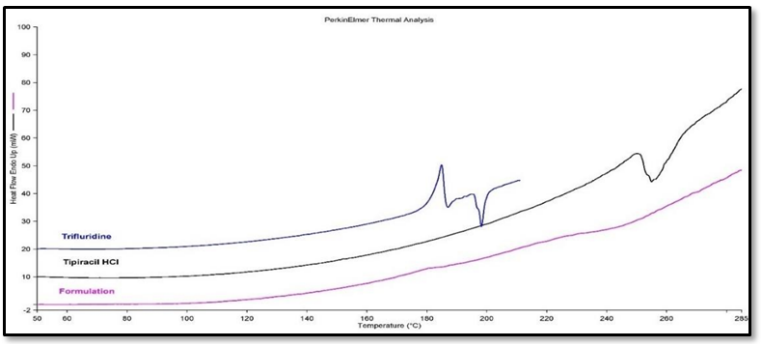

Figure 5: Differential Scanning Calorimetry (DSC) of optimized batch No. 13

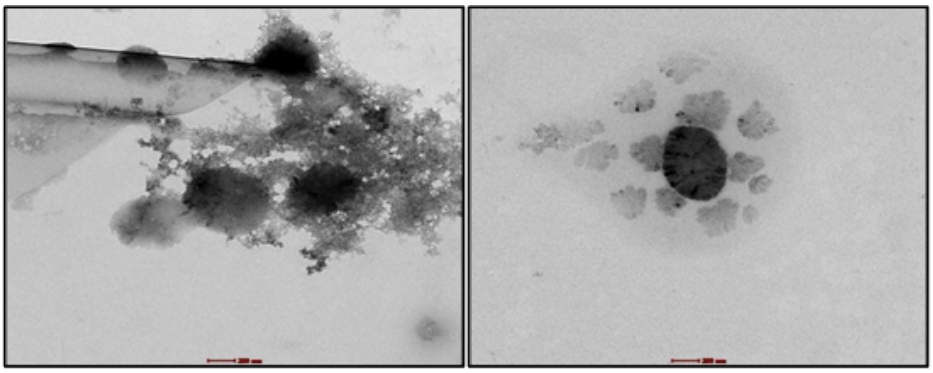

Figure 6: TEM images of the sample. (a): TEM images of the microsphere with low magnification; (b): TEM images of the high magnification

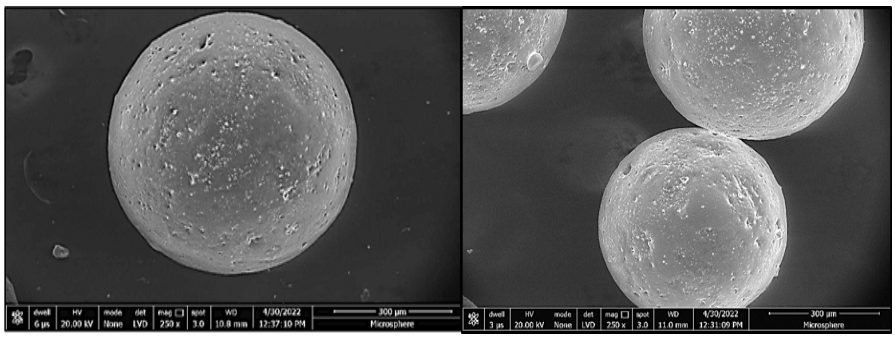

Figure 7: Scanning Electron Microscopy (SEM) for optimized batch B13

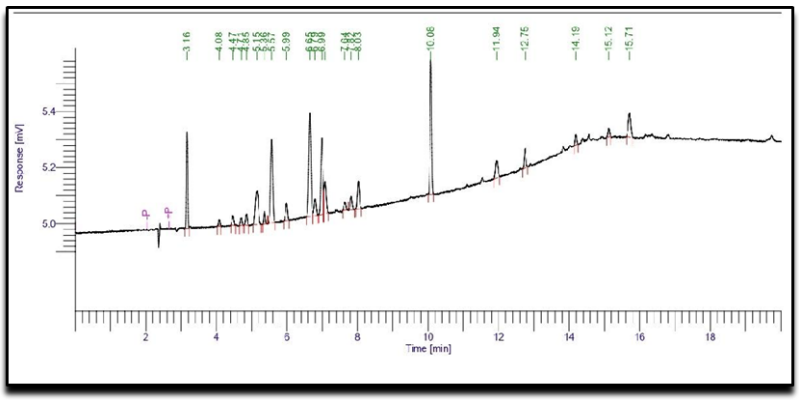

Figure 8: GC Scan of Batch No. B13 formulation

Table 2: Stability study of optimized floating microsphere (B13) (n=3)

Parameters	Initial		After 3 months		After 6 months	
	TPI	TFD	TPI	TFD	TPI	TFD
%Entrapment Efficiency	76.5% ±0.20	77.0 % ±0.15	75.85%± 0.12	76.2 5% ± 0.6	75.65% ± 0.41	76.20% ±0.42
Particle size	300 µm		293µm		290 µm	
Buoyancy lag time(s)	39 ± 0.57		42 ± 0.55		45 ± 0.57	
Total buoyancytime (hrs.)	>12		>12		>12	

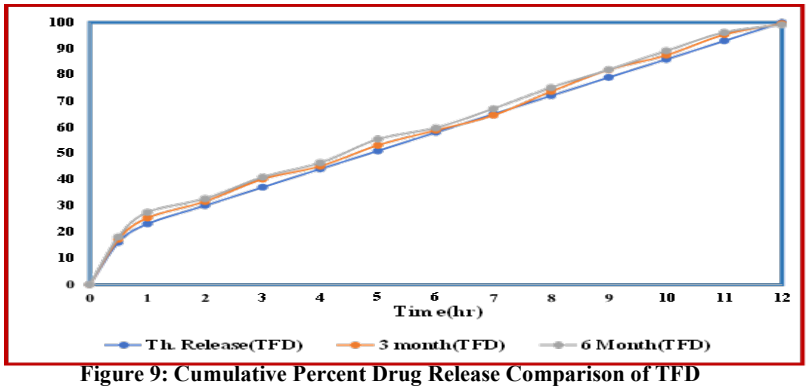

Figure 9: Cumulative Percent Drug Release Comparison of TFD

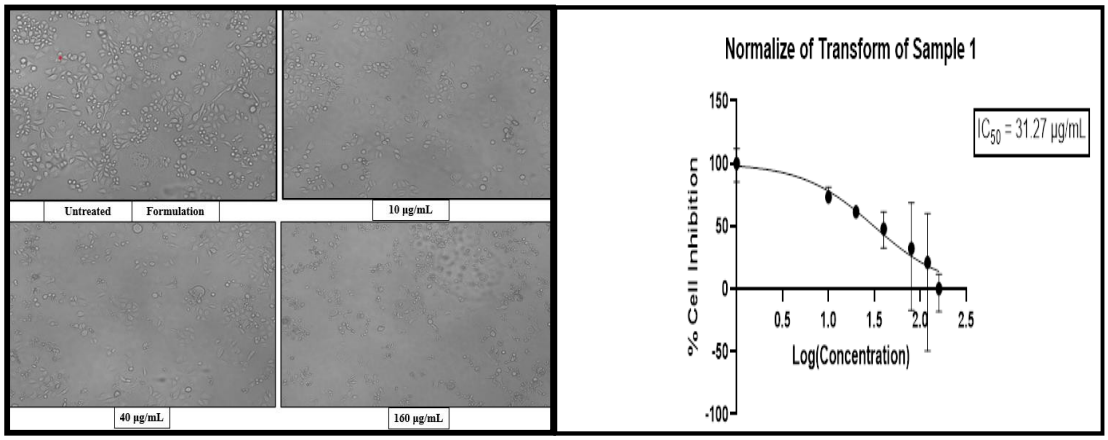

Figure 10. (a) Microscopic images of cytotoxicity effect observed at AGS cells control and treated with different concentrations for formulation (b) Graph representing % cell viability versus logarithm of theconcentration and IC 50 values obtained for formulation

DISCUSSION:

Before the development of the gastroprotective floating microsphere of selected drugs, pre-formulation studies were carried out. Identification of the drugs was done by doing their physical evaluation and also by performing the Fourier transform infrared spectroscopy (FTIR) study. The results verified the purity of procured drugs. The final optimization of the floating microsphere was done by applying a Box Behnken Design, using HPMC K 15 M: EC 45 (X1), DCM: Acetone(X2) and RPM (X3) as independent variables. The % entrapment efficiency (Y1), time required for 50% drug release (T50%) (Y2) and, particle size (Y3) was taken as dependent variables. The design was employed and evaluated using the Design Expert software 13 by running 15 experiments. The % EE of the optimized batch was 76.5% and 77 %, T50% was 5.39 and 5.06 hours, particle size was 300 µm, and Buoyancy lag time was 40 sec. and cumulative percent drug release was 100.30 %. MDT value is used to characterize the drug release rate from the microspheres and the retarding efficiency of the polymer. Statistical analysis of the Box-Behnken design batches was performed by multiple

regression analysis using Microsoft Excel. To evaluate the contribution of each factor at different levels to the response, a two-way analysis of variance (ANOVA) was performed using Design Expert software 13 For evaluation and comparison of dissolution profiles, the dissolution profiles were analysed using dissimilarity factors f1 and similarity factors $f2$ and T-test. The data were fitted into the Korsmeyer et al. model to validate the release process. They showed high linearity (r = 0.982 to 0.0.994), with the slope or exponential value (n) ranging from 0.50 to 0.733, showing a coupling of diffusion and erosion mechanisms—so-called anomalous diffusion. However, this n number tends to indicate that diffusion is the dominant mechanism of drug release for this formulation. The drug release pattern upto12 hours in Batch No. 13 was found identical to the predicted theoretical drug release profile of TP and TFD (f2 = 96-TP, 94.15-TFD and f1 = 1.30-TP, 1.15- TFD). The applied statistical model was validated by deriving two checkpoint batches from overlay graphs using design expert software. The findings of checkpoint batches were compared with predicted values given by the software and showed similar values with slight or negligible error (less than 5%). FTIR and DSC studies carried out for optimized formulation confirmed compatibility between the drug and excipient. SEM revealed the spherical and smooth surface of the microsphere. The average size estimated by TEM was found 303 nm with a spherical shape microparticle. Gas chromatographic studies were performed to check the amount of residual solvent. The results indicated that DCM was within the limits (less than 600 ppm), and acetone and dichloromethane residue were within the limits for the prepared floating microsphere. As a result, the developed formulation is thought to be safe for use in the treatment of gastric cancer with improved patient compliance. Accelerated stability studies of optimized floating microspheres were performed as per the ICH guidelines, and the results indicated that the optimized formulation was stable with no substantial changes in physicochemical attributes. The cytotoxicity of TFD/TP-loaded floating microspheres was investigated using an AGS human gastric carcinoma cell line by MTT assay, studying their effect on cell cytotoxicity. Cytotoxicity was determined by following the addition of 10, 20, 40, 60, 120, and 160 µg/ml, which were compared to placebo, TFD, and TP for 24 h in an AGS cell. They obtained 60.05, 56.46, 55.46, and 47.57% cell survival for floating microsphere, placebo, TFD, and TP in the AGS cell line. The inhibitory potency of the pure drug on the AGS cell line was compared by using the IC50 value. The

IC50 results are given in Figure TFD/TP inhibited AGS cell growth with IC50 values of 113.3, 31.27, 23.24, and 32.76 µg/mL, respectively. Drug-loaded microspheres showed very significant results compared with the pure drug solution. Pure drug and drug-loaded microspheres (B13) both showed cytotoxicity against AGS cells. Hence, the formulation can be effectively tested for its anticancer activity. The studies conducted in the present research work gave promising results. The anticancer drugs, which need to be in the stomach for better absorption, can be prepared as floating microspheres. It can be concluded that the drug delivery of such drugs can be improved, which increases the bioavailability of the drug. Also, the duration of the drug's action can be extended, resulting in a possible reduction in dose, fewer side effects, a low overall cost of therapy, and, hence, better patient compliance.

CONCLUSION:

Drug delivery to the stomach has become attractive to researchers whose main interest is the treatment of upper GIT diseases and disorders. Stomach cancer is one of the most causative diseases responsible for a considerable number of deaths across the world. The present study aimed to formulate and evaluate floating microspheres for targeting combined drug delivery of Trifluridine/ Tipiracil HCl to the stomach. The result of in vitro dissolution studies demonstrated that HPMC K 15M and Ethyl Cellulose 45 can be used successfully to achieve sustained release of Trifluridine/ Tipiracil HCl. The drug release pattern and stomach retention time were governed by polymer type, concentration and other processing parameters. Among all the batches, Batch no B13 formulated using HPMC K15 (68 mg), EC 45 (67mg), and DCM: acetone (30 ml in the ratio of 1:1) formulated at 1500 Revolution per Minute (RPM), shows nearly similar In-vitro drug release profile with desired theoretical drug release profile.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

ACKNOWLEDGMENTS:

The authors are thankful to the Dharmsinh Desai University Nadiad, India and Pioneer Pharmacy College, Vadodara, India, for providing the laboratory support. I also thank SICART, A, for providing me with technical and infrastructural support.

REFERENCES:

[1] Hua S: Advances in oral drug delivery for regional targeting in the gastrointestinal tract-influence of physiological, pathophysiological, and pharmaceutical factors, Frontiers

in pharmacology. 2020 Apr 28; 11:524.

[2] Birajdar, A.A., Deshmukh, M.T. and Shete, R.V: A Review on Gastro-Retentive Floating Microspheres, Journal of Drug Delivery and Therapeutics, 2021, 11, 11, 31-138.

[3] Tripathi J, Thapa P, Maharjan R, Jeong SH: Current state and future perspectives on gastro retentive drug delivery systems, Pharmaceutics, 2019 Apr 20;11(4)193.

[4] Rashmitha, V. & S, Pavani & Rajani, T: An Update on Floating Drug Delivery System: A Review, International Journal of Advances in Pharmacy and Biotechnology. (2020). 6. 9.

[5] CHMP extension of indication variation assessment report, European Medicines Agency Science Medicines Health, 2019.

[6] Hani U, Osmani RA, Yasmin S, Gowda BJ, Ather H, Ansari MY, Siddiqua A, Ghazwani M, Fatease AA, Alamri AH, Rahamathulla M: Novel Drug Delivery Systems as an Emerging Platform for Stomach Cancer Therapy. Pharmaceutics. 2022 Jul 29;14(8):1576.

[7] Kang C, Dhillon S, Deeks ED: Trifluridine/Tipiracil: A Review in Metastatic Gastric Cancer. Drugs. 2019 Sep;79(14):1583-1590.

[8] Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F: Gastric cancer. Lancet. 2020 Aug 29;396(10251) 635- 648.

[9] PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 6256, Trifluridine; [cited 2023 Mar. 16]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Trifluridine

[10] Johnston FM, Beckman M: Updates on Management of Gastric Cancer. Curr Oncol Rep. 2019 Jun 24;21(8), 67.

[11] PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 PubChem Compound Summary for CID 9903778, Tipiracil hydrochloride; [cited 2023 Mar. 16]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Tipiracil-hydrochloride

[12] Rathor S, Ram A. Porous microsphere of 5flouru uracil: a tool for site-specific drug delivery in gastric cancer. Int J. of Current Pharm Research. 2011;3(1):38- 42.

[13] Abdellatif AA, Ali AT, Bouazzaoui A, Alsharidah M, Al Rugaie O, Tolba NS. Formulation of polymeric

nanoparticles loaded sorafenib; Evaluation of cytotoxicity, molecular evaluation, and gene expression studies in lung and breast cancer cell lines. Nanotechnology Reviews. 2022 Feb 28;11(1):987-1004.https://doi.org/10.1515/ntrev-2022-0058.

[14] Reddy KR, Mutalik S, Reddy S. Once-daily sustained-release matrix tablets of nicorandil: formulation and in vitro evaluation. AAPS pharmscitech. 2003 Dec;4:480-8.

[15] Khan KA. The concept of dissolution efficiency. Journal of pharmacy and pharmacology. 1975 Jan;27(1):48-9. https://doi.org/10.1111/j.2042-7158.1975.tb09378.x.

[16] Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. The AAPS journal. 2010 Sep;12:263-71. https://doi.org/10.1208/s12248-010-9185-1.

[17] Gupta R, Shanthi C, Mahato AG. Characterization of Captopril-Ethyl Cellulose Microspheres by Thermal Analysis. Int. J. Drug Dev. Res. 2010;2:394-8.

[18] Wang W, Tu Y, Wang L, Liang Y, Shi H. Transmission electron microscopy and Raman characterization of copper (I) oxide microspheres composed of nanoparticles. Applied surface science. 2013 Jan 1;264:399-403.https://doi.org/10.1016/j.apsusc.2012.10.033.

[19] Zhang S, Wu D, Zhou L. Characterization of controlled release microspheres using FIB-SEM and image-based release prediction. AAPS PharmSciTech. 2020 Jul;21:1-4. https://doi.org/10.1208/s12249-020-01741-w.

[20] Noorbasha K, Shaik AR. Determination of residual solvents in paclitaxel by headspace gas chromatography. Future Journal of Pharmaceutical Sciences. 2021 Dec;7:1-7. https://doi.org/10.1186/s43094-021-00186-7.

[21] Shim H, Sah H. Assessment of residual solvent and drug in PLGA microspheres by derivative thermogravimetry. Pharmaceutics. 2020 Jul 4;12(7):626. https://doi.org/10.3390/pharmaceutics12070626.

[22] Costa P, Lobo JM. Modeling and comparison of dissolution profiles. European journal of pharmaceutical sciences. 2001 May 1;13(2):123-33. https://doi.org/10.1016/S0928-0987(01)00095-1.

[23] Tyagi LK, Kori ML. Stability study and in-vivo evaluation of lornoxicam-loaded ethyl cellulose microspheres. Int J Pharm Sci Drug Res. 2014;6(1):26- 30. https://ijpsdr.com/index.php/ijpsdr/article/view/290.

[24] Abdellatif AA, Ali AT, Bouazzaoui A, Alsharidah M, Al Rugaie O, Tolba NS. Formulation of polymeric nanoparticles loaded sorafenib; Evaluation of cytotoxicity, molecular evaluation, and gene expression studies in lung and breast cancer cell lines. Nanotechnology Reviews. 2022 Feb 28;11(1):987-1004.https://doi.org/10.1515/ntrev-2022-0058.

[25] Gaur PK, Mishra S, Kumar A, Panda BP. Development and optimization of gastroretentive mucoadhesive microspheres of gabapentin by Box–Behnken design. Artificial cells, nanomedicine, and biotechnology. 2014 Jun 1;42(3):167-

[26] Vimalkumar E, Kamaraj C, Gandhi PR. Dexamethasone-loaded PLGA microspheres: effective and sustained anticancer drug delivery system. Pharmacology, Toxicology and Biomedical Reports. 2017;3(3). https://doi.org/10.5530/PTB.2017.3.11.

[27] Kaushik D, Sardana S, Mishra D. In-vitro characterization and cytotoxicity analysis of 5-Fluorouracil loaded chitosan microspheres for targeting colon cancer. Ind J Pharm Educ Res. 2010 Jul 1;44(3):274-82.