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Oral Formulation of Anticancer Agents for Colon Cancer

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Authors' contributions

This work was carried out in collaboration between both authors. Author DP designed the study, performed the statistical analysis, managed the analysis of the study, read and approved the final manuscript. Author KP wrote the draft of the manuscript, literature searches, analytical method developed by HPLC, performed cell line study. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims/Objective: To develop and estimate enteric-coated capsules containing mucoadhesive Microspheres of Capecitabine and Oxaliplatin to treat Colon cancer.
Study Design: Box Behnken.
Place and Duration of Study: Department of Pharmaceutics, Parul Institute of Pharmacy and Research, Parul University, Vadodara, between 2017 to 2021.
Methodology: Capecitabine and Oxaliplatin are used as antineoplastic agents and can be delivered via the oral route of administration. For the estimation of drugs Analytical method has been developed by HPLC. Box Behnken design has been used to optimize Drug: polymer ratio (1:2), Inlet temperature 170ºC, and crosslinking agent with a 0.5 ml 1% Gluteraldehyde solution.
The microspheres were successfully prepared by using the spray drying technique and evaluated.
Results: The results of optimized Capecitabine microspheres were obtained as Particle size 87.91 µm ± 0.274,% yield 57.21± 1.5,% Mucoadhesion 57.21± 1.5,% entrapment efficiency 82.16± 0.725.

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The results of optimized Oxaliplatin microspheres were obtained as Particle size 99.88µm±0.034,% yield 56.0± 0.088,% Mucoadhesion 87.0± 0.80,% entrapment efficiency 82.61±0.085. The drug content of Capecitabine and Oxaliplatin in the filled capsule was 94.67% ±0.32 and 93.45%±0.712, respectively. % Drug release of Capecitabine and Oxaliplatin in Phosphate buffer pH 7.4 was found to be 94.83±0.22 and 96.94±0.11 respectively after 8 hrs. Stability study at $40^0C±2^0C$ / 75 ± 5 % RH revealed that there was no significant change in disintegration time, drug content and % CDR during 6 months. So, prepared formulation was stable during stability study. MTT assay has been performed on the formulation of Capecitabine and Oxaliplatin microspheres for assessing the % viability of both the drugs on the Caco-2 cell line.

Conclusion: The present study confirmed that prepared mucoadhesive microspheres filled with enteric-coated capsules have an antitumor effect on colon cancer cells. The formulation induced high cell death within 48 hours, and less cell viability was obtained compared to API. Six months accelerated Stability study indicates that formulation is fairly stable at storage conditions.

Keywords: Capecitabine; oxaliplatin; colon cancer; mucoadhesive microspheres; spray dryer.

ABBREVIATIONS

Caco2 : Cancer coli-2
MTT : 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO : Dimethyl Sulfoxide
DMEM : Dulbecco's Modified Eagle Medium
NCCS : National Centre for Cell Science
Caco2 : Cancer coli-2
IC_{50} : half maximal inhibitory concentration
ELISA : enzyme-linked immunoassay

1. INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth to invade or spread to other parts of the body. Benign tumours do not spread to other parts of the body [1]. Colon cancer is the cancer of the epithelial cells lining the colon. Colorectal cancer is mainly divided into different stages according to the invasiveness and metastatic ability of the tumour. Diagnosis of colorectal cancer can be made by sigmoidoscopy or by colonoscopy with biopsy confirmation of cancer tissue. Treatment of colorectal cancer range from surgery in the early stages to palliative care in the most advanced stages [2]. Capecitabine is currently used as first-line therapy in patients with metastatic colorectal cancer [3]. Oxaliplatin is an anticancer ("antineoplastic" or "cytotoxic") chemotherapy drug. Oxaliplatin is used to treat colon or rectal cancer that has spread (metastasized); it is often given in combination with other anticancer drugs (fluorouracil and leucovorin) [4,5].

Capecitabine was developed as a prodrug of Florourosil, with the goal of improving tolerability and intratumor drug concentrations through tumor-specific conversion to its only active metabolite, FU, by thymidine phosphorylase. Higher levels of this enzyme are found in several tumors and the liver, compared with normal healthy tissue [3]. Capecitabine is metabolized to 5-FU which in turn is a Thymidylate synthase inhibitor, hence inhibiting the synthesis of thymidine monophosphate (ThMP), the active form of Thymidine which is required for the synthesis of DNA [1].

Capecitabine is currently use as first-line therapy in patients with metastatic colorectal cancer when single-agent fluoropyrimidine therapy is preferred. The drug is also approved for use as a single agent in metastatic breast cancer patients who are resistant to both anthracycline- and paclitaxel-based regimens or in whom further anthracycline treatment is contra indicated Improved tolerability and comparable efficacy compared with IV FU/LV in addition to oral administration make Capecitabine an attractive option for the treatment of several types of cancers as well as the focus of future trials [3].

Oxaliplatin is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. Oxaliplatin is classified as an "alkylating agent."

Alkylating agents are most active in the resting phase of the cell. These drugs are cell-cycle non-specific. There are several types of alkylating agents [4].

Microspheres are small spherical particles with diameters in the micrometer range (typically 1 µm to 1000 µm). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials [6,7]. Mucoadhesive

microspheres enhance the intimate contact with the mucus layer and drug targeting to the absorption site by anchoring bacterial adhesions, plant lectins, antibodies etc [7,8]. Mucoadhesive Microspheres prolong the residence time of the dosage form at the site of absorption. Due to an increased residence time, it enhances absorption and hence the therapeutic efficacy of the drug. Delivery via the oral route, increase in drug bioavailability due to first-pass metabolism avoidance. The drug is protected from degradation in the acidic environment in the gastrointestinal tract: improved patient compliance - ease of drug administration. Faster onset of action is achieved due to the mucosal surface having an enormous blood supply and good blood flow rates. Therefore, site-specific activity, reduced systemic side effects (systemic cytotoxicity), reduced dose and toxicity, Increased stability, and provided constant and longer therapeutic effect. Reduces the frequency of daily administration and thereby improves patient compliance. Improve the absorption of drug hence improve the bioavailability of drug and reduce the chances of adverse effects. The morphology of Microspheres permits a controllable variability in degradation and drug release [7,8]. Oral administration of drugs is one of the most convenient and patient-accepted methods of drug delivery. However, the gastrointestinal microenvironment presents many delivery challenges, including the acidic conditions of the stomach, the proteolytic activity of the gastrointestinal tract due to the presence of digestive enzymes, and the high density of bacterial species. While intravenous administration of chemotherapeutics is common practice, the oral route provides an anatomical advantage for delivering such agents. It permits direct access to the luminal tissue affected by many diseases. One promising method for oral drug delivery involves mucoadhesive biomaterials such as chitosan [9].

Enteric-coated HPMC Capsule Shell has been commercially available to the dietary supplement industry as a vegetarian alternative to gelatin. As HPMC is often used as a pre-coating material for enteric-coated tablets, it may be expected that the application of enteric-type polymers to a capsule made from HPMC would result in suitable Polymer to Polymer adhesion and compatibility [10].

The main objectives of the study are focused on decreasing the Drawbacks associated with the Current Therapy of the Colon Cancer, through targeting of the Antineoplastic agents at the site of the Tumor.

Synergistic effect: When introduced in Combination.

By Formulating Mucoadhesive Microspheres, Provide Site-specific Delivery and release the drug at the tumor site. Mucoadhesive microspheres Prolongs the residence time of the dosage form at the site of absorption. Due to an increased residence time it enhances absorption and hence the therapeutic efficacy of the drug.

pH sensitive capsule protect drug throughout the GI tract.

Reduction of systemic adverse effects: due to site specific drug release, the systemic side-effects of both the drugs will be reduced.

Patient compliance as both the drugs are delivered in single formulation and convenient route of administration.

2. MATERIALS AND METHODS

2.1 Materials

Capecitabine was obtained as a gift sample from BDR Pharmaceuticals International Private Limited. Vadodara (Gujarat, India). The Oxaliplatin was purchased from Taj Mahal Chemicals Pvt. Ltd. Mumbai, India. Chitosan was purchased from Chemdynes Corporation, Vadodara. Glacial acetic acid and Glutaraldehyde were purchased from Sulab Reagents, Vadodara, HPMC Capsule Shell purchased from Qualicaps Europe, S.A.E. Caco2 cell line obtained from NCCS Pune, culture media DMEM obtained from Himedia. MTT dye, DMSO, Trypsin, Triton were obtained from Himedia. HPLC make Agilent, Software: Empower 2, Spray Dryer make ELECTROLAB ultima, Dissolution Apparatus USP make: Electrolab, TDT-8L, Disintegration apparatus make: Electrolab, ED-2L, FTIR Spectrometer make: Bruker-alpha Spectrophotometer, Japan.

2.2 Methodology

2.2.1 Differential scanning Calorimetry (DSC)

DSC analysis was conducted using a Thermal Analyzer. Samples, including Capecitabine, physical mixture of Capecitabine and polymer, Oxaliplatin, physical mixture of Oxaliplatin, and

polymer, were weighed into an aluminium pan, which was sealed with a pinhole-perforated cover. The samples were purged with dry nitrogen at a flow rate of 20 mL/min. Heating curves were record at a scan rate of 10°C/min from 30 to 400. Heating Curves of Drugs and the Physical mixture of Polymers were recorded [11,12].

2.2.2 Method of preparation for Capecitabine microspheres

Dissolve chitosan in 1% v/v glacial acetic solution. Then add 150mg Capecitabine and dissolve in 10 ml water. Sonicate the solution for 5 min, then mix both the solutions and add 1% 0.5ml glutaraldehyde solution. Stir at 500 rpm/min for 30 min, then spray dried at inlet temperature 170°C, outlet temperature 120°C, and the flow rate was 5 mL/min using a spray drier. Collect dried microspheres, weigh and evaluate [13,14].

2.2.3 Method of preparation for Oxaliplatin microspheres

Dissolve chitosan in 1% v/v glacial acetic solution. Then add 50mg Oxaliplatin and dissolve in 10 ml water. Sonicate the solution for 5 min, then mix both the solutions and add 1% 0.5ml glutaraldehyde solution. Stir at 500 rpm/min for 30 min, then spray dried at inlet temperature 170°C, outlet temperature 120°C, and the flow rate was 5 mL/min using a spray drier. Collect dried microspheres, weigh and evaluate [13,14].

2.3 Evaluation Parameters of Mucoadhesive Microspheres

2.3.1 Surface Morphology

Shape and surface morphology was studied with Scanning electron microscopy (SEM) [14].

2.3.2 Particle size

The particle size of the microspheres was determined by using the optical microscopy method. A small amount of dry microspheres was suspended in distilled water. A small drop of the suspension was placed on a clean glass slide. The slide containing suspended microspheres was mounted on the microscope stage, and 100 particles were measured using a calibrated ocular micrometer. The process was repeated three times for each batch prepared [14,15].

2.3.3 Flow Properties

The flow properties of microspheres were investigated by determining the angle of repose, bulk density, tapped density, Carr's and Hausner's ratio. Each parameter was calculated three times for each batch prepared, and results were averaged.

The angle of Repose: Angle of repose (θ) was measured according to the fixed funnel of Banker and Anderson. A funnel with the end of the stem cut perpendicular to the axis of symmetry is secured with its tip at a given height of 1cm (H) above graph paper placed on a flat horizontal surface. The microspheres were carefully poured through the funnel until the apex of the conical pile so formed just reached the tip of the funnel. Thus, the R being the radius of the base of the microspheres conical pile:

$$\tan\theta = \frac{H}{R}$$

$$\theta = \tan^{-1}\frac{H}{R}$$

Where, θ = Angle of repose, H = Height of pile, R = Radius of the pile [13,14].

Carr's Index and Hausner's Ratio: Poured density was determined by placing the exact quantity 'M ' of microsphere into a graduated cylinder and measuring the volume' V ' occupied by the microspheres.

$$\text{Poured Density} = \frac{M}{V}$$

Tapped density was determined by placing a graduated cylinder containing a known quantity (M) of the prepared microspheres on a mechanical tapping apparatus operated for a fixed number of taps until the bed volume reached a minimum.[14,15].

$$\text{Tapped Density} = \frac{M}{V}$$

The Carr's Index and Hausner's ratio were calculated using the formula [14,15].:

$$\text{Carr}'\text{s index } (\%) = \frac{\text{Tapped Density} - \text{Poured Density}}{\text{Tapped Density}} \text{X } 100$$

$$\text{Hausner}'\text{s ratio} = \frac{\text{Tapped Density}}{\text{Poured Density}}$$

2.3.4 Percentage yield

The microspheres were evaluated for percentage yield. The % yield was calculated by the formula:

$$\%\ \text{Yield} = \frac{\text{weight of microspheres recovered}}{\text{weight of Drug+weight of the polymer}} \text{X } 100$$ [14,15].

2.3.5 Mucoadhesion: *In-Vitro* Wash-off test for Microspheres

An *in-vitro* wash-off test evaluates the mucoadhesive properties of the microspheres. A 2 cm x 2 cm piece of chicken intestine mucosa was tied onto a glass slide (3inch by 1inch) using thread. Fifty microspheres were spread onto the wet rinsed tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus is operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated intestinal fluid USP (PBS pH 7.4). At the end of 8 hours, the number of microspheres still adhering onto the tissue is counted [14,15].

$$\%\, mucoadhesion = \frac{\text{No.of microspheres adhered (after 8 hrs)}}{\text{No.of microspheres applied}} \text{ X } 100$$

2.3.6 % Entrapment Efficiency

150mg Capecitabine or 50 mg Oxaliplatin loaded core microspheres was weighed and washed with 10ml of phosphate buffer of pH 6.8 to remove the surface-associated drug. then microspheres were kept in a phosphate buffer of pH 7.4 for digestion for 24 h and sonicated for 1 h at room temperature, from that, 1ml of sample is withdrawn and diluted 1000 times using phosphate buffer pH 7.4 and quantified by HPLC. Entrapment efficiency is determined by using the formula [14,15].

$$\%\ \text{Drug Entrapment} = \frac{\text{Actual content}}{\text{Theoretical content}} \text{X } 100$$ [14, 15].

2.4 Evaluation Parameter of Enteric coated HPMC Capsules: [16,17,18]

2.4.1 Appearance Capsules

The enteric-coated capsules were evaluated as per pharmacopoeial tests for their appearance, average weight, disintegration (PBS pH 7.4), average weight of empty capsule shell, net content, drug content, *in vitro* drug release.

2.4.2 Drug content

Transferred one intact Capsule into 100mL volumetric flask and added 75mL of Phosphate buffer pH 7.4, sonicate for 45 minutes, checked visually if capsules are disintegrated, and then shook for 30 minutes, made up 100ml volume with Phosphate buffer pH 7.4 and mixed well and filtered through 0.45µ PVDF filter. Further pipetted out 5mL of this filtrate to 50mL and made up to volume with Phosphate buffer pH 7.4 to prepare 150µg/mL of Capecitabine and 50 µg/mL of Oxaliplatin solution and injected into the HPLC system.

2.4.3 *In-vitro* drug release of capsule

In-vitro drug release studies were conducted using a modified USP type 1 dissolution apparatus at 37°C and 75 rpm/min in 90 mL of phosphate-buffered saline (PBS), pH 7.4. At predetermined time intervals, 1 mL samples were taken, and an equivalent volume of fresh PBS was added to maintain a constant volume. Drug concentrations from collected samples were measured using an HPLC. The zero-order kinetics was carried out by plotting the square root time against percent drug release.

2.4.4 Stability study: [19]

Stability studies were conducted as per the International Conference on Harmonization (ICH) guidelines. The samples were stored at 40°C ± 2°C/75 ± 5% relative humidity (RH) for six months. The samples were withdrawn and evaluated for the drug content and in-vitro release at pre- determined time intervals. The variations were analyzed and compared with the freshly prepared formulations. All samples were taken in triplicates (n = 3).

The stability study has been performed and result of the stability study indicated that there was not much difference observed in disintegration time, drug content, and % drug release before and after the storage period at 40 ± 2°C/75% RH ± 5% temperature and relative humidity. This indicates that formulation is fairly stable at storage conditions.

2.5 *In-vitro* Cell Viability Study [20-24]

The MTT assay was performed to assess cell cytotoxic potential of Different formulations of Oxaliplatin and Capecitabine using Caco-2 cell line. MTT assays were performed to check and compare the cytotoxicity of formulation with API solution of the respective drug, in colon cancer cell line Caco-2 by measuring IC50 values.

Cancer Cells (Caco-2) ($5x10^3$) were plated in 96 well plates in 200 µL of MEM medium per well

and incubated for 24 h. Cells were incubated with different concentrations of test solutions for 48 h. The medium was removed from all the wells, and wells were fed with 200 µL of fresh complete medium. 100 µL of MTT solution was added to each well plate and incubated for 4 hrs. Cell plates were centrifuged at 3000 rpm/min for 10 min &culture media was discarded. Each cell was treated with 200 µL of DMSO solution. DMSO solution was added to dissolve MTT formazan crystals. DMSO solution was measured at 540 nm with a microplate reader immediately. Capecitabine and Oxaliplatin formulations and API were diluted with serum-free DMEM to prepare various formulation concentrations at 50mg Oxaliplatin and 150 mg Capecitabine. Cells treated with 200 µL of fresh in complete medium (DMEM) were used as negative control (100% viability will be assumed from the absorbance of wells containing these cells). Cell viability was calculated, and Viability plots were plotted by plotting % viable cells against the treatment.

2.5.1 Statistical analysis and optimization of formulation

Various formulation parameters may have an impact on the various characteristics of the product. Thus, the first attempt was made to evaluate the effect of these parameters on the formulation of mucoadhesive Microspheres. The Design Expert (Version 13) program was used for the design of the experiment and analysis of this and for drawing of three-dimensional response surface and contour plots. Drug: Polymer Ratio %, Inlet Temperature °C, amount of 1%, glutaraldehyde solution was taken as formulation factors which statistically analysed have major impact on the microspheres like entrapment efficiency, % yield, and % mucoadhesion. Box behnken designs applied to optimize Capecitabine mucoadhesive microspheres and Oxaliplatin Mucoadhesive Microspheres individually. The batches and results of Capecitabine microspheres and Oxaliplatin microspheres are shown in Table1, Table 2, Table 3 and Table 4.

3. RESULTS AND DISCUSSION

3.1 Differential scanning Calorimetry (DSC)

Differential scanning calorimetry is used to define the melting point of drug substances by endothermic peak in the curve to find DSC curve, the sample run at 30 to 400 °C temperature. The Endothermic peak of both drugs was observed in the DSC curve of the drug-polymer mixture at a specified temperature. The DSC graphs are shown in Fig. 1, Fig. 2, Fig. 3 and Fig. 4.

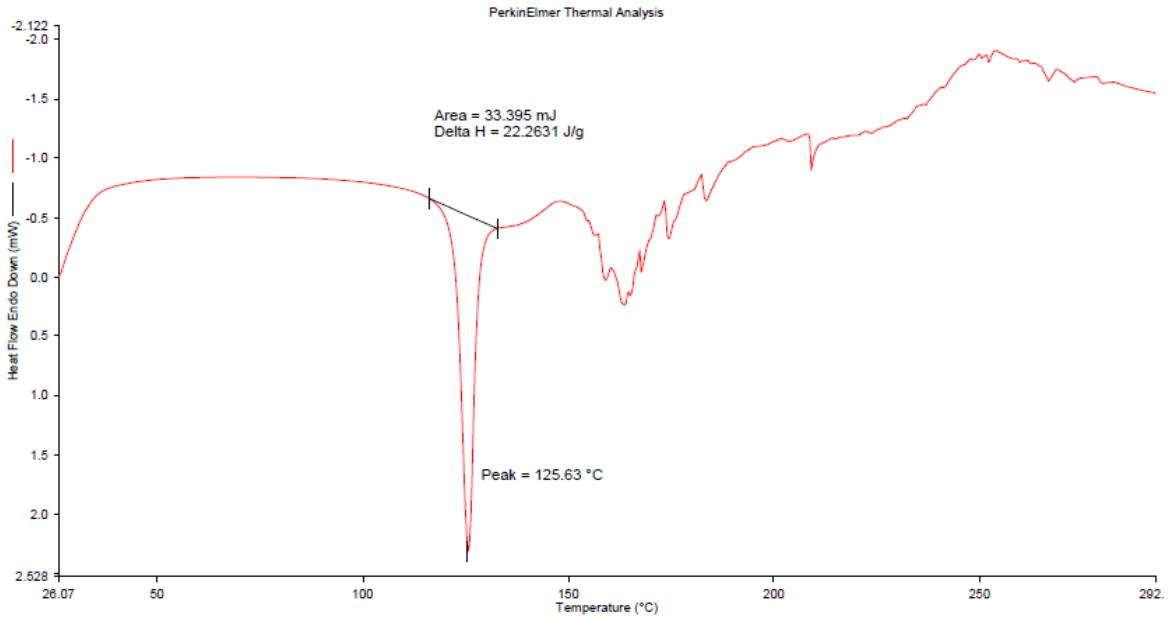

Fig. 1. DSC curve of Capecitabine

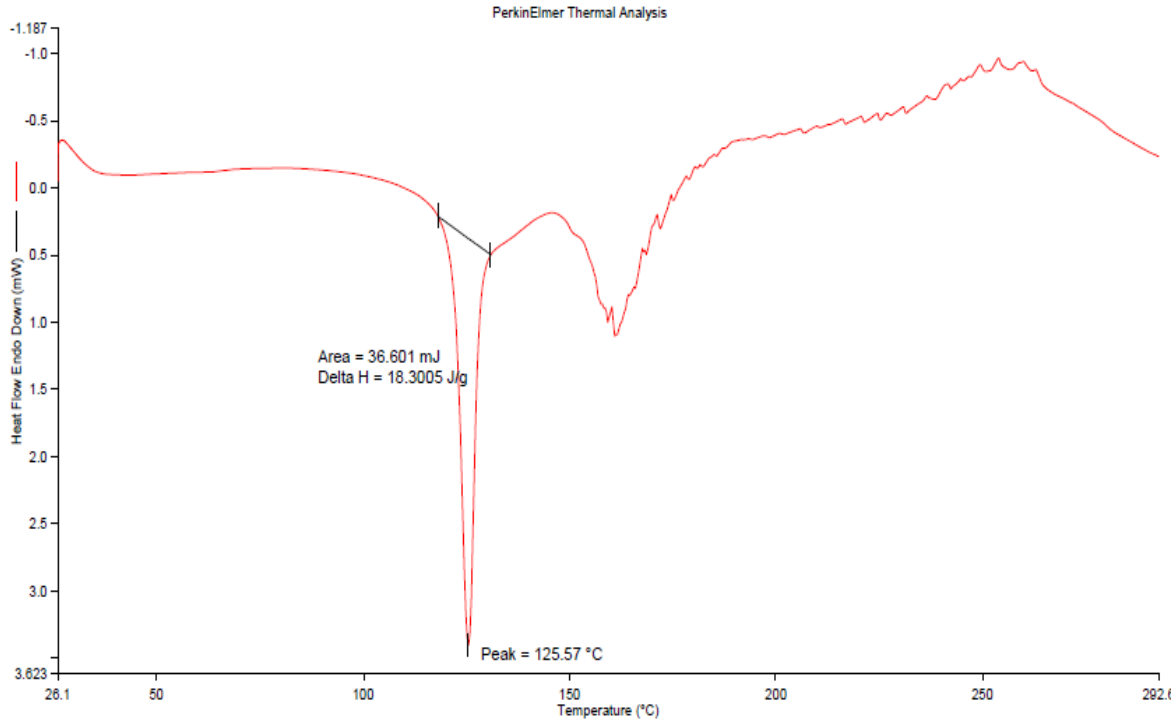

Fig. 2. DSC curve of Capecitabine with Polymer mixture

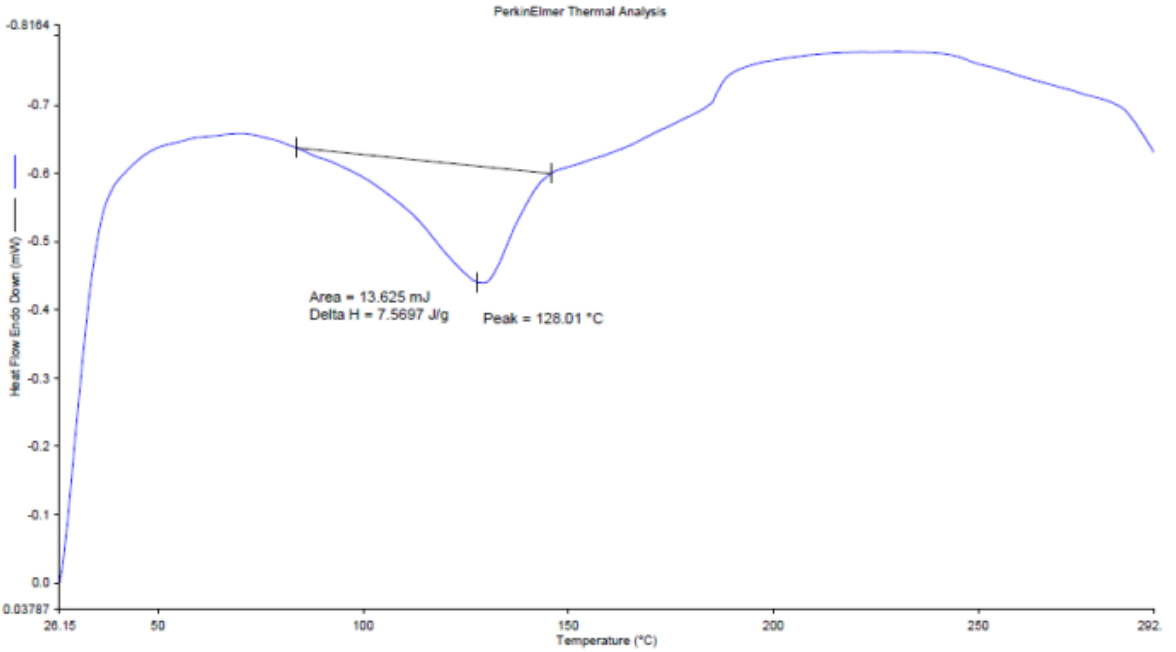

Fig. 3. DSC curve of Oxaliplatin

3.2 Scanning Electron Microscopy (SEM)

The morphology of Capecitabine and Oxaliplatin Mucoadhesive microspheres were examined using Scanning electron microscopy, which shown spherical morphology, narrow size distribution. It is shown in Fig. 5. and Fig. 6.

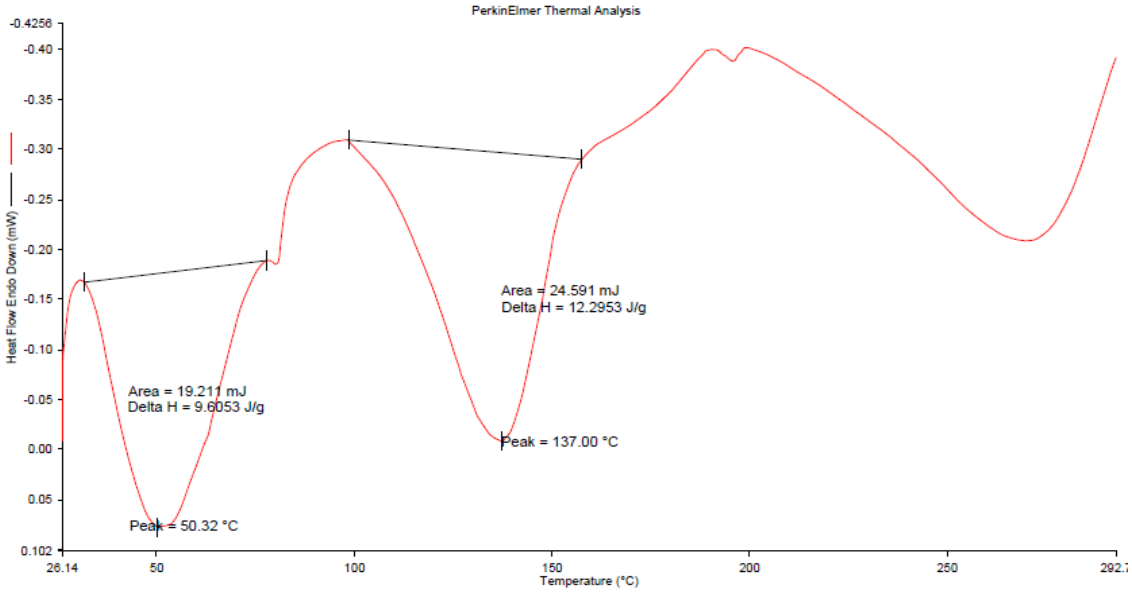

Fig. 4. DCS curve of Oxaliplatin with Polymer mixture

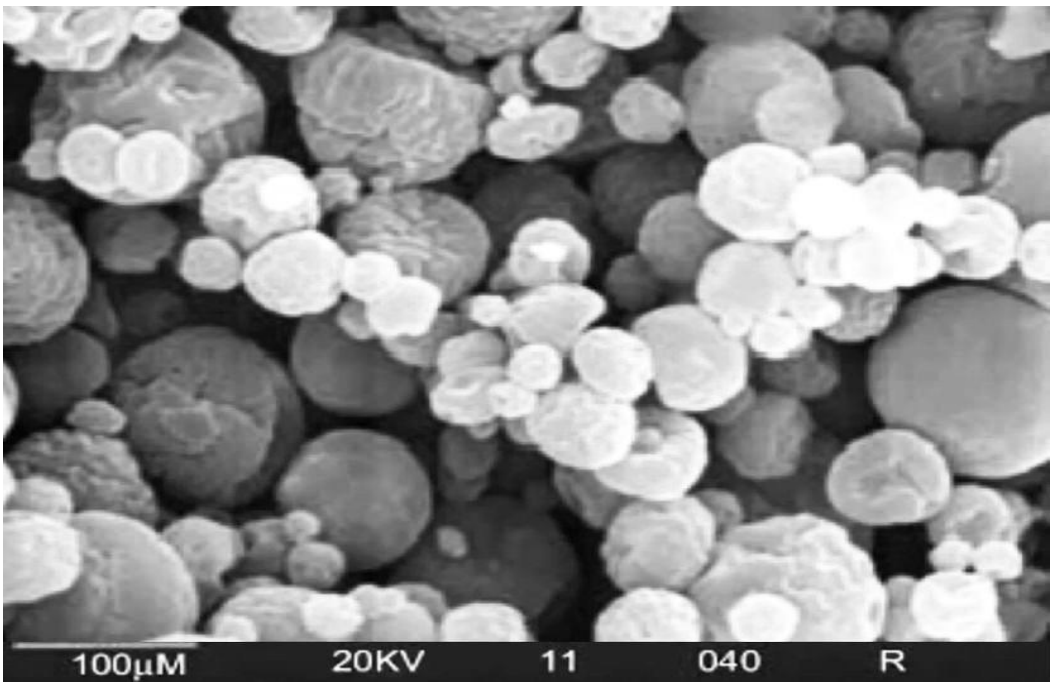

Fig. 5. SEM image of Capecitabine Microspheres

3.3 Results of Evaluation of Mucoadhesive Microspheres

Capecitabine Mucoadhesive Microspheres, % yield, % mucoadhesion, % entrapment efficiency results are shown in Table 1.

Particle size measurement, Flow property, tapped density, Carr's index, Hausner's ratio of Capecitabine mucoadhesive microspheres are shown in Table 2.

From the results of above evaluation of batch **F12** was optimized because of having good Mucoadhesion property, % entrapment efficiency, % yield, swelling Index and this batch was filled into capsule.

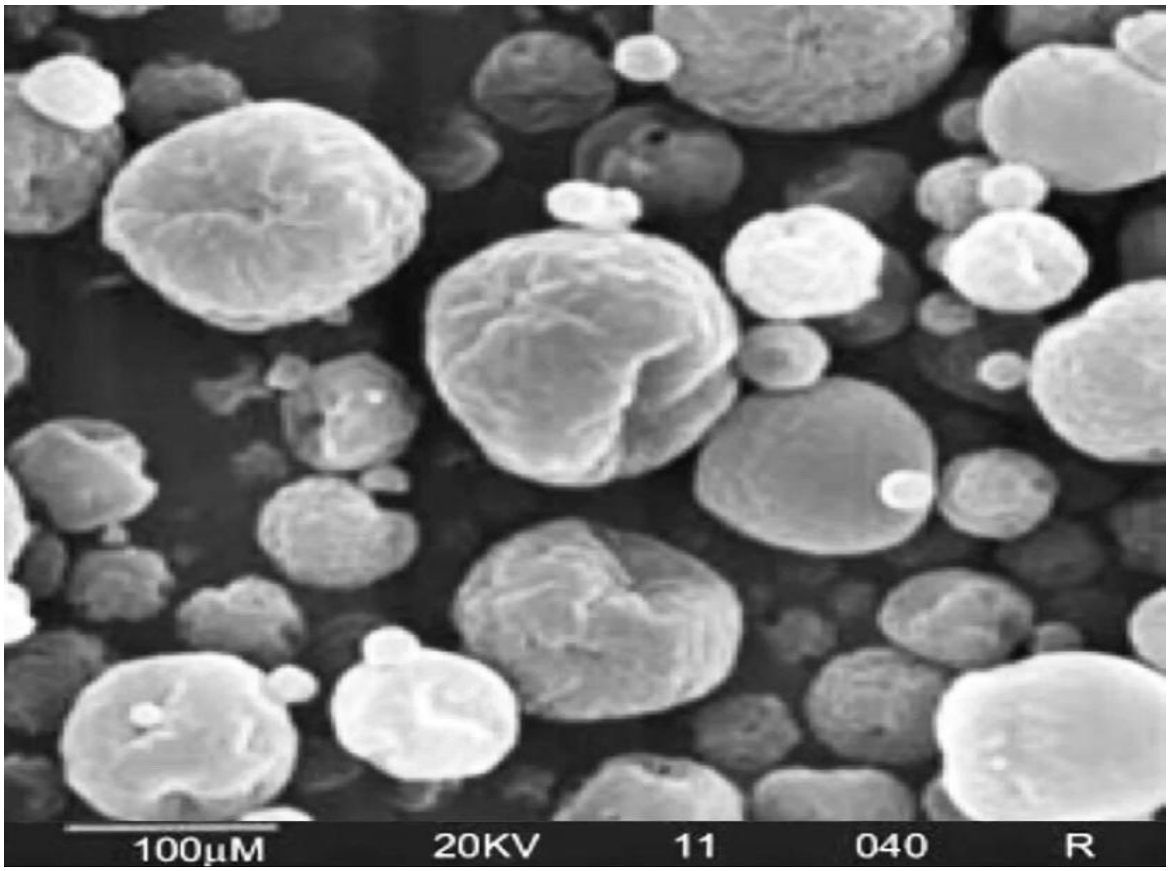

Fig. 6. SEM image of Oxaliplatin Microspheres

Table 1. Results of Optimization of Capecitabine Microspheres

Batch	Factor 1 Drug: Polymer Ratio %	Factor 2 Inlet Temperature ºC	Factor 3 Amount of 1% Glutaraldehyde solution (ml)	Response 1 % Yield	Response 2 % Mucoadhesion	Response 3 % Entrapment Efficiency
F1	1:1.5	170	1.25	43.78± 0.95	64± 0.19	72.28± 0.476
F2	1:2	170	0.5	50.2± 0.82	84±0.17	80.21± 0.125
F3	1.15	160	2	46.21± 0.95	61±0.15	78.63± 0.553
F4	1:1.5	180	0.5	49.2± 1.25	84± 0.19	81.00± 0.607
F5	1:1	160	1.25	32.21± 0.81	65± 0.15	68.2± 0.817
F6	1:1	180	1.25	44.22± 0.94	70±0.56	67.44± 0.330
F7	1:2	160	1.25	43.21± 0.81	66± 0.21	78.35± 0.210
F8	1:1.5	180	2	54.8± 1.63	66± 0.19	79.46± 0.572
F9	1:1.5	160	0.5	46.4± 1.62	80± 0.18	80.01± 0.757
F10	1:1	170	0.5	43.34± 0.95	79± 0.18	77.06± 0.674
F11	1:1.5	170	1.25	47.24± 1.5	70± 1.22	65.34± 0.360
F12	1:2	170	0.5	57.21± 1.5	84± 0.21	82.16± 0.725
F13	1:1.5	170	1.25	36.54± 1.25	78± 0.17	74.83± 0.45
F14	1:2	180	1.25	56.2± 0.81	72± 1.23	78.83± 0.832
F15	1:1	170	2	43.21± 0.81	61± 0.18	60.56± 0.757

**% is a percentage; ml is milliliter, ºC is degree Celsius*

3.4 Statistical Analysis of Capecitabine microspheres

Graphical presentation of effect of factors on variable is shown in Fig. 7. (A),(B),and (C).

Fig. 7. (A) Indicates the effect of drug: polymer ratio and inlet temperature on % yield.

Fig. 7. (B) Indicates the effect of amount of gluteraldehyde and drug polymer ratio on % mucoadhesion.

Fig. 7. (C) Indicates the effect of amount of gluteraldehyde and drug polymer ratio on % entrapment efficiency.

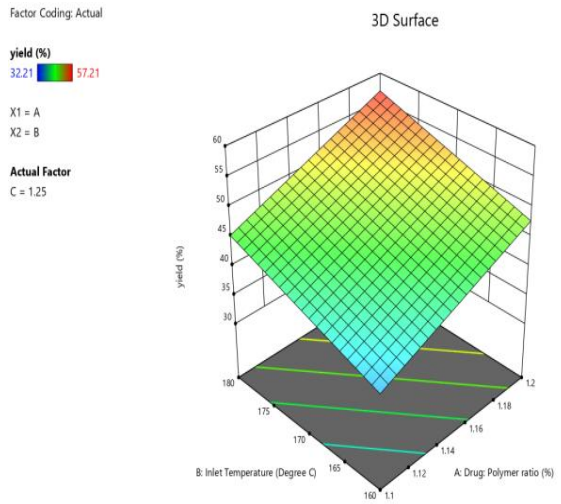

Fig. 7. (A)% Yield

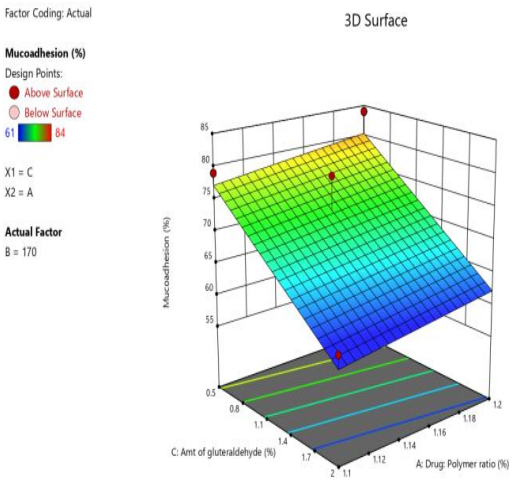

Fig. 7. (B) % Mucoadhesion

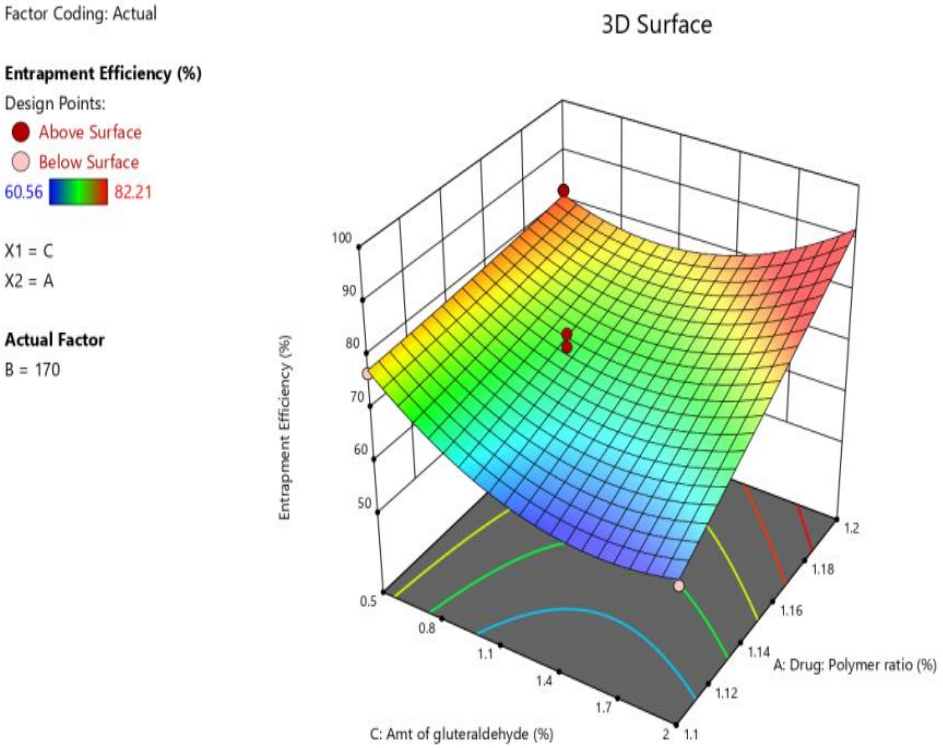

Fig. 7. (C) % Entrapment Efficiency

Table 2. Results of optimization of Capecitabine Microspheres: Particle Size Determination and flow Property Measurement

Batch Name	Particle size µm	Bulk density g/ml	Tapped density g/ml	Carr's index	Hausner's ratio	Angle of repose
F1	76.81 ± 0.47	1.04±0.008	1.19±0.011	12.60±0.036	1.14±0.009	31.5±0.081
F2	88.48± 0.698	1.11±0.009	1.25±0.012	20.0±0.049	1.12±0.008	32.12±0.083
F3	109.6 ±0.70	1.25±0.012	1.42±0.016	11.97±0.035	1.13±0.009	33.00±0.090
F4	85.12 ±0.675	1.05±0.009	1.17±0.012	10.25±0.028	1.11±0.009	34.0±0.091
F5	76.8 ±0.655	0.96±0.007	1.18±0.012	18.64±0.049	1.16±0.010	26.13±0.076
F6	80.96 ± 0.782	1.02±0.008	1.23±0.013	17.07±0.046	1.20±0.012	28.55±0.079
F7	88.68 ± 0.600	0.99±0.008	1.16±0.012	13.79±0.038	1.17±0.010	22.41±0.071
F8	121.17 ± 0.50	1.06±0.008	1.18±0.012	10.16±0.028	1.11±0.009	29.0±0.079
F9	86.16 ± 0.950	1.13±0.009	1.28±0.013	11.71±0.029	1.13±0.009	32.21±0.083
F10	77.01 ± 0.237	1.10±0.009	1.24±0.012	11.29±0.029	1.12±0.008	28.0±0.079
F11	108.52 ± 0.92	1.11±0.009	1.28±0.013	13.28±0.035	1.15±0.009	32.0±0.082
F12	87.91 ± 0.274	1.12±0.009	1.31±0.013	14.50±0.036	1.17±0.010	28.10±0.079
F13	89.73 ± 0.496	1.09±0.010	1.23±0.012	11.38±0.025	1.13±0.009	31.30±0.081
F14	81.84 ± 0.666	1.02±0.008	1.15±0.012	11.30±0.025	1.13±0.009	26.17±0.076
F15	82.3 ± 0.655	1.08±0.010	1.26±0.012	14.28±0.041	1.17±0.010	25.23±0.075

** µm is a micrometer, g/ml is gram per milliliter*

Oxaliplatin mucoadhesive microspheres % Yield, % Mucoadhesion, % Entrapment Efficiency results are shown in Table 3.

Table 3. Results of Optimization of Oxaliplatin Microspheres

Batch	Factor 1 Drug: Polymer Ratio %	Factor 2 Inlet Temperature ºC	Factor 3 Amount of 1% Glutaraldehyde solution (ml)	Response 1 % Yield	Response 2 % Mucoadhesion	Response 3 % Entrapment Efficiency
F1	1:1.5	180	2	49.65±0.043	55±0.95	80.21±0.076
F2	1:1	160	1.25	35.43±0.056	72±0.81	67.41±0.18
F3	1:1.5	170	1.25	47.55±0.076	68±0.79	66.21±0.058
F4	1:1.5	160	0.5	41.32±0.058	82±0.63	73.56±0.39
F5	1:1	170	0.5	38.32±0.039	84±0.80	66.35±0.040
F6	1:2	180	1.5	52.21±0.040	71±0.62	81.23±0.056
F7	1:1	180	1.25	50.32±0.041	62±0.68	56.2±0.058
F8	1:1.5	170	1.25	38.32±0.081	72±0.95	67.44±0.082
F9	1:1.5	170	1.25	37.45±0.039	72±0.79	76.43±0.076
F10	1:1.5	180	0.5	49.34±0.039	84±0.64	74.23±0.042
F11	1:2	170	2	48.56±0.043	61±0.79	81.2±0.043
F12	1:1	170	2	44.21±0.085	56±0.68	78.22±0.086
F13	1:2	160	1.25	53.8±0.088	72±0.68	82.43±0.088
F14	1:1.5	160	2	47.54±0.076	67±0.80	78.32±0.083
F15	1:2	180	0.5	56.0±0.088	87±0.80	82.61±0.085

**% is a percentage; ml is milliliter, ºC is degree Celsius*

Particle size measurement, Flow property, tapped density, Carr's index, Hausner's ratio of Capecitabine Mucoadhesive microspheres are shown in Table 4.

3.5 Statistical Analysis of Oxaliplatin Microspheres

Graphical presentation of effect of factors on variable is shown in Fig. 7. (A),(B),and (C).

Fig. 8.(A) Indicates the effect of drug: polymer ratio and inlet temperature on % yield .

Fig.8.(B) Indicates the effect of amount of gluteraldehyde and drug polymer ratio on % mucoadhesion.

Fig. 8.(C) Indicates the effect of amount of gluteraldehyde and drug polymer ratio on % entrapment efficiency.

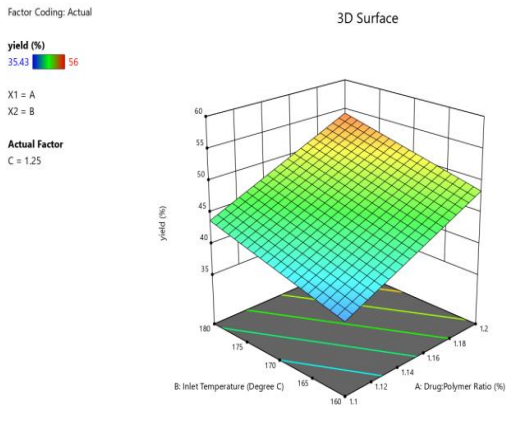

Fig. 8. (A)% Yield

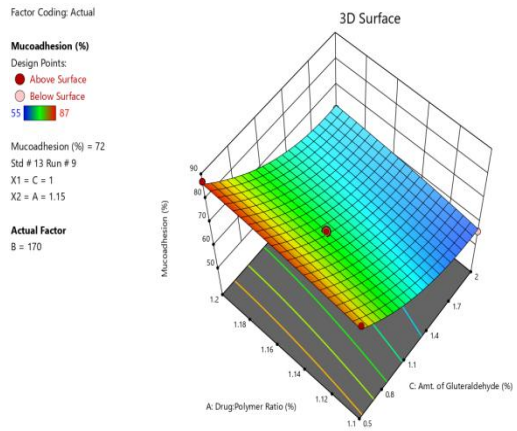

Fig. 8. (B) % Mucoadhesion

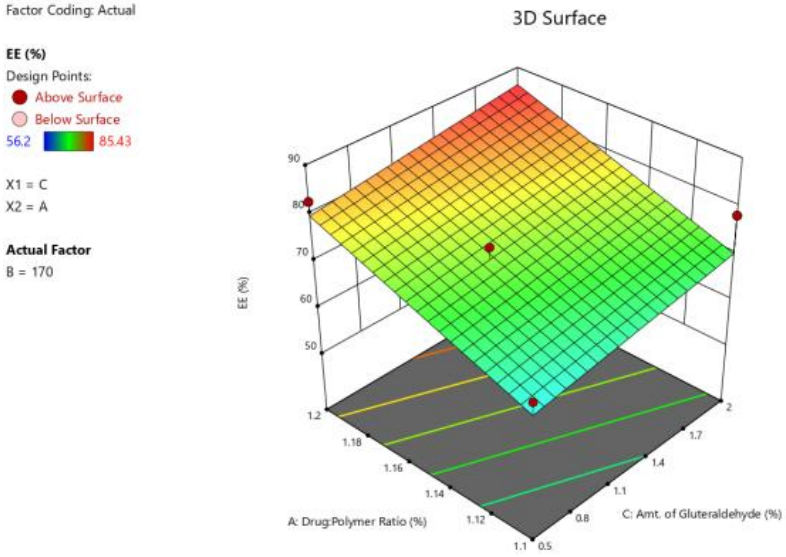

Fig. 8. (C) % Entrapment Efficiency

Fig. 8. Contour plots of Oxaliplatin microspheres (A)% yield (B) % mucoadhesion (C) % entrapment efficiency

3.4 Evaluation Parameters for Microspheres Filled Enteric-Coated Capsules

The final optimized microspheres filled enteric-coated capsules were evaluated as per pharmacopoeial tests for its appearance, average weight, disintegration (PBS pH 7.4), average weight of empty capsule shell, net content, drug content, and results are recorded in Table 5.

Table 4. Results of Optimization of Oxaliplatin Microspheres: Particle Size determination and flow property Measurement

Batch Name	Particle size µm	Bulk density g/ml	Tapped density g/ml	Carr's index	Hausner's ratio	Angle of repose
F1	102.23±0.035	1.25±0.012	1.42±0.008	11.97±0.008	1.14±0.009	25.45±0.008
F2	98.53±0.028	1.13±0.011	1.26±0.007	10.31±0.007	1.11±0.008	18.92±0.004
F3	83.25±0.026	1.02±0.010	1.22±0.007	16.39±0.017	1.19±0.010	24.12±0.007
F4	108.33±0.033	1.14±0.011	1.35±0.008	15.55±0.016	1.18±0.011	22.80±0.006
F5	123.14±0.046	1.25±0.012	1.41±0.008	11.34±0.012	1.13±0.013	26.14±0.009
F6	86.23±0.028	1.16±0.011	1.36±0.007	14.70±0.013	1.17±0.015	23.70±0.004
F7	115.11±0.038	1.04±0.010	1.23±0.006	15.44±0.015	1.18±0.016	21.55±0.004
F8	98.50±0.028	1.11±0.011	1.29±0.007	13.95±0.008	1.16±0.015	24.34±0.005
F9	110.63±0.035	1.08±0.010	1.22±0.006	11.47±0.006	1.13±0.014	32.22±0.012
F10	68.31±0.018	1.12±0.011	1.45±0.009	22.75±0.018	1.29±0.018	28.76±0.008
F11	74.42±0.021	1.09±0.011	1.24±0.007	12.09±0.005	1.14±0.009	32.0±0.013
F12	109.51±0.032	1.13±0.012	1.28±0.007	11.71±0.012	1.13±0.013	28.44±0.010
F13	88.12±0.031	1.05±0.010	1.33±0.008	21.05±0.017	1.26±0.017	22.34±0.008
F14	76.66±0.022	1.23±0.012	1.43±0.009	13.98±0.008	1.16±0.015	27.10±0.007
F15	99.88±0.034	1.08±0.010	1.22±0.007	11.47±0.012	1.13±0.013	28.30±0.007

**µm is a micrometer, g/ml is gram per milliliter*

Table 5. Results of evaluation parameters for filled capsule

Sr no.	Parameters	Results	Limit
1.	Appearance	Opaque White cap /Opaque White body hard enteric-coated HPMC capsule, Size "0"	Opaque White cap /Opaque White body hard enteric-coated HPMC capsule, Size "0"
2.	Average Weight	797.2mg	800mg ± 7.5mg
3.	Length For Cap: (±0.30mm) For Body: (±0.30mm)	10.72mm 18.44mm	(10.42mm to 11.02mm) (18.14mm to 18.74mm)
4.	Closed joined length	21.7mm	(21.4mm to 22.00mm)
5.	Disintegration (PBS pH 7.4)	6.5min	NMT 30 min
6.	The average weight of empty capsule Shell	68mg	70mg± 9mg
7.	Net content	729.2mg	730mg± 7.5%
8.	Drug content Capecitabine	94.67% ±0.32	85% to 115%
9.	Drug Content Oxaliplatin	93.45%±0.712	85% to 115%

**mg is milligram, mm is millimeter, NMT is not more than, min is minute, % is a percentage, PBS is phosphate buffer saline.*

The graphical presentation of % CDR of Capecitabine and Oxaliplatin in the capsule is shown in Fig. 9.

The highest % drug release of Capecitabine and Oxaliplatin was found as 94.83 ±0.22 and 96.94 ±0.11 respectively after 8h. The correlation coefficient (R^2) of Capecitabine and oxaliplatin and the zero-order model was found 0.9518 and 0.944, slightly higher when compared to the peppas plot and higuchi's plot for the final selected optimized batch of capsule. Hence drug release from the preparation followed zero-order kinetics, which indicated that drugs released from the capsule were in a controlled manner.

3.5 Stability Study

The stability study has been performed result of the stability study indicated that there was not much difference observed in disintegration time, drug content, and % drug release before and after the storage period at 40 ± 2°C/75% RH ± 5% temperature and relative humidity. This indicates that formulation is fairly stable at storage conditions.

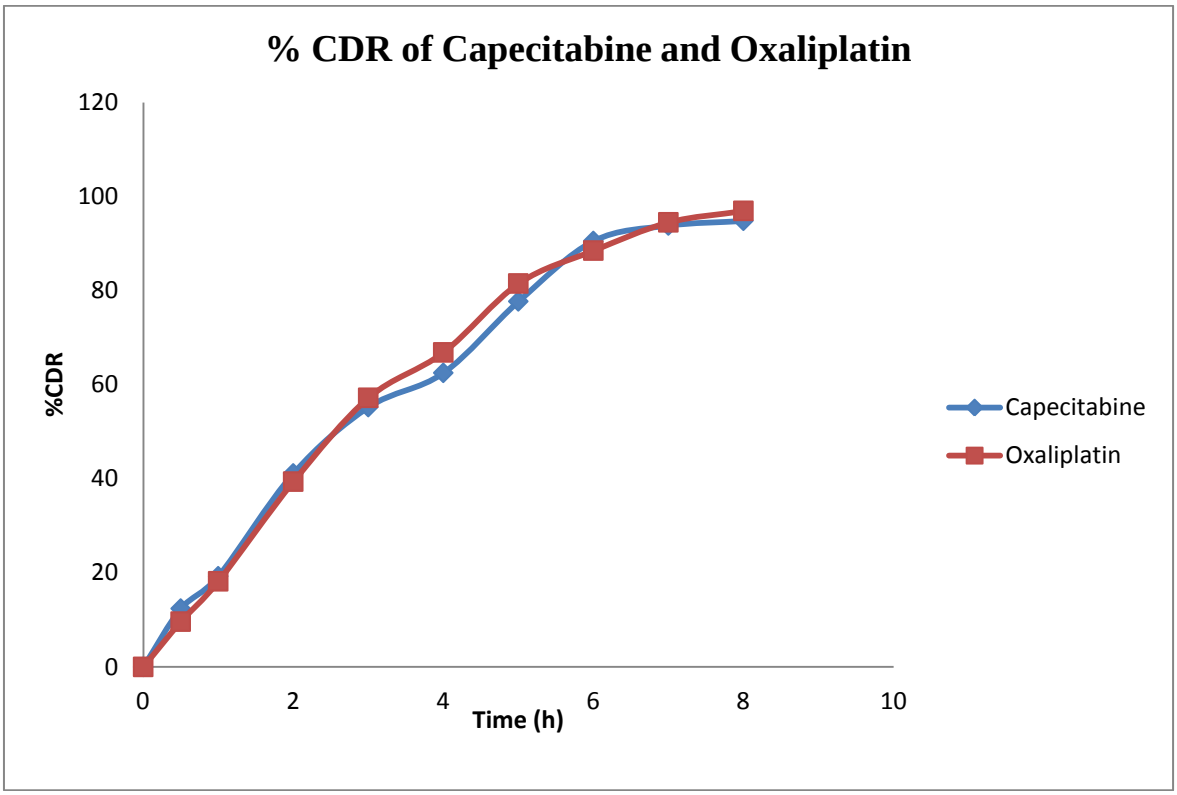

Fig. 9. Cumulative Percent drug release from enteric-coated Capsule containing Capecitabine and Oxaliplatin Mucoadhesive Microspheres

Table 6. Results of Stability data for filled capsule

Sr no.	Parameters	Initial	1 Month	3 Month	6 Months
1	Appearance	Complies	Complies	Complies	Complies
2	Average Weight	795.60mg ±0.37	795.80mg± 0.5	796.45mg ±0.32	796.10mg ±
3	Net content	730.2mg±0.47	729.54mg±0.34	732.18mg±0.21	718.80mg±0.51
4	Disintegration (PBS pH 7.4)	6.8 min±0.65	6.9 min±0.25	7.0 min±0.70	7.55 min±0.66
5	Drug content of Capecitabine	96.77%±1.12	93.33%±0.05	94.55%±0.05	93.85%±1.23
6	Dissolution of Capecitabine (after 8 hr)	94. 66% ±0.32	93. 16%±0.22	93.40%±0.77	92.51%±0.27
	Dissolution of Oxaliplatin (after 8 hr)	96.87%±0.62	96.60%±0.22	92.78%±1.12	92.29%±1.31

% is a percentage

3.6 *In-vitro* Cell Viability Study

MTT assay has been performed on the formulation of Capecitabine and Oxaliplatin microspheres for assessing the % viability of both the drugs on the Caco-2 cell line. Comparison of API with Formulation revealed that formulation produces less cell viability as compared to individual API. The absorbance of the produced formazan is proportional to the number of damaged or dying cells. The viability of the treated cells depends on the cytotoxicity of the drugs. The results are shown in Table 7.

The graph of % cell viability v/s treatment is shown in Fig. 10.

Table 7. Results of percent cell viability

Sr. no.	Treatment	48 h Incubation Period	
		Mean Absorbance*	% Cell Viability*
1	Control (Only cells with fresh media)	0.856±0.002	100
2	Only media with no cells	0.015±0.009	1.75
3	50 mg Oxaliplatin (50µg/ml)	0.154±0.007	17.99
4	150 mg Capecitabine (150µg/ml)	0.251±0.003	29.32
5	Microspheres of Oxaliplatin (F15) 50µg/ml)	0.094±0.015	10.98
7	Microspheres of Capecitabine F12 (150µg/ml)	0.068±0.003	7.94
8	Triton X100	0.003±0.010	0.35

**Readings are considered using a replica of 8 wells for each treatment, % is a percentage, µg/ml is microgram per milliliter, MS is a short form of microspheres*

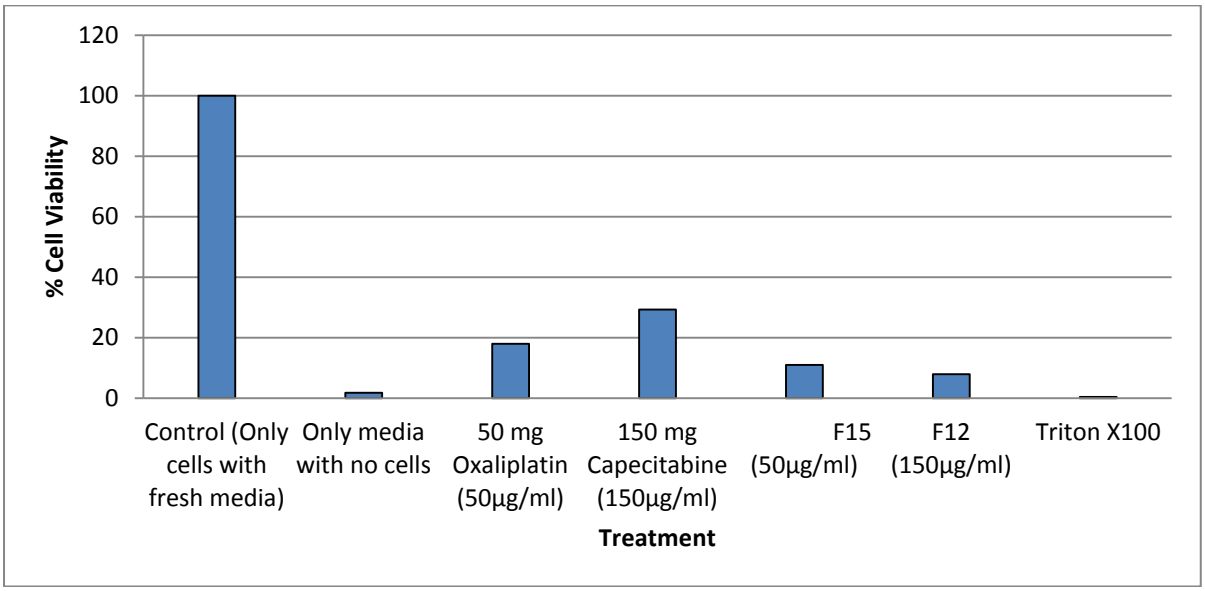

Fig. 10. Graph of % Viability V/S Treatment

4. CONCLUSION

The results of experiment show that the high % mucoadhesion is due to increase in drug: polymer ratio and decrease in % gluteraldehyde and no impact of inlet temperature on mucoadhesion. Increase % yield due to increase in inlet temperature and increase in drug: polymer ratio there is no more impact of amount of gluteraldehyde on % yield of prepared formulation. The mucoadhesion strength and *in-vitro* drug release were dependent on the concentration of polymer and cross- linking agents. Through the experiment optimized the final drug: polymer ratio, inlet temperature and amount of crosslinking agents. The mucoadhesive microspheres adhere to the chicken intestine mucosa for more extended period and it proven by *In-Vitro* Wash-off test for Microspheres. Based on formulation and evaluation parameters, have concluded that both Capecitabine and oxaliplatin mucoadhesive microspheres filled enteric-coated capsules have good effect on colon cancer than other single individual dosage form. Based on % yield, Entrapment efficiency, % mucoadhesion batch F12 of Capecitabine microspheres and batch F15 of Oxaliplatin microspheres was optimized batch. Capecitabine and Oxaliplatin mucoadhesive microspheres containing capsule was found to be suitable for colon cancer treatment. This oral formulation holds great potential for treating disease with patient compliance in combination form, more convenient, prolong the residence time. MTT Assay has been performed on Formulation of Capecitabine and Oxaliplatin Microspheres for assessing the % Viability of both the drugs on Caco-2 Cell Line. Comparison of API with formulation revealed that formulation produces less cell Viability as compared To Individual API. Finally it would conclude that the new

combination of oral formulation for Capecitabine and Oxaliplatin overcome the problem associated with side effect, parenteral route of administration, give cytotoxic effect. Two drugs administered in a single formulation hence patient compliance. The stability study indicates that formulation is fairly stable at storage conditions.

Apart from this, compared with available literature Goutam Kumar Jena et al. [13] that microspheres were successfully prepared and optimized with maximum drug entrapment and minimum particle size. The optimized microspheres coated with Eudragit S100, having 62.5% entrapment efficiency and 100% drug release in phosphate buffer pH 7.4 in 24 h. whilst prepared mucoadhesive Capecitabine and Oxaliplatin microsphere prepared by using spray drying technology with chitosan as polymer having more entrapment efficiency and lower the particle size.

Moreover available literature Aleksandra M et.al and Rudra P. et al. [9,25] they have designed a novel “particle in a particle” formulation where oxaliplatin was first loaded into nanoparticles composed of lipid like polymeric molecules which were later encapsulated in micro-sized alginate based particles. We believe that this combinatorial approach allowed for an improved and targeted delivery of the drug to the lower gastrointestinal tract where the tumor cells reside. This study helps to conclude oral delivery of oxaliplatin can provide good therapeutic effect.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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