PREPARATION AND EVALUATION OF 5-FLUOROURACIL SOLID DISPERSION FORMULATIONS FOR THERAPEUTIC MANAGEMENT OF COLORECTAL CANCER (CRC)

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ABSTRACT

Solid dispersion technology (SD) involves dispersion of poor water soluble active pharmaceutical ingredients (API) in a solubility enhancing polymer with the utmost goal of improving oral bioavailability of the API. This study is aimed at production and evaluation of SD formulations of 5-Fluorouracil (5-FU) for colon delivery using hot melt solid dispersion method (SD). The solid dispersions of 5-FU were prepared by hot melting method; The SD formulations were characterized using a scanning electron microscopy, USP dissolution apparatus type 2, and MTT assay. The SEM revealed that all SD formulations particles were in amorphous state, non-spherically shaped with size ranging between 98 -112µm. The FTIR spectral show no chemical interactions between the excipients and 5 FU. The yield (PY), drug entrapment efficiency (DEE) and the drug loading (DL) values were high enough to support commercial scale up of the technique. SD2 had the highest DEE, cumulative drug released and DL values. This may be responsible for the improve cytotoxicity against HT115 cells. The releases of 5 FU in all the formulations follow both Fickian’s and non-Fickian’s kinetics which are also pH responsive with no sign of dose dumping. This study demonstrated successful production of pH responsive 5 FU solid dispersions, by hot melt technique. The release follows Korymeyer–Peppas kinetic models and selectively delivered 5 FU to the colon which improved cytotoxicity activity against CRC.

KEYWORDS: SOLID DISPERSION, COLORECTAL CANCER AND 5-FLUOROURACIL

INTRODUCTION

The colon and rectum are jointly refers to as the large bowel, which is the last portion of the digestive system and its about 150 cm long. Development of cancer within this portion of the digestive track is generally referred to as colorectal cancer (CRC), [1]. The incidence of colorectal carcinomas varies significantly throughout the world, and it is adjudged the third leading cause of cancer-related deaths in men and women in the United States, and may be responsible for about 50,630 deaths in 2018, [2]. Environmental, nutrition and genetic are key player in CRC aetiology; genetic susceptibility ranges from well-defined inherited syndromes, e.g. familial adenomatous polyposis, to ill-defined familial aggregations, [3].

Recent data suggest two main pathways: a mutational pathway, which involves inactivation of tumor suppressor genes such as APC; and microsatellite instability which occurs in hereditary nonpolyposis colon cancer (HNPPCC) and a proportion of sporadic carcinomas, [4].

5-Fluorouracil (5FU), is a pyrimidine analog and an antimetabolite clinically useful in the treatment of various neoplastic diseases such CRC. In-vivo, 5FU is converted to its active metabolite 5-fluoroxyuridine monophosphate (F-UMP) which is incorporated into RNA synthesis and progressing by replacing uracil one of the four building block of RNA, thereby inhibiting cell growth. Similarly, 5FU
second active metabolite, 5-fluoro-2 deoxyuridine-5-O-monophosphate (F-dUMP) inhibit thymidylate synthesis resulting in depletion of thymidine triphosphate (TTP) one of the four nucleotide essential for DNA synthesis, [5].

5FU is widely used in the treatment of CRC alone or in combination with other treatment options such as, surgery and radiotherapy, [6,7]. However its only available as injectable solution due to its water poor solubility resulting on poor oral bioavailability. This study is aimed at formulating and evaluating a novel formulation of 5FU that is orally active and can achieved selective delivery of 5FU to the colorectal cells using solid dispersion technology. Solid dispersion technology (SD), entails uniform dispersion of poor water soluble drug (API) in biologically inert but hydrophilic polymer or mixture of polymers using various techniques such as hot melting, precipitation etc). The use of SD to achieved increased dissolution and excellent oral bioavailability of low aqueous soluble drugs (PWSD), abound in the literatures,[8,9]. It is however critical that the polymers to be used as carrier system in SD formulations must have good water solubility to be effective, since the dissolution rate of the drug is dependent on the dissolution rate of the carrier polymer. Therefore the success of SD formulation depends on judicious selection and fabrication of appropriate drug carriers, [10].

MATERIALS AND METHODS

Materials

5-Fluorouracil was obtained from Sigmal Chemical, Germany, Polyethylene glycol 4000 from LOBA Chemie and Ethyl Cellulose from Qualikems Laboratories.

Methods

<table>
<thead>
<tr>
<th>Table 1: Batch formulation of 5FU SD by hot melting technique</th>
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<tr>
<td>Ingredients</td>
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<td>-------------</td>
</tr>
<tr>
<td>5FU</td>
</tr>
<tr>
<td>PEG 4000</td>
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<tr>
<td>ETHOCEL</td>
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</table>

SD Yield

The amount of SD obtained from each batch was used to calculate the percentage yield of the microsphere SD using equation 1.

Microsphere SD Yield = \( \frac{XF}{XI} \times 100 \) Eqn.1

Where XF = weight of obtained SD and XI total weight of all starting materials (drug + polymers)
Morphological study

The surface morphology of the microspheres was observed by SEM (JEOL model JSM840A, Japan). The SD formulations were deposited on carbon grid and sample sputtered with gold and acceleration voltage adjusted to about 10 kV, images of microspheres were taken by random scanning of the stub.

Determination of drug loading & entrapment efficiency

The amount of 5 FU loaded within each formulation was determined with the aid of a UV/VIS spectrometer operating at 266 nm (Beckman 220 Instruments, Fullerton, CA, USA). Known quantity of SD was weighed and transferred into a 100 ml volumetric flask containing 50 ml phosphate buffer solution (PBS, pH 7.4). To ensure complete solubility of the drug, the dispersion of SD in PBS was sonicated for about 120 seconds using a probe sonicator (PCI Analytics, Mumbai India). The volume was made up to 100 ml with PBS, before filtering and analyzing for 5 FU content. The drug loading and the drug entrapment efficiency were calculated from equation 2 & 3 below:

Drug loading (% W/W) = \( \frac{\text{amount of drug in SD}}{\text{weight of SD}} \times 100 \) …Eqn 2.

Drug entrapment efficiency = \( \frac{\text{Drug estimate in SD}}{\text{Initial weight of drug used to prepared SD}} \times 100 \) .... Eqn. 3

In-vitro drug release studies

The amount of 5FU release per time were determined in two different pH conditions; simulated gastric fluids pH 4.5 and simulated intestinal fluid pH 7.5 maintained at 37 ± 0.5°C. Korsmeyer – peppas model, [12] (log cumulative percentage of drug released vs. log). The R² values for each mathematical model were calculated.

Drug release kinetic and mechanism studies

Data obtained from dissolution studies were fitted to various kinetic equations. The kinetic models used were zero order (cumulative percentage of drug unreleased vs. time in min), first order (log cumulative percentage of drug remaining vs. time), Higuchi’s model, [11] (cumulative percentage of drug released vs. square root of time) and Korsmeyer – peppas model, [12] (log cumulative percentage of drug released vs. log). The R² values for each mathematical model were calculated.

Drug stability studies

Stability of all batches of SD were assessed by storing the formulations at 45 ± 2 °C and 75 ± 5% RH for 45 days. At the end of study period, formulations were evaluated for physical change, drug content and percentage cumulative drug released after 12 hours (PCDR _12_).

Methylthiazolyldiphenyl-tetrazolium bromide (MTT) Cell proliferation assay

The assay is based on non-radioactive fluorescent of 3-(4, 5- Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) for quantitative determination cell death as a result of cytotoxicity of the administer formulations. The ability of the cancer cell line (HT115) to produce oxidoreductase enzymes reflects the number of viable cells. Under test conditions, MTT is reduced to formaza, which is purple colour fluorescent which is then analyzed calorimetrically, [13, 14 &15]. A 96 well plate was utilized with HT115 cell line grown to about 75-80% confluence in Dulbecco’s modified eagle’s medium (DMEM) containing 2mM of glutamine and 15% of Foetal Bovine Serum (FBS) maintained at 37°C in a humidified CO₂ incubator (Model MCO-15AC; Sanyo Electric Biomedical Co. Ltd., Osaka, Japan. The cells were plated at a density of 4×10³ cells/well (optimal seeding density) and after 12 hours of incubation, the medium in the wells was replaced with fresh medium containing prepared 5-FU SD formulations. At about 48 hours of incubation, MTT dye solution was added to each well and the incubation was continued for another 4 hours. The medium in each well containing unbound MTT and death cells was removed by suction. The formazan crystals were solubilized with 100 µL dimethylsulfoxide (DMSO) and the solution vigorously mixed to dissolve the reacted dye. The absorbance of each well was determined by reading the, optical density (OD) values at 595nm.
using DMSO as a blank. A plot of cell viability against the concentration was constructed and the concentration required for a 50% inhibition of viability (IC\textsubscript{50}) was determined from graphically.

**Statistical Analysis**

All determinations were conducted in triplicate; data are presented as the average mean ± standard deviation. The significance of the difference between treatment groups was evaluated using unpaired Student's two-tailed t-test. P≤0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

The use of Fourier transform infrared spectroscopy to study possible chemical interactions between drugs and excipients pre date this present study, [15,16, & 17]. The FTIR spectra of pure PEG4000, ETHOCEL, and 5FU are as presented in Fig.1.

![Figure 1: FTIR spectral of, A= ETHOCEL, B= 5 FU, C = PEG4000 and D =SD formulation](image)

The FTIR spectra exhibited characteristic peaks indicating the purity of the samples used. Comparative analysis of FTIR peaks of 5FU with that of SD formulation shows no new additional
peaks and no observed disappearance of any of the peaks. The minor shifting of some peaks observed may be due to physical interactions such as hydrogen bonding between the hydrogen atom of the CH$_3$ of the 5 FU and one of the ion pairs of the oxygen atom in PEG 4000. ETHOCEL and PEG 4000 are therefore useful in the construction of 5 FU SD formulations since there are no chemical interactions between the drug and the excipients. The yields and the loading capacities of SD formulations were high enough to support commercial production, (table 2). Drug entrapment efficiency (DEE) is used to adjudge the intrinsic ability of polymer or polymer mixture to effectively incorporate drug molecule within its structure. DEE was high enough to support its use as drug delivery vehicle without dose dumping as low DEE values are associated with indiscriminate dose dumping, some adverse drug reaction and unachieved therapeutic goals, [18,19]. SD2 had the highest DEE, and this may be responsible for its corresponding improved cytotoxic activity against HT115 cell line. The scanned microscopy (figure 2) shows that the all the SD formulations are rod like shaped and are in amorphous state with no well-defined lattices structure and shape. Drugs in amorphous state had been reported to exhibit better dissolution profile compared to those in crystalline states, [20, 21]. Four mathematical models, each describing the release mechanism of the entrapped drugs were used to evaluate the in-vitro release data. Model with the highest co-efficiency of variance (R$^2$) value is considered to be the predominant mechanism of release of the drug from the formulation, [22]. Higuchi equation relates diffusion release mechanism which is the case with most swellable polymers, [11, 23], while, Korsmeyer – peppas equation is an expression of both Fickian and non-Fickian drug released kinetics from swelling and non-swelling polymers, [12, 24]. The R$^2$ values for the formulations are presented in table 3. Korsmeyer –Peppas values were highest in all formulations of SD, which shows the release of 5 FU was through both Fickian and non-Fickian mechanisms.

Table 2: Some SD Formulations Parameters

<table>
<thead>
<tr>
<th>Code</th>
<th>DEE (%)</th>
<th>DLC (%)</th>
<th>PY (%)</th>
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<tbody>
<tr>
<td>SD1</td>
<td>94.7</td>
<td>82.7</td>
<td>89.1</td>
</tr>
<tr>
<td>SD2</td>
<td>99.0</td>
<td>88.9</td>
<td>89.2</td>
</tr>
<tr>
<td>SD3</td>
<td>95.3</td>
<td>82.7</td>
<td>89.2</td>
</tr>
<tr>
<td>SD4</td>
<td>95.6</td>
<td>82.7</td>
<td>89.2</td>
</tr>
</tbody>
</table>

Keys: DEE = Drug Entrapment Efficiency, DL = Drug Loading, PY = Percentage Yield

Table 3: R$^2$ values for SD formulations

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Zero order ($R^2$)</th>
<th>First order ($R^2$)</th>
<th>Higuchi order ($R^2$)</th>
<th>Korsmeyer – peppas ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1</td>
<td>0.88</td>
<td>0.89</td>
<td>0.92</td>
<td>0.99</td>
</tr>
<tr>
<td>SD2</td>
<td>0.89</td>
<td>0.89</td>
<td>0.92</td>
<td>0.99</td>
</tr>
<tr>
<td>SD3</td>
<td>0.91</td>
<td>0.91</td>
<td>0.96</td>
<td>0.98</td>
</tr>
<tr>
<td>SD4</td>
<td>0.91</td>
<td>0.90</td>
<td>0.96</td>
<td>0.99</td>
</tr>
</tbody>
</table>

The released of 5 FU in both SIF and SGF were as presented in figure 3 (a & b). There were marks significant differences (p ≤ 0.05) in the amount of 5 FU released in both media. The maximum cumulative released in SGF (pH 1.2) was 6.6 % while that of SIF was 98.7 %.

The accelerated stability data for SD formulations were presented in table 4. The physical attributes remain unchanged and there were no significant changes in the drug contents and percentage cumulative drug released at 12 hours (PCDR$_{12}$). The stability success may be attributed to polymers type rather than the ratio of the polymer blend.
Colon drug delivery systems such as SD formulations under current investigation are developed to preferentially release the active drugs at the colon of which various formulation techniques like using natural and synthetic polymers that interact with one or more aspects of gastrointestinal (GI) physiology to effectively release the drug molecule. Some formulations however utilized the differences in the pH along the GI tract (pH sensitive) to affect the release of the drug preferentially in the colon, [25]. The presence of colonic microflora, and enzymes, may also be used to achieve colon delivery of some pharmaceutical products, [26, 27]. All SD formulations show increased cumulative drug release in SIF compared to their releases in SGF, Figure 3 (a & b).
This selective drug release in SIF is significantly important, (p ≤ 0.05), and it’s one major goal of this present study. The pH responsive release will offer targeted colon delivery of 5 FU, increased availability of 5 FU at the colon for effective uptake by the tumor cells and ultimately reduces the clinically observed adverse drug reaction mostly due to indiscriminate distribution of 5 FU after parenteral administration. The ranking order for cumulative drug released in SIF was, SD2 ≥ SD3 ≥SD4 ≥SD1 implying that SD2 with drug, polymer ratio of 1:2 achieving highest in-vitro release and dissolution profile which may be as a result of it highest values of drug entrapment and drug loading capacity, table2.

MTX (mixture of 5FU and excipients) shows significantly low cytotoxic activity at all concentrations when compared to the SD formulations, (figure 4) (p ≤ 0.05). The inhibition of HT115 cell line is dose dependent with more cell deaths with increase concentrations of 5 FU. SD2 formulation shows most remarkable cell deaths at all concentrations, (figure 3) this may be connected to its remarkable highest cumulative drug release, DEE and DLC values. Similar dose dependent cytotoxicity actives were reported for Cisplatin loaded liposome, [28]. Tamoxifen nanoparticle, [29] and Fulvestrant liposomes, [15]

CONCLUSION

Targeted delivery of 5FU is achieved through SD formulations development by hot melt technique. This novel colon delivery is pH sensitive and the drug released is in a sustained manner without dose dumping. The ratios of the drug to polymer blend significantly affect the cumulative amount of drug going into solution. The improved cumulative drug released obtained in SD2 invariably contributed to its highest cytotoxic activities. Data obtained also suggested that the formulations are expected to retain and remain stable throughout the shelf life.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

We the authors declared no conflict of interest in this investigation

REFERENCES


