

Preparation and Evaluation of 5-Fluorouracil Loaded Microsponges for Treatment of Colon Cancer

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Abstract

5-Fluorouracil (5-FU) has a wide anticancer activity versus several types of solid tumors. The activity of 5-FU can be improved and its toxicity can be diminished by enhancing the relative specific accumulation in the tumor regions. The aim of this work was to develop Eudragit RS100 based 5-FU microsponges (MS) for treatment of colon cancer. Oil in oil emulsion solvent diffusion method was used for the preparation of 5-FU sustained release Eudragit RS100 MS. MS were characterized for their encapsulation efficiency, production yield, drug polymer interaction and drug release profiles. Shape, surface morphology visualized by scanning electron microscope (SEM) and particle size of MS was investigated using laser light scattering technique and. Eventually, HCT 116 and CACO2 cell lines were used for determination of cell viability by MTT assay. The results showed that all prepared MS were spherical in shape with several pores on their surfaces. The production yield was in (62.76% ± 1.06% and 93.80% ± 1.75%), encapsulation efficiency was in (71.80% ± 1.62% and 101.3% ± 2.60%) and particle size was in (53.11 μm ± 41.03 nm and 118.12 μm ± 48.21 nm). Fourier transform infrared revealed that there is no chemical interaction between 5-FU and Eudragit RS100. MS loaded 5-FU was more effective than 5-FU itself as shown by cell viability assay. The results demonstrated that 5-FU with Eudragit RS100 was successfully formulated as sustained release manner and could be a substitution delivery method of 5-FU for oral anticancer treatment.

Keywords: 5-Fluorouracil; Microsponges; Eudragit RS100; Cell viability; HCT116 and CACO-2 cell lines

Introduction

Nowadays, chemotherapy, radiation, and surgery are classical treatment methods for cancer, but they have stark mental and biochemical side effects which predominantly destroy the healthy cells of patients. 5-FU is one of the most potent chemotherapeutic drugs which is efficient against several types of cancer such as skin cancer, breast cancer, pancreatic cancer, stomach cancer and is more effective in the curing of colorectal cancer [1,2]. 5-FU is a fluorinated pyrimidine antimetabolite converted within the cells into 5-fluorodeoxyuridine monophosphate which constrains the synthesis of DNA by inhibiting the cellular enzyme thymidylate synthetase leading to blocking the conversion of deoxyuridine acid to thymidine [3,4].

The key challenge related to the oral administration of 5-FU is the unconventional oral bioavailability due to which the intravenous administration of this drug is truly in clinical usage. However, intravenous administration leads to the quick distribution and elimination of 5-FU with an instant half-life of 8-20 minutes [5]. It was reported that 5-FU rapidly cleared from the plasma due to its rapid metabolism with a mean half-life 6.3 minutes and with regular peak plasma at 13.4 μg/mL. Whereas oral administration of 5-FU had a normal peak plasma level of 8.3 μg/ml which happened 10-20 minutes post-dosing with a short half-life of 7.2 minutes on account of the extreme first pass catabolism [6]. Moreover, dihydropyrimidine dehydrogenase enzyme which accountable to catabolism of 5-FU in the liver is furthermore found in high concentration in the gastrointestinal tract epithelium, while is not found in colon carcinomas, which enhance the susceptibility of colon cancers to 5-FU [7]. Also 5-FU can be PEGylated using short or long chain poly ethylene glycol, this idea depended on results obtained in our lab after PEGylation of octreotide [8]. Notwithstanding, the oral administration of 5-FU has a great variability in plasma levels which

represent an important factor to oral as compared to administration intravenously as reported previously [9].

Conversely, intravenous administration of 5-FU yields severe systemic toxic effects of gastrointestinal (GIT), blood complaints, and skin diseases, due to the 5-FU cytotoxicity on healthful human cells. Hence, the need to formulate targeted delivery of 5-FU through oral route would not only diminish the exposure of healthy cells to the drug but also provide an efficient and harmless curative effect for colon cancer with a lower dose and reduced duration of therapy [5]. It was reported that the therapeutic effect of 5-FU can be augmented and its harmful effects can be suppressed by the precise accumulation of the anticancer agent in the tumor regions with continued exposure of the cells to this drug [10]. To extend the exposure time of cancer cells to 5-FU, the drug delivery was formulated by incorporation into microsphere formulation.

Microsponges (MS) are micro-sized particles with high porosity and unique ability for encapsulation of a wide multiplicity of pharmaceutical active ingredients. MS delivery system is used to target and modify the release of active ingredients from pharmaceutical formulations, enhance the stability and decrease the side effects [11]. MS have high load activity reach up to 90% entrapment efficiency [12], self-sterilizing

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with average pore size of 0.25 μm where bacteria cannot penetrate [13] and they release the drugs in a sustained manner [13-15].

MS may efficiently be taken up by the macrophages in the colon, thus showed effective localized drug action at the site of action [5]. These characteristics make MS more advantageous over many drug delivery systems such as microcapsules and liposomes [14-16]. Moreover, targeting can be improved by altering the physicochemical properties such as size of the formulated NPs [17-21] or by attaching targeting ligand *in vitro* as well as *in vivo* [22-26]. Furthermore, the formulated NPs need to be identified using different analytical methods which are a novel approach for cancer targeting [20,25,27]. It was reported that a dual-sensitive core/shell hybrid nanospheres, consisting of gold nanorods (GNR) as the core and poly (N-isopropyl acrylamide-co-methacrylic acid) as the shell were synthesized. The resulted nanospheres revealed significant applications of in controlled release of anticancer agents and have a synergistic end product of chem-photothermal therapy [27]. Silver and quantum dots hybrids microspheres were developed as a novel near-infrared photoluminescence (NIR PL) which showed highly selective and sensitive NIR PL responses to CA125 antigen, over other potential interferences [28,29].

This manuscript studied and developed new formula novel 5-FU loaded microsponges by Oil in Oil emulsion solvent diffusion method. These 5-FU loaded microsponges can be incorporated in specific formulations as colon targeting for treatment of colon cancer. These prepared 5-FU loaded microsponges are more effective than 5-FU on the cell lines. Our study depended on the formulation of 5-FU as MS in the form of Oil/Oil emulsion method to enhance the entrapping efficiency of more than 95%.

The purpose of the present study was to prepare Eudragit RS 100-MS containing 5-FU using an emulsion solvent diffusion method for colon cancer treatment. The study investigated the effect of D:P ratio, magnesium stearate: solvent ratio and solvent: polymer ratio on the physicochemical characteristics of the prepared MS. The formulated MS were characterized for the particle sizes, entrapment efficiency and *in vitro* release. By comparing the *in vitro* release rate of 5-FU from MS, the best formula to be incorporated in colon-specific formulations has been selected for colon cancer targeting. Furthermore, the formulated MS were incubated with cell lines (HCT16 and CACO2) for determination of the internalization of 5-FU and for studying the toxicity of the formulations on the cell lines of colon cancer.

Materials and Methods

Materials

5-Fluorouracil was purchased from, Applichem for pharmaceuticals co., (Gmbh, Germany). Eudragit RS 100, phosphate buffer saline (PBS) MTT stain and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, USA). Light liquid paraffin and Magnesium stearate were purchased from El-Gomhoria Chem. Co. (Cairo, Egypt). Acetone, hydrochloric acid, orthophosphoric acid potassium dihydrogen phosphate, n-hexane and disodium hydrogen phosphate, were purchased from El-Nasr Chemical Co. (Cairo, Egypt). Fetal bovine serum (FBS), DMEM media, and penicillin/streptomycin (PS) were purchased from Gibco (USA). Human colorectal cancer cells CACO-2 and human colon carcinoma cells HCT116 were purchased from VACSERA CO., Dukki, Egypt. The refined water utilized for all experiments was obtained using a distilled water purification system from double steam distillation device (Gesellschaft für GmbH, Germany). All glasswares were thoroughly washed with freshly

prepared aqua regia, extensively rinsed with water several times and oven-dried at 50°C before use.

Preparation of Microsponges

5-FU-MS were prepared using oil in oil emulsion solvent diffusion technique [12]. Eudragit RS 100 was dissolved in acetone, once a clear solution was obtained, 500 mg of 5-FU was added in addition to magnesium stearate (3% w/v solvent) and the whole mixture was kept in the ultrasonic bath adjusted at a 90-kHz frequency for 5 minutes to obtain homogenous dispersion as shown in Table 1. Then the mixture was poured into 150 ml of liquid paraffin formerly cooled to 10°C \pm 0.5°C while the use of mechanical stirrer for 45 minutes. The oil in oil emulsion formed was progressively warmed to 35°C \pm 5°C and was stirred at this temperature for another 30 minutes. During this period of time, the acetone was entirely removed by diffusion into liquid paraffin and evaporated through the air/liquid interface. The solidified MS were purified, washed five times with 60 ml of n-hexane, dried at room temperature for 12 hours and stored in a desiccator for further investigations (Figure 1).

Variation of preparation parameters

Many parameters were taken into consideration such as drug-polymer ratio, polymer-solvent ratio, and magnesium stearate-solvent ratio. Three D:P ratios, 1:1, 1:2, and 1:3 were investigated in the preparations F1, F2, and F3, respectively. Two other concentrations of magnesium stearate were studied, 1.5% w/v used in formulations F4, F6 and 5% w/v in F5 and F7. The polymer/solvent ratio initially used in the preparation of MS was 1:5. However, this ratio was altered to 1:10 in formulations F8, F10 and 1:20 in formulations F9, F11, (Table 1).

Determination of mean particle size

The particle size studies were carried out using laser light scattering technique (Horiba LA-300 Light Scattering Particle Size Distribution Analyzer, Kyoto, Japan). The aqueous microsponges' suspension was first put into the sample chamber equipped with an ultrasonic probe to aid in the dispersion of cohesive particles. The MS was continuously re-circulated from the sample chamber via the sample flow cell. The achieved uniform dispersion was examined to measure particle size distribution. Particle size distribution was analyzed using the inbuilt software. Each sample was measured at three times and the average particle size was calculated as mean diameter [13,16,17].

Determination of encapsulation efficiency (EE)

The EE percent was determined by accurate weighing a quantity of

Preparations (F)	Eudragit RS 100 (g)	Acetone (ml)	D:P ratio	P:S ratio	% w/v Mg stearate ratio
F1	0.5	2.5	01:01	01:05	3
F2	1	5	01:02	01:05	3
F3	1.5	7.5	01:03	01:05	3
F4	0.5	2.5	01:01	01:05	1.5
F5	0.5	2.5	01:01	01:05	5
F6	1	5	01:02	01:05	1.5
F7	1	5	01:02	01:05	5
F8	0.5	5	01:01	01:10	3
F9	0.5	10	01:01	01:20	3
F10	1	10	01:02	01:10	3
F11	1	20	01:02	01:20	3

Table 1: The composition of different MS prepared by O/O emulsion solvent diffusion method.

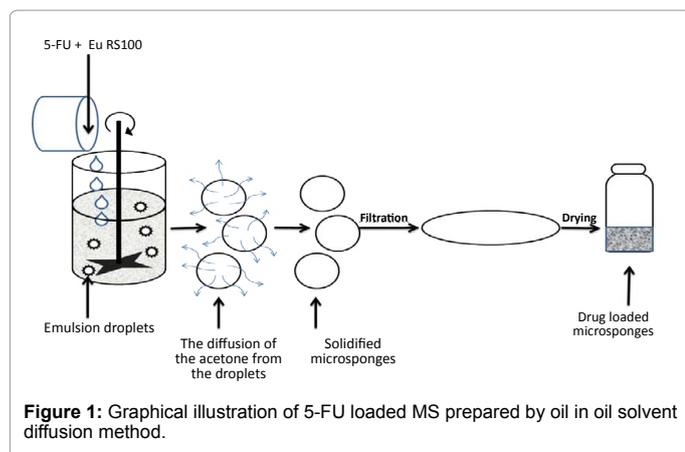


Figure 1: Graphical illustration of 5-FU loaded MS prepared by oil in oil solvent diffusion method.

MS equivalent to 10 mg of 5-FU. Drug concentration was determined spectrophotometrically using (UV-1601 Shimadzu Co. Kyoto, Japan) [30]. 5-FU is sparingly soluble in water (12 mg/ml), so 10 mg of drug (5-FU) equivalent of microsponges was crushed carefully in a glass mortar and transferred to a 100 ml volumetric flask using phosphate buffer pH 6.8. The volumetric flask was completed to the volume with phosphate buffer pH 6.8 then agitated in ultrasonic bath for 1 hour. The sample was filtered and the drug concentration was determined spectrophotometrically at 267 nm using phosphate buffer pH 6.8 as a blank. The concentration was calculated using the standard calibration curve of 5-FU in phosphate buffer pH 6.8 and EE% was calculated using the following equation:

$$EE\% = \frac{\text{Actual amount of drug}}{\text{Theoretical amount of drug}} \times 100$$

Eudragit RS 100 and magnesium stearate are water insoluble so these is no interference of the excipients.

Infrared Fourier transform spectroscopy analysis (FTIR)

5-FU, Eudragit RS 100, 5-FU-Eudragit RS100 physical mixture and optimized formula (F3) were subjected to infrared (IR) analysis, the samples were scanned in the range from 400 cm^{-1} to 4000 cm^{-1} and recorded on an FTIR (SSP-10 A Shimadzu Co., Japan), using the KBr disk technique [30].

Scanning electron microscopy

Scanning electron microscope (SEM) was used for investigation of the shape and surface morphology of MS. The MS were fixed with carbon-Glue and covered regularly with gold palladium within argon atmosphere. Samples were then investigated with a JEM-100 S, (Japan), scanning electron microscope [12].

In vitro drug release study

USP type II dissolution test apparatus (Paddle) was used for *in vitro* dissolution studies of 5-FU-MS. 50 mg of the 5-FU equivalent of MS was placed in 900 ml phosphate buffer (pH 6.8) stirred at 50 rpm and the temperature was kept at $37^\circ\text{C} \pm 0.5^\circ\text{C}$ [31]. At predetermined time interval, 5 ml samples were pulled out and substituted with an equal volume of fresh buffer. The concentration in the pulled out samples was determined spectrophotometrically at 267 nm. Each sample was implemented in three times and expressed as mean values \pm standard deviation.

Kinetic data

The dissolution profile of each formulation was fitted to various models such as zero order kinetics, first order kinetics, Higuchi, and Korsmeyer-Peppas to assess the kinetics of drug release from prepared 5-FU-MS [32].

Cell culture

Human colorectal cancer cells CACO-2 and human colon carcinoma cells HCT116 were maintained in DMEM cell culture media containing 10% FBS and 1% penicillin/streptomycin. Cells were grown at 37°C in a 5% CO_2 humidified atmosphere. The effect of the F2, F3, and 5-FU on cell viability was investigated using MTT cell viability assay [33]. The principles of MTT assay are based on the cleavage of the insoluble tetrazolium salt to a detectable colored formazan product by succinate-tetrazolium reductase enzyme; that exists in the mitochondria and it is only active in the viable cells. The produced insoluble formazan is directly proportional to the activity of the mitochondrial dehydrogenase enzyme and consequently to the portion of the viable cells. The intracellular formazan dye resulting from the metabolically active cells can be solubilized and quantified by measuring its absorbance using ELISA reader [34]. Briefly, the cells were cultivated in 96-well plates (5×10^3 / well) in 100 μl of DMEM media. On the following day, the experimental media F2, F3, and 5-FU were added and then incubated for 24 hours. After addition of fifty μl of MTT solution (2 mg/ml in PBS), every well was incubated for 4 hours. Each well were received 150 μl of DMSO, after, careful removal of the media, and then after shaking the absorbance was measured at 570 nm using an ELISA reader (Molecular Devices, Sunnyvale, USA).

Results and Discussion

Spherical, uniform, regular and free flowing MS were successfully prepared by oil in oil emulsion solvent diffusion method. Investigations proved that, when the drug to polymer ratio was increased, the entrapment efficiency was increased due to decrease the total drug lost at the steps of the fabrication process. The fact that more amount of drug was present per unit polymer so F1 (1:1, drug to polymer ratio) has higher drug content than F3 (1:3, drug to polymer ratio). The production yield was very high due to the existence of the nonpolar liquid paraffin in the extrinsic phase which inhibits the escape of ingredients used in the formulation of MS [35].

Magnesium stearate was used as stabilizer to overcome the problem of coalescence flocculation of particles during solvent diffusion by the reduction of the phase tension between the formed microsponges and liquid paraffin [36,37]. The influence of Mg stearate concentration (1.5, 3 and 5% w/v) on the characteristics of the prepared MS showed, that low concentration (1.5% w/v) produced aggregated MS. The microsponges obtained by using medium concentration of magnesium stearate (3% w/v) were spherical in shape, uniform, regular and free flowing. However, increasing magnesium stearate concentration to 5% led to neutralization the electric charge of Eudragit RS-100 due to assembling of magnesium stearate on the surface of MS that produced aggregation of MS [38]. It has been reported that magnesium stearate reduced the interfacial tension and prevented the electrical charges and flocculation during the preparation of microsponges in general [12]. The variation of the magnesium stearate concentration (1.5, 3 and 5%) in the formulations did not have any influence on the release profiles of the drug.

The decrease in polymer/solvent ratio resulted in a significant decrease in the particle size of prepared MS. This observation was

imputed to the fact which when the dispersed phase with higher viscosity poured into the dispersion medium of high viscosity (liquid paraffin), the emulsion was barely broken into small droplets, and the larger ones were formed. It was reported that the particle size of microparticles was proportional to the dispersed phase viscosities. In such a way, increasing the dispersed phase viscosity resulted in increasing the particle size of microparticles (Table 2) [26-28]. 5-FU were prepared previously by another group as MS [39], using the technique W/O/W emulsification method. This method showed a good formulation but with lower% EE, which showed maximum% EE of about 59%. This is due to the aqueous solubility of 5-FU, 5-FU has a certain solubility of around 12 mg/ml water, these in turn make the loaded 5-FU microsponges prepared oil in water emulsion contained very low drug content as 5-FU escaped to aqueous external phase. Our study showed that the formulation of 5-FU as MS in the form of oil/oil emulsion method has a higher% EE of more than 95%, this is due to the insolubility of 5-FU in external phase, this make our formula has a better% EE than the other one.

The results showed that the increase in polymer concentration resulted in increasing the particle size of MS. When acetone diffuses out, almost the dispersed phase is turned into solid MS and separated particles appear. Therefore, in high drug to polymer ratios, less polymer amounts surrounded the drug and decreased the particle sizes of MS. When the dispersed phase with elevated viscosity teemed into the dispersion medium bigger droplets were formed and mean particle sizes increased [40].

Infrared Fourier transform spectroscopy analysis (FTIR)

FT-IR spectroscopy is an indication of the functional groups in the scanned samples either of the polymers or drug. Since IR spectroscopy measures the sample's absorbance spectrums rapidly and directly with a controllable sampling depth and with little or no preparation of the sample [30-34]. For this reason, FT-IR was applied to confirm the compatibility of all used additives with 5-FU. FTIR spectrum of 5-FU showed a distinctive peak appear in 3000 cm^{-1} to 2900 cm^{-1} ; represents C-H stretching, bands in the region 1429 cm^{-1} to 1660 cm^{-1} corresponding to the C=C and C=N ring vibrations. The peaks at about 1348 cm^{-1} due to pyrimidine compound vibration. The peaks at 1180 cm^{-1} and 1246 cm^{-1} corresponded to the C-O and C-N vibrations, respectively [41]. Eudragit RS 100 showed a C=O stretching band around 1726 cm^{-1} [42]. The FTIR scanning of the physical mixture of 5-FU with Eudragit RS 100 and the selected formula (F3) showed the characteristic fingerprint of both drug and polymer. These observations explained that there is no chemical interaction or changes took place during the mixing formulation of MS with added materials (Figure 2).

Formula No.	% Production Yield \pm SD	% EE \pm SD	Mean particle size (μm) \pm SD (nm)
F1	87.20 \pm 2.10	95.44 \pm 2.67	67.05 \pm 24.74
F2	87.70 \pm 3.22	91.18 \pm 0.19	92.57 \pm 29.06
F3	92.21 \pm 1.78	88.24 \pm 1.22	118.12 \pm 48.21
F4	76.50 \pm 2.26	101.90 \pm 2.60	80.50 \pm 36.08
F5	62.76 \pm 1.06	97.90 \pm 2.98	68.00 \pm 39.85
F6	82.47 \pm 1.80	93.00 \pm 3.07	103.10 \pm 29.95
F7	89.80 \pm 2.00	90.76 \pm 1.05	91.80 \pm 32.75
F8	90.00 \pm 3.01	90.90 \pm 4.74	60.40 \pm 38.33
F9	87.10 \pm 2.14	77.80 \pm 0.19	53.11 \pm 41.03
F10	91.50 \pm 0.94	74.80 \pm 2.67	89.80 \pm 30.09
F11	93.80 \pm 1.75	74.58 \pm 1.62	85.40 \pm 34.00

Table 2: Production yield, percentage recovery (drug content) of different Microsponges and mean particle size.

Scanning electron microscopy (SEM)

The scanning electron images of the selected MS (F3) was observed by SEM analysis that, the MS were finely spherical and uniform (Figure 3a). More detailed image indicated several pores on the MS surfaces (Figure 3b). The numerous pores present in microsponges surface were induced by the diffusion of the solvent from the emulsion droplets during the formation of the MS [43]. These pores enable the dissolution medium to penetrate the into MS to dissolve and release the entrapped drug from microsponges [44].

In vitro release study

In vitro release of pure 5-FU and different 5-FU-MS is illustrated in Figure 4. The release of 5-FU from MS was characterized by an immediate burst release followed by a moderately slow release. The initial rapid release is attributed to the dissolution of drug adsorbed in microspunge's surfaces. The later relatively slow release rate could be attributed to release and diffusion of the drug from the interior of MS. The drug release was characterized by biphasic pattern matrix diffusion kinetics [45]. Primarily, diffusion of the aqueous dissolution medium into pores of MS results in dissolution of the drug. After that, diffusion of the drug through the microsponges pores into the dissolution medium [44]. The result obtained from Figure 4 showed that the *in vitro* release of 5-FU from the prepared MS was retarded with increasing the polymer/solvent ratio. This due to faster precipitation of the polymer around the drug in higher polymer/solvent concentrations. The fast polymer precipitation due to rapid acetone evaporation led to higher drug content and decreases the diffusion of the drug in the external phase identical results were gained by Kondo et al. [46]. The low drug release rate could be also attributed to the decrease in a number of pores at MS surface at a higher polymer to drug ratios or an increase of internal phase viscosity [47]. The delay in drug release also could be imputed to an increase in the diffusional path length that the drug has to overpass.

Kinetic analysis of release profiles

The release patterns were fitted on various kinetic models such zero, first, Higuchi and Korsmeyer-Peppas. The correlation coefficient of the straight line that has the highest value was used to compare between different models. Table 3 listed the cor-relation coefficients (r) of all formulated MS. The obtained data revealed that the release profiles of formulae from F1 to F9 follow Higuchi diffusion model while release profiles of formulae of F10 and F11 follow Peppas diffusion model. The diffusion mechanism of drug release was due to the presence of porous structure on the surface of MS which enables easy penetration of the release media that dissolves the 5-FU and it diffuses through the diffusion paths (pores).

Cellular toxicity

IC_{50} values are very dependent on the stipulations under which they are measured. Generally, the concentration of inhibitor is higher; the more drug activity will be lowered. IC_{50} value increases as 5-FU concentration increases. Furthermore depending on the type of inhibition other agents may affect IC_{50} value; for ATP-dependent enzymes, IC_{50} value has an interdependency with the concentration of ATP. IC_{50} values were used for comparison of the potency between two drugs [48]. The IC_{50} of a drug can be determined by building up a dose-response curve and inspecting the effect of different concentrations of the formula on contrary 5-FU activity. IC_{50} values can be calculated for a certain new formula by measuring the concentration that needs to inhibition of half of the maximum biological response of the 5-FU [48].

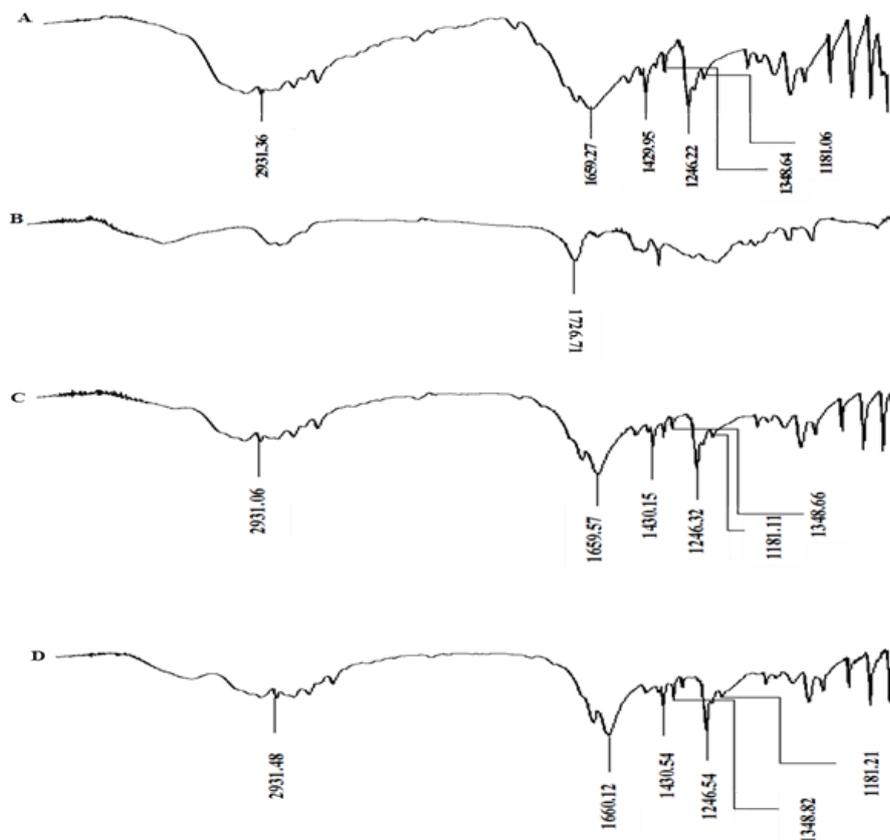


Figure 2: FTIR spectroscopy of (A) 5-FU, (B) Eudragit RS 100, (C) 5-FU_Eudragit RS 100 in physical mixture and (D) F3 (1:3, drug to polymer ratio).

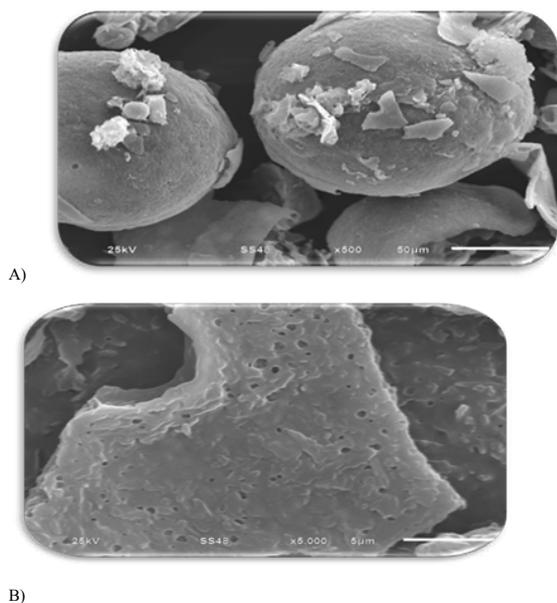


Figure 3: Scanning electron micrographs of 5-Fluorouracil-Eudragit RS 100 microsponges (F3), a) the whole microsponges, b) high magnification power of microsponges showed the characteristic pores.

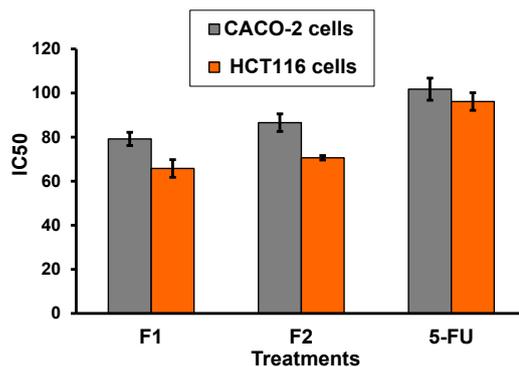


Figure 4: IC₅₀ of different formulae on CACO2 and HCT116 cell lines.

The effect of formulations F3 and F2 compared to the effect of the drug (5-FU) on human colorectal cancer cells CACO-2 and HCT116 was investigated by MTT assay and the results (Figure 5). The cell viability experiment was designed as a tool for evaluation the efficacy of the different concentrations of drug samples used in the current research. The control sample was already used to calculate the IC₅₀ but not included in the histogram, as this control cell showed no data. For more confirmation the experiments were repeated 5 times. MTT

Correlation Coefficient	Formula No.											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	
Zero	0.71	0.8	0.9	0.567	0.656	0.878	0.87	0.743	0.771	0.713	0.832	
First	0.93	1	1	0.859	0.957	0.988	0.99	0.97	0.97	0.944	0.965	
Higushi diffusion	0.94	1	1	0.929	0.97	0.99	0.99	0.984	0.987	0.938	0.955	
Korsemey-er-Peppas	0.94	0.9	0.9	0.923	0.949	0.975	0.97	0.952	0.957	0.959	0.985	
Order	Diffusion model								Kors.-Pepp			

Table 3: The kinetics parameter correlation coefficient (r) of zero, first, Higuchi and Peppas models for different microsponges formulations.

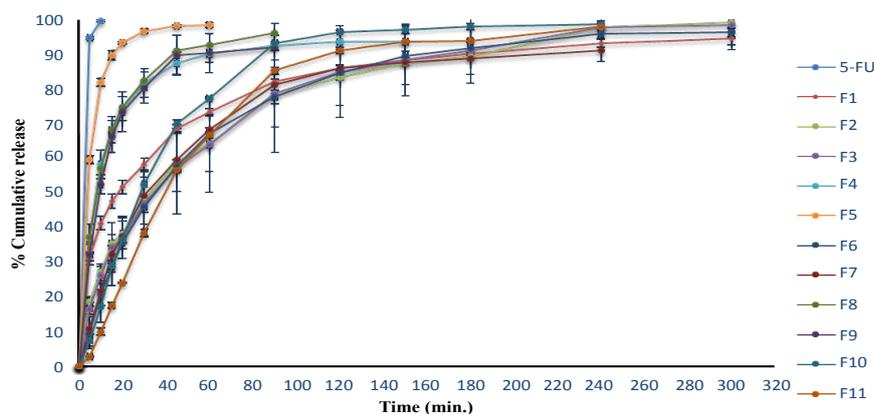


Figure 5: *In vitro* release of 5-Fluorouracil from Eudragit RS 100 microsponges

measure the cell viability and as you know the non-viable cells are died, so the two are the same. In CACO-2 cell line, F3 was effective than F2 and drug itself. Quantitatively, IC_{50} was 79.2, 86.6 and 101.2 ng/ μ l for formulations F3, F2, and 5-FU, respectively. At the same time, in HCT116 cell line the IC_{50} was 65.8, 70.6 and 96.2 ng/ μ l for F3, F2, and 5-FU, respectively indicate that F3 still the most effective formula. Comparing between the two cell lines (CACO-2 and HCT116) the given formulae F3, F2, and 5-FU exerted more toxicity on HCT116 versus those of CACO-2.

Our results showed that F3 was the most toxic formula on both cell lines (CACO-2 and HCT116) as indicated by less IC_{50} .

Conclusion

From the previous results, it was possible to conclude that oil in oil solvent diffusion represented a successful method of encapsulation of 5-FU with relatively high encapsulation efficiencies. The addition of magnesium stearate is essential for the successful formulation of MS. The release profiles showed that all formulations obey the diffusion mechanisms, F3 was more effective on colorectal cell lines than F2 and 5-FU.

Future prospectus

On account of 5-FU is the first drug of choice in the treatment of colon cancer, our plan in the future is to formulate the tablet having MS containing the 5-FU as a colon specific delivery system in a specific oral dosage form used for colon targeting for treatment of colon cancer.

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