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FORMULATION OF 5-FLUOROURACIL MICROSPONGES AS COLON TARGETED DELIVERY SYSTEM USING 3^2 FACTORIAL DESIGN

Ahmed U. Ali, Mahmoud El-Badry and Tahani H. Elfaham*

Department of Pharmaceutics, Faculty of Pharmacy, Asyut University, Asyut 71526, Egypt

5-Fluorouracil is widely used for treatment of colorectal cancer and is provided as intravenous bolus or infusion because it has erratic oral bioavailability. Diarrhea and myelosuppression are potential and the major side effects of the intravenous administration route. This work was aimed to develop colon-targeted delivery of 5-Fluorouracil microsponges so that, the oral bioavailability enhanced and the side effects could be potentially reduced. Quassi-emulsion solvent diffusion method was used to prepare 5-Fluorouracil microsponges using polyethylene glycol as an emulsifier. Different formulae were prepared with different composition and processing factors. The entrapment efficiency 5-Fluorouracil in these formulae ranged from 17.81 to 78.61%, the particle size of the prepared microsponges ranged from 87 to 229 µm and the % cumulative drug released after 24 hours ranged from 47.7 to 98.58%. The prepared microsponges were subjected to compatibility studies as Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). Thus 5-fluorouracil microsponges considered as a promising system for the colon-specific delivery that has potential for future use as an anticancer therapy for colorectal cancer.

INTRODUCTION

Colorectal cancer is the third most common cancer and the fourth most common cancer cause of death globally, accounting for roughly 1.2 million new cases and 600 000 deaths per year[1]. Colorectal cancer is a disease that is manifested by the formation of adenomatous polyps and malignant cells in the colon[2]. These abnormal cells creating tumors are characterized by unregulated replication and the capability of spreading to other sites[2]. Many drugs are used for treatment of colorectal cancer including: Oxaliplatin, Irinotecan and (Fluorouracil (5-FU) in combination with leucoverin) which are administered by intravenous route and capecitabine, a prodrug of 5-FU which is taken orally[3].

5-Fluorouracil and its derivatives i.e., Capecitabine[4], Doxifluridine[5], Tegafur[6], and Eniluracil[7] represent first-line drugs for the treatment of gastrointestinal cancers. 5-Fluorouracil is most commonly administered intravenously. Oral preparations as syrups, tablets and solutions have been used, although in most cases the absorption 5-FU is impredictable by the oral route because it has erratic absorption as the levels of dihydropyrimidine dehydrogenase enzyme (DPD) are variable in the gastrointestinal tract[8]. After oral administration, the responses appear to be lower and shorter when compared with intravenous administration[9]. Diarrhea and other gastrointestinal disorders adverse reactions develop due to the damage of the intestinal epithelium at the time of drug absorption, intestinal villi atrophy and lose were observed[10&11]. In addition, the maximal tolerated dose (MTD) is determined by myelosuppression induced leukopenia and neutropenia[9&12&13] which could be avoided by maintaining systemic blood drug concentrations low. So, a drug delivery system by which the drug is mainly released in the colon, would allow reduction in gastrointestinal disorders. In addition, 5-FU absorption from the colon does

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*Corresponding author: Tahani H. Elfaham, E-mail: Tahany.elfaham@pharm.au.edu.eg

not depend on active transport[14&15], thus the excessive absorption of 5-FU into the systemic circulation is inhibited and myelosuppression is reduced.

The colon as a site for drug delivery, offers some advantages e.g. a nearly neutral pH, reduced digestive enzymatic activity, a longer transit time, and a much greater responsiveness to absorption enhancers[16]. Several strategies for targeting orally administered drugs to the colon include; glycoside conjugates[17], covalent linkage of a drug with a carrier which includes azo bond conjugates[18] and glucuronide conjugates[19], coating with pH-sensitive polymers which utilizes the fact that pH of the human GIT increases progressively from the stomach (pH 1-2 which increases to 4 during digestion), small intestine (pH 6-7) at the site of digestion and it increases to 7-8 in the distal ileum[20], bioadhesive systems[21], formulation of timed released systems[22] and osmotic controlled drug delivery systems[23]. Certain polysaccharides from plant origin such as inulin, amylose, pectin and guar gum remain unaffected in the presence of gastrointestinal enzymes and facilitate the way for the formulation of colon targeted drug delivery systems[20].

Microsponges are polymeric microspheres with surface pores.They are tiny sponge like spherical particles which consists of plenty of interconnecting spaces within a non-collapsible structure[18]. This system can entrap wide range of active materials due to the presence of several inter connected pores, and can adsorb high quantity of active ingredients on its surface[24]. Formerly, microsponges were used for topical application as they can reduce the irritation of various actives e.g. antiacne, sunscreens and rubifacients and so that, it seemed harmless and can prevent excessive accumulation of ingredients within the epidermis and the dermis[25]. Microsponges can also increase the time required for absorption of drugs as they get entrapped on the surface of the colon[26] and thus could be potentially used as colon-targeted drug delivery system.

Several drugs were formulated as microsponges for the oral (colon targeted)delivery of including: paracetamol[27], flurbiprofen[28], dicyclomine[29], meloxicam[30] and ketoprofen[31].

MATERIALS AND METHODS

Materials

5-Fluorouracil (5-FU) was purchased from AvansCureLifeSciences Private Limited, China. Eudragit RS100 (ERS-100) was obtained from Röhm, Germany. Dichloromethane was purchased from S D Fine-Chem Limited, India. Triethyl citrate (TEC) was obtained from Alfa Aesar, Germany.Tribasic sodium phosphate was obtained from Oxford laboratory chemicals, India. Polyethylene glycol (PEG) (Mwt 4000) from El- Nasr Company, Egypt. Hydroxypropylmethyl cellulose (HPMC) hard capsules.

Methods

Preparation of microsponges

The microsponges containing 5-FU were prepared by the quasi-emulsion solvent diffusion method[17]. To prepare the inner phase, Eudragit RS 100 was dissolved in 10 ml of Dichloromethane and Triethyl citrate (TEC) 1% w/v as a plasticizer was added. Then, 5-FU (100 mg) was added to the solution and were dispersed under ultrasonication for 5 minutes at 35°C. The inner phase was then poured into the PEG 4000 solution (0.02% W/V) in water (outer phase) with stirring. After 4 hours of stirring, the microsponges were formed and the contents were filtered through filter paper to separate them. The microsponges were dried in open air at 25°C for 12 h to obtain the final product.

Optimization of microsponges via 3^2 factorial designs

The 3^2 full factorial design was utilized to study the effect of each factor on the responses as well as the interactions between these factors on the response variables. The dependent variables which we selected were the encapsulation efficiency (y1), mean particle size (y2) and % cumulative drug released from the microsponge formulations (y3). Preliminary experiments were carried out in establishing the preparation method; some factors had been taken in consideration such as, the concentration of triethyl citrate, the concentration of poly ethylene glycol, temperature of the environment, stirrer type and the volume of dichloromethane. From

these preliminary experiments we had chosen the concentration of (TEC) as 0.5%, the concentration of polyethylene glycol to be 0.025%, the temperature of the environment to be the room temperature, the mechanical stirrer and the volume of dichloromethane to be 5 ml and found that conditions are optimum conditions for the preparation of the microsponges and A series of formulations were prepared by 3^2 full factorial design using (Minitab 17.3.1. software) where, the polymer content (x1) and stirring rate (x2) are the independent variables(the amount of 5-FU, volume of dichloromethane and the amount of triethyl citrate were kept constant).The composition of the different formulations is shown in table 1.

Characterization of the prepared microsponges

Determination of the entrapment efficiency

20 mg of drug loaded microsponges were weighed and were suspended in 20 mL phosphate buffer pH 6.8 for 12 hrs with continuous stirring at 500 rpm. The samples were filtered analyzed at 266 nm against blank (microsponge formulations without drug treated in the same way) using UV spectrophotometer (UV 1700, Shimadzu)[24]. The product yield (PY), actual drug content and the entrapment efficiency for all formulations were calculated according to the following equations.

Product Yield (%)

$$= \frac{\text{Practical mass (microsponges)}}{\text{Theoretical mass (polymer + drug)}} \text{ x } 100 \qquad \text{(A)}$$

Actual content of drug %

$$= \frac{\text{Mac}}{\text{Mm}} \text{ x } 100 \qquad \text{(B)}$$

Entrapment efficiency %

$$= \frac{\text{Mac}}{\text{Mth}} \text{ x } 100 \qquad \text{(C)}$$

Where M_{ac} is the actual amount of drug in the weighed quantity of microsponges, M_m is the weighed quantity of microsponges, and M_{th} is the theoretical amount of 5-FU in microsponges.

Fourier transform infrared spectroscopy (FTIR)

Fourier transformInfrared spectroscopy (FTIR) measurements were performed using a Hitachi 295 spectrophotometer (Hitachi, Tokyo, Japan) using the KBr disc method. The samples were scanned over the range of 4000 to 400 cm^{-1}. Infrared spectroscopic analysis was run for drug (5-FU), polymer, physical mixture of the drug and the polymer at 1:1 ratio and for the microsponge formulations.

Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) measurements were performed using differential scanning calorimeter calibrated with indium (ShimadzuDTG-60/DTG-60A, Japan). Samples of 5-FU, Eudragit RS100, physical mixture and drug-loaded microsponge-based formulation were studied employing differential scanning calorimeter. The analysis was performed on 3-5 mg sample sealed in standard aluminum pan. Thermo grams were obtained at a scanning rate of 10°C/min. Each sample was scanned between 20-300°C.

Scanning electron microscopy

The morphology of microsponges was examined using a scanning electron microscope (GEOL 5400, USA) operating at 15 kV. Dried microspheres were coated with gold–palladium alloy for 45 s under an argon atmosphere before observation. SEM photograph was recorded at magnification of ×150 and 500.

Particle size determination

The particle size of the prepared microsponge formulations (FMS-1- FMS-9) was determined using Laser scattering particle size distribution analyzer (Horiba LA-300, Germany).

***In-vitro* drug release from the prepared microsponges**

The method adopted by (Orlu *et al*., 2006) for the in-Vitro release studies of the drug from the prepared microsponge was utilized. Weighed amounts of microsponge formulations (equivalent to 20 mg drug) were subjected to *in-vitro* release studies, 20 mg of free drug were taken as a control. Dissolution test was conducted in USP rotating paddle

apparatus (ERWEKA DT 720, USA) with a stirring rate of 50 rpm at 37±0.5°C for 24 hrs. Initially drug release was carried out using 250 ml of 0.1 N hydrochloric acid pH 1.2 (as the release medium) for 2 hrs, then the pH was shifted to 6.8 by addition of 100 ml of 0.1 M tribasic sodium phosphate dodecahydrate (TBS)for the next 22 hrs. Samples were withdrawn at regular time intervals: 0.5, 1, 2, 4, 6, 8, 10, 12, 16 and 24 hrs and were compensated with equal volume of fresh dissolution medium to maintain the sink conditions and withdrawn samples were analyzed spectrophotometrically at 266 nm.

***In-vitro* drug release from acid resistant capsules**

The selected microsponge formulations were packed into acid resistant hydroxyl-propylmethyl cellulose (HPMC) hard capsules and were subjected to *in-vitro* release studies. *In-vitro* release studies were carried out in USP basket apparatus (ERWEKA DT 720, USA) with stirring rate 50 rpm at 37±0.5°C, the same procedure used in the *in-vitro* drug release from prepared microsponges was applied.

RESULTS AND DISCUSSION

Nine microsponge formulae of 5-FU were prepared by the quasi-emulsion solvent diffusion method which seemed a promising method for the preparation of 5-FU microsponges being easy, reproducible and rapid method. The polymer used was Eudragit RS100 and Triethyl citrate was used as a plasticizer. The composition of 9 formulae is shown in table 1

Processing factors affecting microsponge formulation

Effect of polymer content on the encapsulation efficiency and microsponge size

The production yield, actual drug content and enctrapment efficiency (EE) of the prepared microsponge formulations FMS1-FMS9 were calculated and cited in table 2.

The entrapment efficiencies of the prepared 5-FU microsponges formulations were found to range from 17.81 to 78.61%. By increasing the ratio of polymer : drug from 6:1 to 10:1, the % entrapment efficiency was increased significantly ($p< 0.05$).

The microsponge size of the prepared formulations was measured and found to range from 87.04 to 273.23 µm (Table 3). At the same stirring rate, as the drug : polymer ratio increases, the particle size also increases.

Thus, increasing the polymer content has a positive effect on both the EE and the particle size of the prepared microsponges. That is because, as the polymer content increases, the viscosity of the internal (organic) phase increases which handicaps the easy diffusion of the solvent and more time is required for droplet formation giving rise to larger droplets entrapping larger amounts of the drug. These results are in agreement with those obtained by (Gupta *et al*., 2015)[32] who prepared enteric coated HPMC capsules plugged with 5-FU loaded microsponges and reported that, a higher concentration of polymer or a lower level of organic solvents produced a more viscous dispersion, which formed larger droplets and consequently larger microsponges obtained.

Table 1: Composition of microsponges formulations prepared at different polymer contents and also at different stirring rates using 3^2 full factorial design.

Microsponge formulation	Amount of Drug(mg)	Polymer content (mg) (x1)	Stirring Speed (rpm) (x2)	Dependent variables
FMS-1	100	600(-1)	500(-1)	Entrapment Efficiency Particle size % Cumulative drug released
FMS-2	100	800(0)	500(-1)	
FMS-3	100	1000(1)	500(-1)	
FMS-4	100	600(-1)	750(0)	
FMS-5	100	800(0)	750(0)	
FMS-6	100	1000(1)	750(0)	
FMS-7	100	600(-1)	1000(1)	
FMS-8	100	800(0)	1000(1)	
FMS-9	100	1000(1)	1000(1)	

Table 2: Effect of Polymer content on the Product Yield, Actual Drug content and % encapsulation efficiency of the prepared 5-FU microsponges.

Formulation Code	Polymer Content (mg) (x1)	Stirring speed (rpm) (x2)	Product Yield %	Actual Drug content%	Encapsulation Efficiency % (y1)
FMS-1	600	500	50.30±1.30	6.34±0.60	40.19±01.75
FMS-2	800	500	66.20±1.03	7.28± 0.35	65.70±01.57
FMS-3	1000	500	71.00±0.36	7.50±0.23	78.61±01.76
FMS-4	600	750	32.28±1.40	5.43±0.36	36.26±01.47
FMS-5	800	750	38.60±0.60	3.45±0.50	60.29±02.12
FMS-6	1000	750	47.70±0.69	4.29±0.21	47.14±02.85
FMS-7	600	1000	22.80±0.650	2.10±0.12	17.81±01.40
FMS-8	800	1000	30.00±01.40	3.36±0.32	31.74±02.80
FMS-9	1000	1000	37.454±2.87	3.15±0.13	36.26±02.07

Table 3: Particle size of different microsponges formulation.

Formulation Code	Polymer content/ mg (x1)	Stirring speed (rpm) (x2)	Particle size (µm) (y2)
FMS-1	600	500	188.84± 3.76
FMS-2	800	500	229.35± 3.65
FMS-3	1000	500	160.44± 2.54
FMS-4	600	750	177.10± 4.87
FMS-5	800	750	209.38± 3.67
FMS-6	1000	750	273.28± 3.43
FMS-7	600	1000	87.04± 4.90
FMS-8	800	1000	102.72±3.67
FMS-9	1000	1000	154.13± 2.98

Effect of stirring rate on the prepared microsponge formulations

At the same polymer : drug ratio, as the stirring rate increases, the entrapment efficiency as well as the particle size decreases (Table 3).

The results showed that the microsponge mean size decreased with an increase in the stirring speed[33&34]. (Sansdrap and Moës 1993)[33] found that, the internal phase is distributed into smaller particles in a response to the force of higher stirring speed resulting in the formation of smaller particles entrapping smaller amount of drug. Another explanation suggests that, the increase in the stirring speed generates greater energy to the system, resulting in an increased breakdown of the formed microsponges and decreased the encapsulation efficiency[35].

Factorial equation design

The combined effect of changing both the polymer content and the stirring rate on the entrapment efficiency and the particle size is illustrated by the factorial equations.

Factorial equation for % entrapment efficiency

The response surface linear model generated for the entrapment efficiency was found to be significant with an F-value of 19.97 ($p< 0.05$).

% Entrapment efficiency=

$$35.2 + 7.90\ x1 - 0.0658\ x2 \quad (D)$$

Where, (x1 is the polymer content, x2 is the stirring rate).

The co-efficient of x1 is positive, indicating that, when the polymer content increased, the entrapment efficiency increased, whereas the negative coefficient of x2 indicates

that entrapment efficiency decreased on increasing the stirring speed. The *P* value for variable x1 and x2 were 0.005 and 0.004 respectively ($p< 0.0500$) indicated that both the independent variables show significant effect on the dependent variable i.e. % entrapment efficiency.

Factorial equation for particle size

The response surface linear model generated for particle size was found to be insignificant with an F-value of 2.38 ($p> 0.05$).

Particle size= 203 + 11.2 x1 - 0.1565 x2 (E)

Where, (x1 is the polymer content, x2 is the stirring rate).

The coefficient of x1 is positive, indicating that when the polymer content increased, the particle size increased, whereas the negative coefficient of x2 indicates that particle size decreased on increasing the stirring speed. The *P* value for variable x1 and x2 were 0.319 and 0.107 respectively ($p> 0.0500$) indicated that both the independent variables show insignificant effect on dependent variable i.e. particle size.

Contour Plot graphs of the responses (% entrapment efficiency and particle size) were generated from these polynomial equations to visualize, the simultaneous effect of two independent variables on the response parameters are illustrated in figures 1&2.

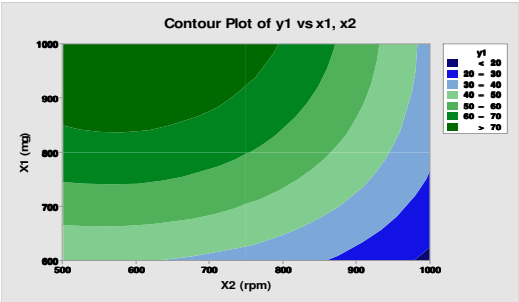

Fig. 1: Contour plot of y1 vs x1, x2.

Abbreviations: y1: % Entrapment efficiency, x1: polymer: drug content, x2: stirring speed.

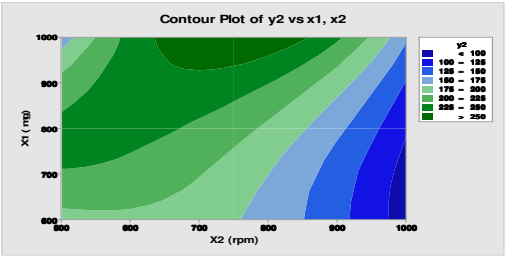

Fig. 2: Contour plot of y2 vs x1, x2.

Abbreviations: y2: particle size, x1: polymer : drug content, x2: stirring speed.

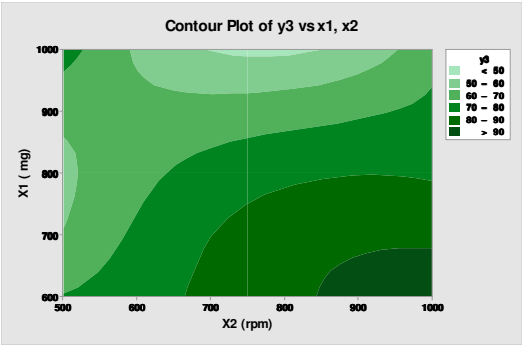

Fig. 3: Contour plot of y3 vs x1, x2.

Abbreviations: y3: % Entrapment efficiency, x1: polymer : drug content, x2: stirring speed.

Characterization of the prepared microsponge formulations

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the pure drug, polymer, physical mixture and the prepared microsponges are shown in figures 4-8. The principal peaks of 5-FU were, NH stretch at 3124 cm^{-1}, C=O stretch at 1722.95 cm^{-1} for the carbonyl group No. 5 and 1660.24 cm^{-1} for carbonyl group No. 2, at 1716 cm^{-1}, CH in plane deformation at 1246.29 cm^{-1} and CH out of plane deformation at 813 cm^{-1} while the characteristic peaks for Eudragit RS100 were, -CH3 bending at 1453 cm^{-1} and C=O at 1731 cm^{-1}. Matching up to FTIR spectrum of 5-FU with the physical mixtures revealed no distinctive changes indicating that Eudragit® RS100 was not involved in intermolecular interaction with 5-FU. In the spectra of the

microsponges formulations, The peaks at 1246.29 cm^{-1} for the CH in plane deformation and at 813 cm^{-1} for the CH out of plane deformation corresponding to the drug still present in all the microsponge formulations while the peak at 1660 cm^{-1} appeared only in formulation FMS-1 (drug : polymer ratio (6:1) and at stirring rate of 500 rpm) but at lower intensity and also in FMS-4 (drug : polymer ratio (6:1) and at stirring rate of 750 rpm) formulation but at lower intensity than in FMS-1 and disappeared in all other formulations prepared with higher (polymer : drug) ratio and at higher stirring rates. Also, the peak for NH binding of 5-FU disappeared indicating a type of hydrogen bonding formation between the drug and the polymer.

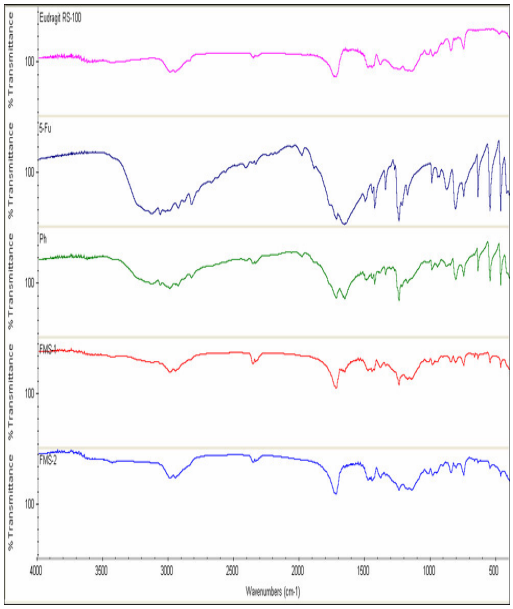

Fig. 4: FTIR spectra of (Eudragit Rs-100, 5-FU, physical mixture (PH) and FMS-1,2).

Abbreviations: 5-FU: 5-Fluorouracil, FMS1, 2: microsponges formulations no 1, 2.

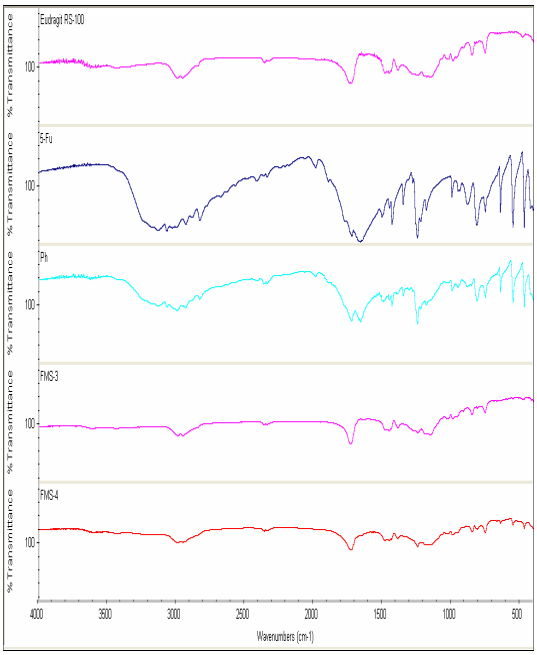

Fig. 5: FTIR spectra of (Eudragit Rs-100, 5-FU, physical mixture (PH) and FMS-3, 4).

Abbreviations: 5-FU: 5-Fluorouracil, FMS1, 2: microsponges formulations no 3, 4.

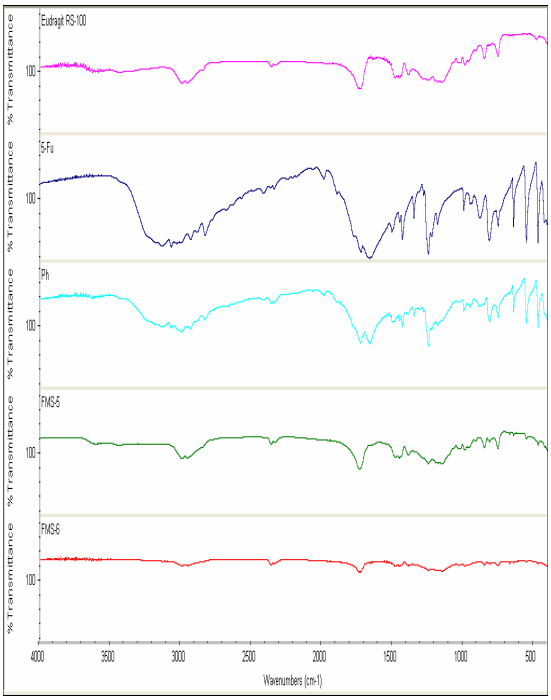

Fig. 6: FTIR spectra of (Eudragit Rs-100, 5-FU, physical mixture (PH) and FMS-5,6).

Abbreviations: 5-FU: 5-Fluorouracil, FMS-5,6: microsponges formulations no 5, 6.

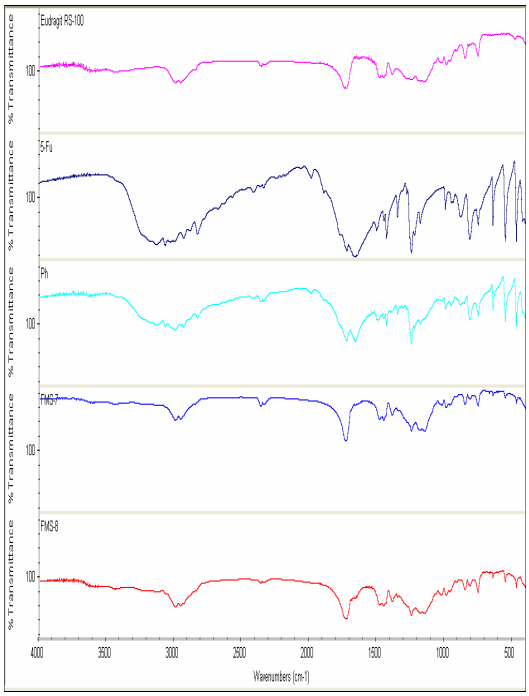

Fig. 7: FTIR spectra of (Eudragit Rs-100, 5-FU, physical mixture (PH) and FMS-7,8).

Abbreviations: 5-FU: 5-Fluorouracil, FMS-7,8: microsponges formulations no 7, 8.

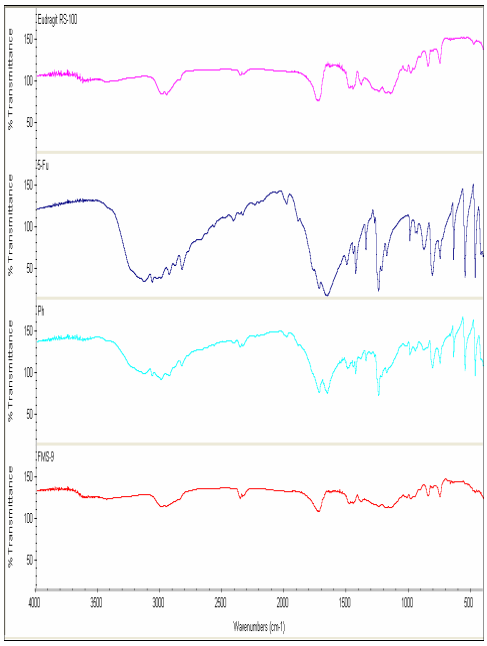

Fig. 8: FTIR spectra of (Eudragit Rs-100, 5-FU, physical mixture (PH) and FMS-9).

Abbreviations: 5-FU: 5-Fluorouracil, FMS-9: microsponges formulation no 9.

Differential scanning calorimetry (DSC)

The DSC thermograms of pure 5-FU, Eudreagit RS-100, physical mixture of 5-FU and Eudreagit RS-100, different microsponges formulations are shown in figure 9. 5-FU shows an endothermic peak at 285°C, Eudragit RS-100 shows no endothermic peak before 300°C. In the physical mixture, the peak of the drug was shifted to 279°C. In the thermograms of microsponge formulations FMS-1-10, the characteristic peak of the drug disappeared completely, indicating the complete inclusion of the 5-FU within the polymer matrix forming the microsponges. These results coincide with those obtained by (Illangakoon *et al*., 2015) who prepared 5-Fluorouracil loaded Eudragit fibers by electrospinning technique using Eudragit S-100.

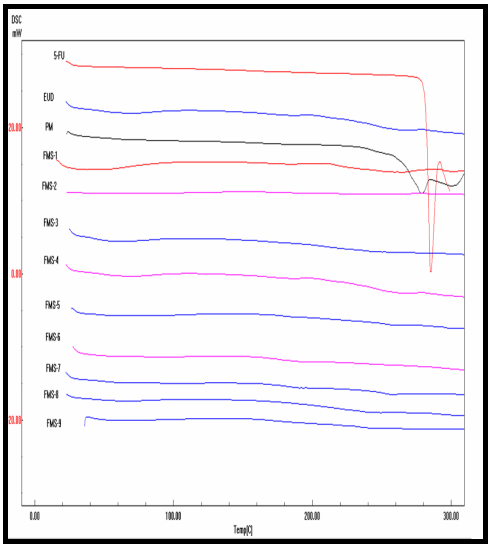

Fig. 9: DSC thermograms of pure 5-FU, Eudragit RS-100, physical mixture of 5-FU and Eudragit RS-100, different microsponges formulations.

***In-vitro* release studies**

The *in-vitro* release curves are shown in figures 10-12. It is found that, the % cumulative drug release (CDR) ranged from 47 to 98% for the different microsponge formulations. About 88% of control sample (free drug) released at the first 30 minutes in the acidic pH (1.2) medium, while the release of drug from microsponges formulations extended to 24 hrs. Generally, at the same polymer : drug ratio, as the stirring rate increases, the % cumulative drug released also increased.

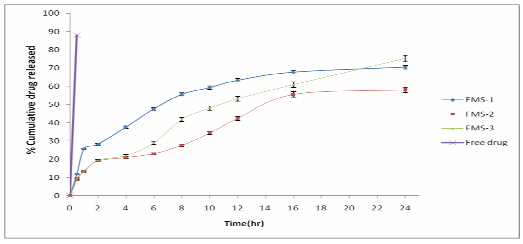

Fig. 10: In vitro release of 5-FU from microsponge formulations (FMS-1-3) at different pH values.

Abbreviations: 5-FU: 5-Fluorouracil, FMS1-3: microspongesformulations 1-3.

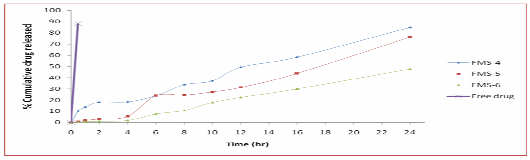

Fig. 11: *In-vitro* release of 5-FU from microsponge formulations (FMS-4-6) at different pH values.

Abbreviations: 5-FU: 5-Fluorouracil, FMS 4-6: microsponges for mulations no 4- 6.

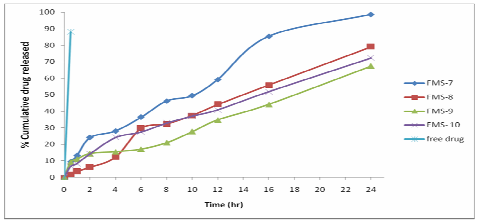

Fig. 12: *In-vitro* release of 5-FU from microsponge formulations (FMS-7-10) at different pH values.

Abbreviations: 5-FU: 5-Fluorouracil, FMS7-10: microsponges formulations 7-10.

The increase in the level of the polymer (Eudragit RS-100) forms larger microsponges, and hence increase the distance which the drug molecules have to traverse. Another explanation suggested by (Illangakoon et al., 2015)[36] is that on increasing the level of the polymer, the amount of drug close to surface with decreased with simultaneous increase in the amount of drug getting entrapped in the polymer matrix, this leads to lowering the rate of drug release from the prepared microsponges. On the other hand, formulae FMS-1 prepared with lower level of Eudragit RS-100 formed smaller sized microsponges which can be associated with higher surface area and shorter path length leading to increase in the release rate. Thus, the extent of drug release depends primarily on the polymer levels and the stirring speed which affects the final size of the particle. (Gupta *et al*., 2015)[32] reported that the release of 5-FU from microsponges is related to the pores throughout the microsponges, which facilitates the rapid penetration of the release medium into the microsponges and helping in diffusion and dissolution of the drug from the polymeric matrix. The kinetics of drug release are shown in table 4. Most of the microsponge formulations exhibited zero order release kinetics, two formulations exhibited Higuchi diffusion.

Table 4: *In-vitro* drug release models for different microsponges formulations.

	Zero order		**First order**		**Diffusion**	
Form. Code	r^2	K mg/hr	r^2	K hr^{-1}	r^2	K $mg/hr^{1/2}$
FMS-1	0.889	2.387	0.938	0.047	0.967	14.386
FMS-2	0.967	2.203	0.970	0.035	0.968	12.222
FMS-3	0.977	2.886	0.996	0.055	0.989	16.186
FMS-4	0.994	3.140	0.958	0.069	0.958	16.755
FMS-5	0.986	3.112	0.952	0.054	0.951	16.630
FMS-6	0.992	2.092	0.984	0.027	0.945	11.035
FMS-7	0.985	3.894	0.932	0.159	0.979	21.455
FMS-8	0.990	3.345	0.985	0.062	0.985	18.441
FMS-9	0.988	2.411	0.963	0.040	0.931	12.602
FMS-10	0.981	2.908	0.950	0.058	0.969	15.909

Factorial equation for % cumulative drug released

The response surface linear model generated for the % CDR was found to be insignificant with an F-value of 3.62 ($p > 0.05$).

% cumulative drug released=

$$94.9 - 5.29\ x1 + 0.0274\ x2$$

The coefficient of x1 is negative, indicating that when the polymer content increased, the % Cumulative drug released decreased, whereas the coefficient of x2 is positive indicates that % Cumulative drug released decreased on increasing the stirring speed. The *P* value for variable x1 and x2 were 0.064 and 0.194 respectively ($p > 0.05$) indicated that both the independent variables show insignificant effect on the dependent variable, % Cumulative drug released.

Contour Plot graph of the response y3 (% cumulative drug released) was generated from these polynomial equation to visualize, the simultaneous effect of two independent variables (x1 and x2) on response y3 and is illustrated in figure 3.

Selection of optimized formulation

Theoretically, formula FMS-10 of the particle size 165.36 µm, of maximum entrapment efficiency 44.67%, and % CDR 8 h of 74.79% and composite desirability of 0.764 was identified as the optimized formulation and was used for development of colon target capsules. Figure 13 shows the optimization plot for FMS-10.

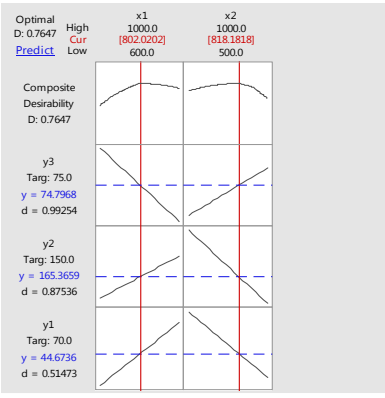

Fig. 13: Optimization plot for FMS-10.

Abbreviations: y1: % Entrapment efficiency, y2: particle size, y3: % cumulative drug released, x1: polymer : drug content, x2: stirring speed, FMS-10: MICROSPONGE formulation no. 10.

***In-vitro* drug release from acid resistant capsules**

The optimum formulation (FMS-10), had been predicted by the Minitab software and formulation (FMS-3) is the best one from the practical point of view, it shows maximum entrapment efficiency and almost the same particle size and the same % cumulative drug release after 24 hrs compared to (FMS-10). These 2 formulae were plugged into acid resistant capsules (equivalent to 20 mg drug) and subjected to *in-vitro* release study using USP basket apparatus and the release curves are shown in figure 14. FMS-3 shows significantly higher % cumulative drug release compared to FMS-10.

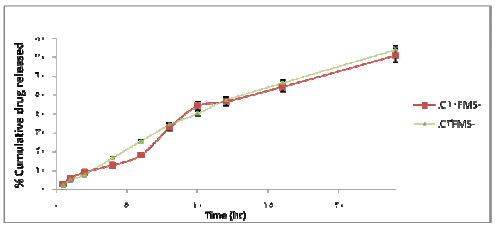

Fig. 14: *In-vitro* release study from (HPMC) acid resistant capsules at different pH values.

Abbreviations: 5-FU: 5-Fluorouracil, FMS-10,3. C: microsponges formulations plugged into hard (HPMC) capsules, HPMC (hydroxy propylmethyl cellulose).

Validation of the experimental design

An extra design check point formulation (FMS-10) was made and the predicted values and experimental values of dependent variables were compared. No significant difference was recorded between the two values (Table 5) thereby establishing validity of the generated model.

Scanning electron microscope

Photographs obtained using the scanning electron microscope (SEM). Figures (15-18) shows that the microsponges are almost spherical in shape with porous surface. These pores vary in size from a formulation to another one. Formula FMS-3 (which prepared with a polymer content of 1000 mg, at a stirring rate of 500 rpm) is an exception of the factorial design sequence of the responses (% cumulative drug release). It was expected to show lower (about 40-45 % cumulative drug release) however, it shows 75.28 % cumulative drug release. This is due to that, formulae FMS-3 (Fig. 16) has larger pore size which accounts for the higher % cumulative drug release.

Table 5: Predicted and experimental responses for FMS-10.

Formulation Code	Polymer content mg(x1)	Stirring speed (X2)	Encapsulation Efficiency % (y1)	Particle size (µm) (y2)	% cumulative drug released (y3)
FMS-10 Predicted value	802	818	44.67	165.360	74.79
FMS-10 Experimental values			41.45 ± 02.45	161 ± 2.34	71.33 ± 2.3
P value			0.085	0.054	0.060

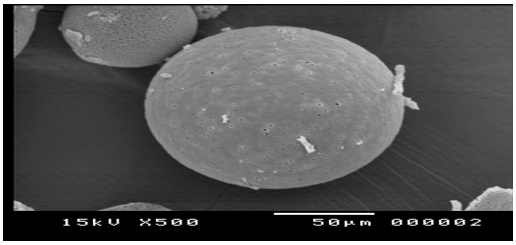

Fig. 15: SEM photo for FMS-3.

Abbreviations: FMS-3: microsponges formulation no. 3.

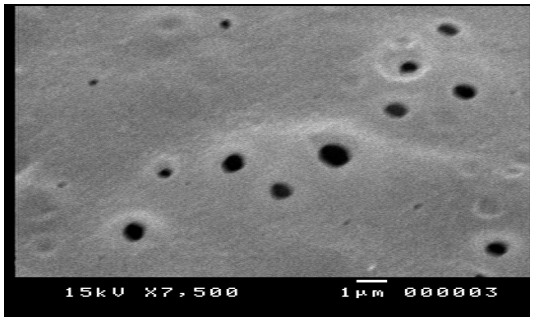

Fig. 16: SEM photo for FMS-3 showing the pores.

Abbreviations: FMS-3: microsponges formulation no. 3.

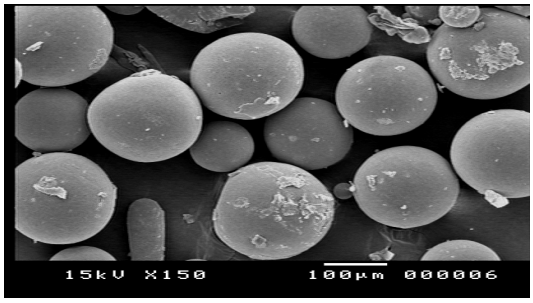

Fig. 17: SEM photo for FMS-10.

Abbreviations: FMS-10: microsponges formulation no. 10.

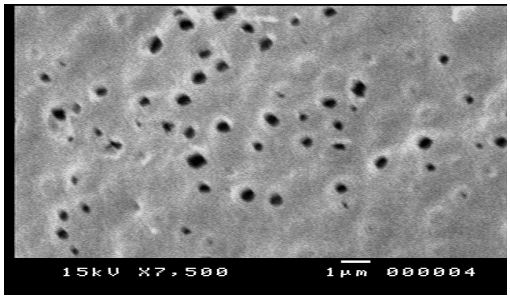

Fig. 18: SEM photo for FMS-10 showing the pores.

Abbreviations: FMS-10: microsponges formulation no. 10.

Another photos obtained by the (SEM) shows the rupture of the microsponge particles during the *in-vitro* release process (Fig. 19) and the cleavage of the particles at the end of release process (Fig. 20). Figure 21 shows the surface of microspong particle with many pores which increase towards the surface and decrease towards the inner surface of the particle, no pores were determined in the core of the particle.

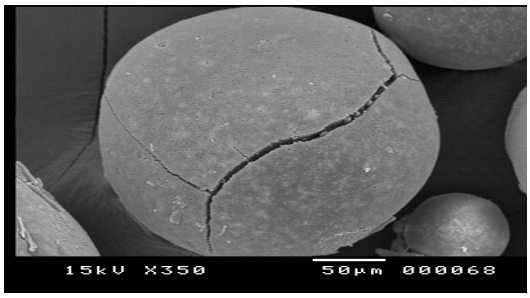

Fig. 19: SEM photo for FMS-10 showing rupture of the particle during the release process.

Abbreviations: FMS-10: microsponges formulation no. 10.

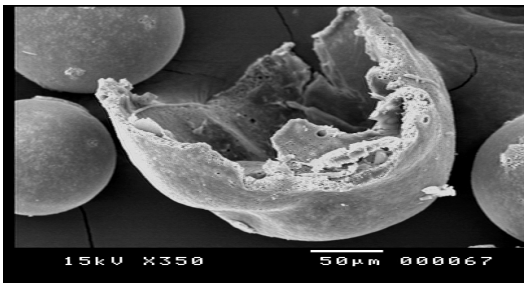

Fig. 20: SEM photo for FMS-10 showing cleavage of the particle at the end of the release process.

Abbreviations: FMS-10: microsponges formulation no. 10.

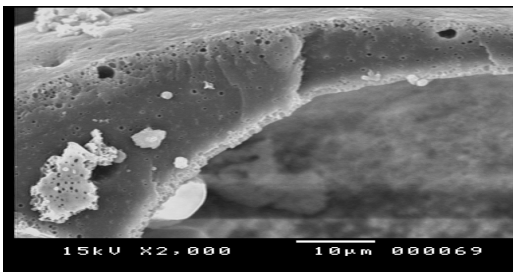

Fig. 21: SEM photo for FMS-10 showing surface of the particle at the end of the release process.

Conclusion

A simple, easily prepared dosage form of 5-FU was developed for colon-targeted delivery for treatment of colorectal cancer. The analysis of factorial design revealed that changing the polymer content and stirring speed have significant effects on the entrapment efficiencies while insignificant effect on both the particle size and the percent cumulative drug released. The prepared colon targeted capsules containing microsponges have the ability to deliver the drug to the colon as well as, controlling the release of the drug for 24 hrs which will ensure higher local effect and reduced systemic side effects associated with the parenteral administration of the drug.

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نشرة العلوم الصيدلية
جامعة أسيوط

صياغة اسفنجيات ٥-فلورويوراسيل كنظام موجه الي القولون باستعمال طريقة ٢٣ لتصميم المتغيرات

أحمد أسامه علي – محمود البدري – تهاني حسن الفحام

قسم الصيدلانيات ، كليةالصيدلة ، جامعة أسيوط ، أسيوط ، مصر

ان عقار الخمسه فلورويوراسيل شاع استخدامه في علاج سرطان القولون الشرجي ويتم استخدامه عن طريق الحقن مره واحده أو بالتنقيط ولا يتم استخدامه عن طريق الفم لان استخدامه بالفم لا ينتج عنه انتظام في الاتاحه البيولوجيه واستخدامه عن طريق الحقن ينتج عنه اسهال وتثبيط نخاع العظم. ان هذا العمل يهدف الي تشييد اسفنجيات من عقار الخمسه فلورويوراسيل موجهه الي القولون وهذا يحسن الاتاحه البيولوجيه للعقار عن طريق الفم وتقلل الاعراض الجانبيه عن طريق الحقن. تم استعمال طريقة الكواسي لانتشار مذيب المستحلب في تحضير اسفنجيات الـ ٥-فلورويوراسيل باستعمال البولي ايثيلين جليكول كعامل استحلاب. تم تحضير صياغات كثيره وذلك بتغيير التركيبه والعوامل المؤثره. تراوحت كفاءة الاحتواء من ١٧,٨١ الي ٧٨,٦١% وتراوح حجم الجزيئات من ٨٧ الي ٢٢٩ ميكروميتر وكذا كان انبعاث العقار من الجزيئات بنسبة ٤٧,٧ الي ٩٨,٥٨%. تعرضت الاسفنجيات المحضره الي قياس الطيف باستخدام متحول فوريه وكذا الي قياس تفصيل مسعر المسح التباييني. وبهذا تعتبر اسفنجيات الفلورويوراسيل كانظمه واعده لتحضير نظام توصيل العقار الي القولون لاستعمالها في علاج سرطان القولون الشرجي.