

Optimization and Scale-up Methodology in Preparing Microsponge Loaded with 5-Fluorouracil (5-FU).

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Research Article

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Optimization and scale-up methodology in preparing microsphere loaded with 5-fluorouracil (5-FU).

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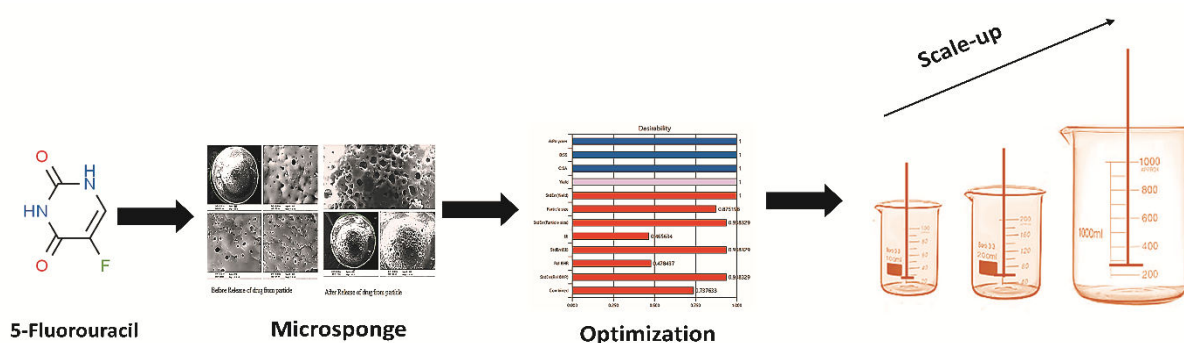
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Highlights

- The development of a new microsphere drug delivery system of 5-fluorouracil used to treat actinic keratosis and colon cancer.
- Optimization technique to maximise encapsulation and drug release rate.
- Scale up method to assure reproducibility of product in higher scale.
- FT-IR, DSC and SEM confirmed the compatibility of drug, and porous morphology of drug-loaded microsphere particles.
- The final form of dosage form showed the shear-thinning rheological property, ideal for drug release from dermal delivery system.

Graphical Abstract



Abstract

The present investigation aims at developing models by response surface methodology (FCCCD) followed by the scale-up method in preparing control release microsphere particles loaded with 5- fluorouracil, a drug used to treat actinic keratosis and colon cancer, and producing a new Dermal Delivery System. The polymer-based (ethyl cellulose and eudragit RS 100) microsphere particles were prepared by the w/o/w double emulsification method. The optimized product was formed with the combination of independent variables levels: polymer (600 mg), stirring speed (1198 rpm) and surfactant (2% w/v), yielding responses as yield (~63.6257%), the average size of particles (~151.563 μm), entrapment efficiency (~75.319 %) and drug release in 8hr (~75.75%), with desirability value of 0.737. The products showed similar responses as obtained in scale-up work. FT-IR, DSC and SEM studies confirmed the drug's compatibility with polymers and porous morphology. Finally, gel embedded optimised product showed shear-thinning rheological property, ideal for drug release from the thixotropic gel.

Keywords: microsphere, 5-fluorouracil, sodium alginate, w/o/w, RSM, scale-up.

1. Introduction

The Microsphere Drug Delivery system is unique among other control release microparticulate systems. It has high loading capacity in myriad microporous channels of its spongy structure, self-sterilising ability, and assuring thermal stability and chemical stability in a wide range of pH, making it flexible in developing improved product forms. Diffusion of active material from the porous structure of microsphere particles is triggered due to its solubility in an aqueous medium such as perspiration in topical drug and cosmetics products (antiseptics, deodorants and antiperspirants). Microsphere particles act as a storehouse of the drug and release drug molecules slowly at a controlled rate. The prolonged-release condition reduces the toxicity and allergic effects and improving patients' compliance.

Besides topical drug delivery, a microsphere delivery system was investigated for colon targeted drug delivery systems earlier, dispensing in capsule [1] and tablet [2]. In previous years, researchers prepared and characterised microspheres entrapping various types of drugs, such as 5 –FU [3,4]; antifungal drugs- ketoconazole, miconazole, fluconazole, terbinafin HCl [5–7], antihypertensive drugs- nebivolol, valsartan [8,9], an anti-inflammatory drug- ketoprofen [10], timolol, used for glaucoma [11], antiviral drugs- valacyclovir, acyclovir [12,13], anthelmintic drug- albendazole [14].

Commercially microsphere delivery system is gaining importance as dermal and cosmetic products. Currently, various dermal products of API loaded microspheres are in the market for the topical application: Tretinoin (product name- Retin-A-Micro) is used in treating acne vulgaris, marketed by Ortho-MCNeil Pharmaceutical, Inc. USA; Fluorouracil (Carac cream, Dermik Laboratories, Inc. USA,); Hydroquinone and retinol (EpiQuin Micro, SkinMedica, Inc. USA), Salicylic acid (Micro Peel Plus/Acne Peel, Biomedical IMPORIUM, South Africa), [15].

The microsphere preparation method consists of emulsification, solvent evaporation and solidification. Types of emulsion (w/o, o/w, w/o/w) and the solvent are chosen depending on the characteristics of drug and polymer used. The type of emulsion is reported as o/w (aqueous external phase) in most of the earlier cases of microsphere preparation which makes the process economic provided drug loss during trial is minimum. Oil in oil type emulsion had been reported by several researchers [3,8,14] using Eudragit RS 100 in the preparation of microspheres. On the other hand, very few reports are based on w/o/w double emulsion, which is specially adapted to make the product more stable [16]. Eudragit RS 100 and ethyl cellulose are two structural components widely shown in earlier literature for porous structure formation. These are Food and Drug Administration (FDA) approved, safe, non-irritating, non-toxic and economic excipients, and widely used in the pharmaceutical industry. Moreover, skin toxicity due to polymer use can be assessed by conducting cell line toxicity studies and in vivo skin irritation studies.

The use of Eudragit polymer had been reported in the preparation of microparticles by several investigators [3,7,8,14,17–21]; use of ethylcellulose polymer was found in several earlier works [5,6,12,16,22–26]; eudragit RSPO by P.M. Barde et al. [27], as a single polymer use. A combination of polymers (ethyl cellulose +HPMC) was used by Yasser Shahzada et al. [10] and Jain S.K et al. (ethyl cellulose +eudragit RL 30D) [4]. The coupling of different polymers offers better control of drug release behaviour. The emulsification process often needs the addition of suspending agents like Na alginate [19] to facilitate the dispersion of polymer droplets in the emulsion. PVA as a stabilising agent in the external aqueous phase had been reported in most of earlier cases. Ahmed U. Ali et al. [28] used PEG 4000 solution (0.02% W/V) in water (outer phase). Besides polymers, plasticiser (triethyl citrate) is used to reduce fragility, and pore inducers/ porogens (hydrogen peroxide or sodium bicarbonate, gelatinised starch) are used for increasing number of pores to accommodate higher amount of drug. Other agent is surface active agent (tween 80) used to emulsify. Apart from microsphere composition, preparation techniques play an important role in regulating the performance of

this delivery system. However, complexity arising due to physicochemical properties of ingredients and fluid dynamics in emulsification method and cost of preparation are the limitations in the production of microsponges.

Preparation of multi particulates by 'emulsion solvent evaporation technique is a complex one as it involves fluid dynamics phenomenon and solidification of emulsion droplets (phase change) leading to loss of its emulsion character finally. Reproducibility is often questionable in repeating experiments on higher scales where it is difficult to maintain similar fluid dispersion dynamics. Therefore, controlling variables should be identified aptly by prior trials. Optimisation by QBD for preparing microparticulate systems is nowadays practised by formulation scientists, and optimised operating variables aid in scale-up design. To enhance reliability and reproducibility of the method, several investigators started attempting simple factorial design [29], Central Composite Design -RSM method by design expert software [1,11,13,16,28–31]. They presented linear as well as quadratic models.

The lack of knowledge /information related to the scaling-up of technology used for preparing polymeric microsphere may hamper/delay the launching of the product into the pharmaceutical market. Very few works have been reported on the scale-up method. In 2013, S. A. Galindo-Rodríguez et al. [32] adopted scale-up technology in small batch size up to 1.5 L producing ibuprofen-loaded nanoparticles. They reported satisfactory results in scale-up with a slight difference in particle size of products. Effectiveness of the scale-up procedure had been reported by L. Sánchez-Silva et al. [33] at pilot plant scale using the optimal formulation of microcapsules polystyrene as found at lab-scale. However, there is a paucity of scale-up data in the literature. In 2012, K. Mitri et al. [34] attempted the scale-up of nanoemulsions (NEs) produced by emulsification and solvent diffusion process. They established two power-law relationships between droplet size and Reynolds number; and droplet size and shear stress; and compared nanoemulsion droplet diameter in laboratory and pilot scale.

The present study is emphasised on optimisation of the method of preparation of a new formulation of microsponges (MS) loaded with hydrophilic drug 5-fluorouracil (5-FU) by w/o/w emulsion solvent evaporation method to ensure reproducibility of the product and extending its potentiality to scale up. The results obtained during this optimisation made a starting point for the second stage of this study. To our knowledge, this is the first report on the scale-up approach in the preparation of 5- FU loaded microsphere delivery system.

5-FU is chosen as the model drug in the present work, one of the most potent chemotherapeutic drugs. 5-FU is a fluorinated pyrimidine antimetabolite structure similar to

that of the pyrimidine molecules of DNA and RNA; 5-FU interferes with nucleoside metabolism and is converted within the cells into 5-fluorodeoxyuridine monophosphate, which constrains the synthesis of DNA, leading to cytotoxicity and cell death.

This study aims to develop and optimise the preparation method of 5-FU loaded microsphere particles by the design expert software, characterise products, and extend its possibility to scale up.

2. Materials and Methods

2.1. Materials

5-Fluorouracil (Yarrow Chem. India), eudragit RS 100 (Yarrow Chem. India), ethylcellulose (Quest Chemicals, Kolkata), sodium alginate (Quest Chem. Kolkata), carbopol 934 (Loba Chemie, Mumbai, India), tween 80 (Quest Chem. Kolkata), dichloromethane, ethanol and triethanolamine (Quest Chemicals) were purchased. All the reagents are of analytical grade.

2.2. Method of preparation of Microspheres

Accurately weighed 50 mg of drug 5-FU was mixed with a specified amount of polymer mixture (ethylcellulose and eudragit RS 100, in the ratio 1:1) and then dispersed in 15 mL of a mixture of solvents (DCM: ethanol, 1:1 v/v) (inner phase). The inner phase was sonicated for 30 minutes to make homogeneous dispersion. 100 mL of aqueous solution of sodium alginate (0.4% w/v) was prepared and then surfactant, tween 80 (0.5-2% w/w) was added to it (external phase). Next, 1 mL of 1% (v/v) of external phase was added to the inner phase and mixed by a cyclo mixer (Remi, CM 101) to prepare w/o emulsion. Then this primary emulsion was added dropwise to the external phase followed by stirring continuously in a mechanical stirrer (Remi motor RQT-124A) at a specified rpm for 4 hr. Upon complete evaporation of solvent during stirring, droplets get hardened, and solid microsphere particles were isolated by filtration (Whatman- 150 mm filter paper). The product was dried in a hot air oven for 6 hr. It was stored in desiccators till further study.

2.3. Method of preparation of gel incorporated with 5-FU-microsphere particles

The gel was prepared with carbopol 934, which is a water-soluble polymer. First, accurately weighed carbopol 934 (0.25% w/v) was mixed with double distilled water (DDW) using a magnetic stirrer at 1200-1400 rpm for 45 min. Then a batch of 10 mg of microsphere particles was incorporated in 1 gm of prepared gel with slow stirring for equal distribution. Then triethanolamine was added 1 to 2 drops to adjust pH 5.5-6. Microsphere particles embedded in carbopol gel were kept overnight, and then it was used for in-vitro release study.

2.4. Experimental Design

Experimental design is the process of planning a study to meet specified objectives. Planning

an experiment properly is essential to get reproducible data. This could eliminate the time-consuming phase, which could not be achieved with the conventional empirical method. Among various designs, the Central Composite Design (CCD) is well suited for fitting a quadratic surface in process optimisation. Response surface methodology, a relation between factors and responses, was used for the experimental design and optimisation with minimum runs of the experiments [35]. This study used 'Design Expert 13 version (Stat-Ease, USA)[36–39] statistical software by selecting 'Face Centered Central Composite Design (FC-CCD)' to generate the run design. After fitting response data in the design table, the software generates ANOVA, fit summary, diagnostics, model graphs (3-D response surface plot and 2-D contour plot). The software analyses the effects of main factors and their interactions on responses. Multiple regression analysis yielded quadratic model (general) relating response variables with the independent variables:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_1X_3 + b_6X_2X_3 + b_7X_1^2 + b_8X_2^2 + b_9X_3^2 \quad (1)$$

where Y_i is the measured response; b_0 is an intercept of the polynomial equation, representing the model's coefficient. b_1 - b_9 represent regression coefficients of main effects (X_1 , X_2 , and X_3), interacting effects (X_1X_2 , X_1X_3 , and X_2X_3) and quadratic effects (X_1^2 , X_2^2 , and X_3^2). A set of 20 experiments as designed by the software was conducted based on 3-factors, 3-levels (amount of polymer (A), stirring speed (B) and concentration of tween 80 (C)) to achieve desired responses (%Yield (R1), Particle size (R2), Entrapment efficiency (R3) and %Release in 8hr (R4). Six replications at the design centre point were utilised to determine the average and residual variance responses variation. The optimised formulation of 5-FU loaded microsphere was selected based on the response targets. Targets were to attain the average value of production yield, minimise particle size, maximise entrapment efficiency (%EE) and maximum release at 8hr, which were set by numerical optimisation. After applying these constraints, several solutions were generated by the software. From these solutions, optimised formulation with maximum desirability function was chosen. Three CPF (Check Point Formulations) were selected from the list of solutions, and experiments were conducted to get actual responses compared with predicted values to validate the models.

The ranges or levels of independent variables were determined through preliminary trials and displayed in Table 1. The value of α was fixed at 1 for face-centred design. Each variable in the design was studied at three different coded levels (-1, 0, 1).

Table 1. Selected independent process control variables and their levels (coded and actual) for the preparation of 5-FU loaded microspheres.

Independent variables	Low (-1)	Medium(0)	High(+1)
Polymer, (mg),(A)	200	400	600
SS(Stirring speed)(rpm) (B)	800	1000	1200
SA(surfactant) (%w/w) (C)	0.5	1.25	2

2.5.Characterisation of API, ingredients and product microsphere:

2.5.1. Study of Fourier Transform Infrared Spectroscopy (FTIR)

IR spectra of drug, polymer, physical mixture of drug-polymer and drug-loaded microsphere give information on functional groups and interaction between drug and polymers used. Infrared spectra of the solid samples were recorded in the solid-state by the KBr disk method over a wavenumber range of 4000–600 cm⁻¹ in an FTIR spectrophotometer (Bruker FTIR, Model- Alpha, Germany).

2.5.2. Differential Scanning Calorimetric analysis (DSC)

DSC analysis measures heat, fusion heat, and enthalpic changes associated with the physical and chemical transition. DSC is used to determine the purity of drugs and to check thermal behavior and crystallinity of samples. 2 - 5 mg of sample was heated in sealed aluminium pans from 30 to 500 °C at a scanning rate of 10°C/min under nitrogen atmosphere. DSC analysis is performed in Pyris Diamond TG/DTA (Perkins Elmer Instruments, Mumbai). DSC thermogram depicts the profile of heat flow vs temperature.

2.5.3. Scanning Electron Microscopy (SEM)

The surface morphology, shape and size of microsphere particles can be analysed using Scanning Electron Microscopy (Carl-Zeiss, SEM, Tokyo, Japan). Particles were mounted on a metal stub with conductive tape. Particles were coated with a thin coating of platinum under reduced pressure.

2.5.4. Determination of yield (%)

Each batch of dried microspheres was weighed accurately, and yield was calculated as a percentage using the following equation:

$$Yield(\%) = \frac{\text{weight of microspheres}}{\text{weight of polymer} + \text{weight of drug}} * 100 \quad (2)$$

2.5.5. Determination of Drug Entrapment Efficiency

Drug entrapment efficiency was determined by adopting the solvent extraction method. First, the amount of the drug was estimated in a UV-VIS spectrophotometer (ANALAB UV – 180). Then, accurately weighed 10 mg of microsphere particles was dissolved in 5 mL of methanol

in a magnetic stirrer for 20 min. After a clear solution was formed, 20 mL of fresh phosphate buffer solution (PBS) was added and heated to 45-50°C. After evaporation of methanol, it was cooled down to 25°C and filtered. The concentration of the drug was determined by UV spectroscopy at λ_{\max} 265 nm after suitable dilutions (PBS 7.4). A standard curve plot calculated the concentration of the drug. The following formula is used to calculate drug Encapsulation efficiency (DEE%),

$$DEE\% = 100 * \left(\frac{\text{Actual drug content of microsponges}}{\text{Theoretical drug content of microsponges}} \right) \quad (3)$$

2.5.6. Particle size analysis

The average particle size of microsponges for 50 particles of each batch (in run design) was measured by optical microscope (GOKO- Miamb, Japan). First, average particle size was determined.

2.5.7. Study of *in vitro* drug release (diffusion) for gel containing microsphere

In vitro, drug release studies were carried out using Franz's diffusion cell (Remco, India) at 37°C±0.5. The study was carried out at two pH conditions, pH 5.5 (mimicking skin condition) and pH 7.4 (mimicking systemic absorption). Cellophane membrane (0.45 µm) was attached at the bottom end of the donor cell. A batch of 1gm of gel containing 10 mg of microsphere formulation was placed in the donor cell. Drug content (amount of drug /amount of polymer) depends on the polymer used for any particular formulation. All the design formulations were prepared with a fixed amount of the drug (50 mg). The receptor cell contains 50 mL of eluting medium (PBS) and is stirred at 450 rpm by a magnetic stirrer. Samples of 5 L were withdrawn at predetermined time intervals, and immediately replaced by the same volume of fresh PBS. The aliquots were assayed in a UV spectrophotometer (ANALAB UV-180) to determine drug concentration at λ_{\max} 265 nm. The cumulative per cent release (CPR) was plotted against time. Each experiment was repeated thrice.

2.5.8. Determination of kinetics of drug release from the gel

To understand the mechanism of drug release from gel loaded with microsphere formulations, the release data were computed to various mathematical models: zero-order equation ($Q_t = Q_o + k_o t$), first-order equation ($\ln Q_t = \ln Q_o + k_1 t$), Higuchi's model ($Q_t = \ln Q_o + k_H \sqrt{t}$), Korsmeyer-Peppas model ($M_T / M_\infty = k_p t^n$) and Hixon Crowell model ($Q_t^{1/3} - Q_o^{1/3} = k_{HC} t$) to evaluate the drug release mechanisms [40]. Where, Q_o = initial amount of drug release; Q_t = amount of drug release at time t; k_o , k_1 , k_H , k_p and k_{HC} are release rate constants of each model equation; M_T/M_∞ = fraction of drug release at time t, and n is release

exponent [40]. The following plots were constructed: Q_t against t (zero order), $[\ln Q_t - \ln Q_0]$ against t (First-order kinetic model), Q_t against $t^{1/2}$ (Higuchi model), $\log (M_t/M_\infty)$ against $\log t$ (Korsmeyer-Peppas model), and cube root of drug amount remaining in dosage form against time (Hixson-Crowell model).

2.5.9. Micrometric properties of microsphere formulations

The specific quantity of particles was poured into a 5 mL graduated measuring cylinder, and the volume of initial packing was noted. The bulk density was determined by dividing the weight of the sample by the volume of initial packing. Tapped density of the particles is the ratio of the mass of the powder to the volume occupied by the particles after it has been tapped for a defined period. Tapping was continued until no further change in volume. It was determined by dividing the weight of the sample by the volume of packing after tapping. Hausner's ratio (H_r) is a number that is correlated to the flowability of a particle. It was calculated by dividing tapped density and bulk density. Carr's index % (CI) is an indication of the flowability of particles through a hopper. The formula calculated it: $(1 - 1/H_r)$.

2.5.10. Study of the rheology of gel :

The topical drug in gel form needs adequate consistency to maximise the contact period between the medication and the skin. This can be accomplished by modifying the nature of the vehicle. Therefore, studying the rheology of the product gel is necessary to know its consistency and rheological behavior [41]. Thus, the rheology of gel loaded with microsphere particles was studied by Rheometer (Anton Paar, Austria). Rheological characteristics plots such as strain against viscosity graph, strain against G' (storage modulus) and G'' (loss modulus) and angular frequency vs G' and G'' were generated using Rheoplus/32 Version 3.

3. Results and discussions:

3.1. Parametric Sensitivity and Optimisation

In the present study, the drug 5-FU was embedded in polymeric microsphere by quasi emulsion solvent evaporation method. Few controlling variables were chosen: polymer amount, stirring speed, surfactant concentration, and their effects on response variables were studied. A design of factors' combinations was generated by Design expert version 13 software. Accordingly, 20 batches of products were prepared, and products were characterized to obtain response variables (yield%, average particle size, drug entrapment efficiency% and drug release % in 8 hr). The statistical software analyses the results to generate ANOVA, fit summary and model equations, various graphs using the linear regression square root method to avoid the lack of fit analysis.

Table 2. Design matrix and measured responses of 5-FU loaded microsphere

Run	A: Polymer Mg	B: SS min ⁻¹	C: SA %	Yield %	Particle size micron	EE %	Rel _{8r} %
1	200	800	0.5	90.64	295.88	70.93	50.28
2	600	1000	1.25	73.90	158.34	51.00	88.93
3	400	1000	1.25	74.88	163.43	68.28	57.57
4	400	1000	2	68.22	218.75	85.38	53.86
5	400	1000	1.25	86.97	141.84	55.83	62.21
6	600	800	2	79.28	270.34	82.36	67.82
7	600	1200	2	63.49	147.035	75.98	72.81
8	400	1000	0.5	70.48	233.99	75.63	50.14
9	400	1000	1.25	76.31	164.86	61.64	62.83
10	200	1200	2	60.00	144.36	79.87	58.87
11	200	1200	0.5	67.89	262.05	55.95	58.87
12	200	1000	1.25	79.68	166.73	55.46	73.37
13	600	800	0.5	72.33	273.90	78.00	71.44
14	600	1200	0.5	60.25	254.07	57.42	89.35
15	400	1000	1.25	78.36	236.41	65.72	65.98
16	400	800	1.25	90.40	236.52	69.29	55.85
17	400	1000	1.25	78.39	229.22	63.12	71.31
18	200	800	2	86.16	276.97	79.42	56.88
19	400	1200	1.25	72.17	168.29	61.39	65.85
20	400	1000	1.25	75.42	223.57	71.23	69.17

In Table 2, controlling factors and corresponding responses as obtained from experimental data were shown, yield% varies in the range of 60- 90.64, average particle size changes in the range of 141.84-295.88, EE% varies in the range of 51-85.38, Rel 8hr % varies in the range of 50.28-89.35. For detailed analysis, the present investigation utilised the linear regression method of square root type amongst all the types for all the cases considering constant k to be zero. The fit summary results suggested that the quadratic source be the best-fitted source. The results for all the cases are displayed in Table 3.

Table 3: Comparison of the Adjusted R^2 and Predicted R^2 for the yield %, average particle size, EE% and Rel 8 hr%

	Source	Sequential p-value	Adjusted R^2	Predicted R^2
Yield	Quadratic	<0.0001	0.9918	0.9706
Particle size	Quadratic	0.0173	0.6394	0.6424
EE	Quadratic	<0.0001	0.9644	0.8530
Rel 8hr	Quadratic	<0.0001	0.9614	0.6522

The test results of the following cases (yield%, average particle size, EE% and Rel 8 hr%) are obtained through the ANOVA table represented in Table 4.1-4.4., which suggests that the quadratic model is the best suited for all the cases.

From the table, it can be implied that F-value is 258.86 for yield %, 4.74 for average particle size, 58.13 for EE% and 53.58 for Rel 8 hr%, with p-values of <0.05 for all the cases. Thus, justifying that only 0.01% of possibilities are there for F-outcomes to develop, owing to noise and the outcomes of p-values, suggest that the model terms are fascinating.

Table 4.1: ANOVA for response surface quadratic model for the generation of yield% from Polymers, SS and SA as model inputs.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.55	9	0.5057	256.86	< 0.0001	significant
A-Polymer	0.3918	1	0.3918	198.98	< 0.0001	
B-SS	3.03	1	3.03	1541.07	< 0.0001	
C-SA	0.0069	1	0.0069	3.50	0.0911	
AB	0.1621	1	0.1621	82.33	< 0.0001	
AC	0.2235	1	0.2235	113.50	< 0.0001	
BC	0.0252	1	0.0252	12.77	0.0051	
A ²	0.0012	1	0.0012	0.6054	0.4545	
B ²	0.1323	1	0.1323	67.19	< 0.0001	
C ²	0.5690	1	0.5690	288.99	< 0.0001	

Table 4.2: ANOVA for response surface quadratic model for the generation of 'average particle size' from Polymers, SS and SA as model inputs.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	52.48	9	5.83	4.74	0.0116	significant
A-Polymer	0.1738	1	0.1738	0.1414	0.7148	
B-SS	16.83	1	16.83	13.69	0.0041	
C-SA	8.39	1	8.39	6.82	0.0259	
AB	0.0637	1	0.0637	0.0518	0.8245	
AC	0.0820	1	0.0820	0.0667	0.8014	
BC	6.70	1	6.70	5.45	0.0418	
A ²	0.8939	1	0.8939	0.7271	0.4138	
B ²	2.02	1	2.02	1.65	0.2284	
C ²	8.19	1	8.19	6.66	0.0274	

Table 4.3: ANOVA for response surface quadratic model for the generation of EE% from Polymers, SS and SA as model inputs.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.79	9	0.7540	58.13	< 0.0001	significant
A-Polymer	0.0021	1	0.0021	0.1616	0.6962	
B-SS	0.8461	1	0.8461	65.23	< 0.0001	
C-SA	1.50	1	1.50	115.64	< 0.0001	
AB	0.0605	1	0.0605	4.67	0.0561	
AC	0.0398	1	0.0398	3.07	0.1104	
BC	0.4338	1	0.4338	33.44	0.0002	
A ²	1.18	1	1.18	91.07	< 0.0001	
B ²	0.0939	1	0.0939	7.24	0.0227	
C ²	2.85	1	2.85	219.99	< 0.0001	

Table 4.4: ANOVA for response surface quadratic model for the generation of Rel 8 hr% from Polymers, SS and SA as model inputs.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.73	9	0.8592	53.58	< 0.0001	significant

A-Polymer	2.43	1	2.43	151.78	< 0.0001
B-SS	0.9307	1	0.9307	58.04	< 0.0001
C-SA	0.0039	1	0.0039	0.2459	0.6307
AB	0.0002	1	0.0002	0.0130	0.9114
AC	0.6126	1	0.6126	38.20	0.0001
BC	0.0348	1	0.0348	2.17	0.1715
A ²	3.50	1	3.50	217.97	< 0.0001
B ²	0.0995	1	0.0995	6.21	0.0319
C ²	1.20	1	1.20	74.85	< 0.0001

Figure 1.a. (2D contour plots and 3D response surface plots) illustrate the effects of two controlling variables, polymer and stirring speed (A against B, C=1.25) on yield%, and stirring speed and SA concentration (B against C, A=400) on particle size. It is evident from Table 2 and Figure 1. a. that with the increase of polymer amount yield% decreases, and with the increase of stirring speed, yield% decreases when third factor SA (surface-active substance) is fixed at the centre point. In the case of yield %, polymer amount is dominating factor. Plots (Figure 1. b) (2D and 3D) of particle size (B against C, A=400) illustrates that the response decreases with the increase of stirring speed (factor B) at any fixed value of factor C. At any specific value of factor B, particle size decreases up to the centre level of C and then increases at a higher level. In the case of particle size factors, SA and SS have more control over particle size.

In the case of EE (%), factors B and C have more control over the entrapment of the drug than that of polymer amount. Figure 1. c (2D contour plots and 3D response surface plots) illustrates the effects of two controlling variables (B against C) on the responses while keeping the third factor (A=400) constant at the centre level. EE% decreases with the increase of SA% up to its centre level then increases, and EE% decreases with the addition of stirring speed (B) at any specific value of the C factor.

In the case of Rel 8hr (%), factors A and C have more significant effects on the release of drugs from microsphere particles. From the plot (figure 1.d) (A against C at B=1000) and the model equation, it was observed that Rel 8hr (%) increases when SA(%) was increased up to its centre level. It decreases at a higher level of C, at any specific level of factor A factor. On the other hand, the increase of A factor decreases release% up to the middle level and increases at a higher level, at any fixed factor C factor.

On further development, Fit statistics represented in Table 3 suggests that the Predicted R^2 is in reasonable agreement with the Adjusted R^2 for the case of yield %, average particle size, EE% as the difference is less than 0.2. But in the case of drug release % in 8 hr, the difference is not in close range, which may indicate a significant block effect or possible complications with the block of data. But as the Adequate Precision (ratio of the signal to noise) is 28.035, which is greater than 4, it suggests an adequate indication and can be used for further process. Coefficients in terms of coded factors, displayed in Table 5.1 – 5.4, the VIF (Variance Inflation Factor) should be less than 10 to be tolerable. When the VIF is 1, it suggests that the factors are orthogonal; if the VIF is greater than 1, it indicates multicollinearity factors. If the VIF is higher, then more severe the correlation of factors results in an intolerable outcome. Therefore, the actual equation of the generation of yield %, average particle size, EE% and drug release % in 8 hr are represented by the equations 4-7 with figures 1. a - 1.d for the generation of yield %, average particle size, EE% and drug release % in 8 hr respectively.

Table 5.1: Coefficients in terms of coded factors for the generation of yield %

Factor	Coefficient	df	Standard Error	95% CI		VIF
	Estimate			Low	High	
Intercept	8.75	1	0.0153	8.72	8.79	
A- Polymer	-0.1979	1	0.0140	-0.2292	-0.1667	1.0000
B-SS	-0.5508	1	0.0140	-0.5821	-0.5196	1.0000
C-SA	-0.0262	1	0.0140	-0.0575	0.0050	1.0000
AB	0.1423	1	0.0157	0.1074	0.1773	1.0000
AC	0.1671	1	0.0157	0.1322	0.2021	1.0000
BC	-0.0561	1	0.0157	-0.0910	-0.0211	1.0000
A ²	-0.0208	1	0.0268	-0.0804	0.0388	1.82
B ²	0.2193	1	0.0268	0.1597	0.2790	1.82
C ²	-0.4549	1	0.0268	-0.5145	-0.3953	1.82

Table 5.2: Coefficients in terms of coded factors for the generation of average particle size

Factor	Coefficient	df	Standard Error	95% CI		VIF
	Estimate			Low	High	
Intercept	13.36	1	0.3812	12.51	14.21	
A- Polymer	-0.1318	1	0.3506	-0.9131	0.6494	1.0000

B-SS	-1.30	1	0.3506	-2.08	-0.5161	1.0000
C-SA	-0.9160	1	0.3506	-1.70	-0.1347	1.0000
AB	0.0893	1	0.3920	-0.7842	0.9627	1.0000
AC	0.1013	1	0.3920	-0.7722	0.9747	1.0000
BC	-0.9150	1	0.3920	-1.79	-0.0416	1.0000
A ²	-0.5701	1	0.6686	-2.06	0.9196	1.82
B ²	0.8579	1	0.6686	-0.6318	2.35	1.82
C ²	1.73	1	0.6686	0.2357	3.22	1.82

Table 5.3: Coefficients in terms of coded factors for the generation of EE%

Factor	Coefficient	df	Standard Error	95% CI		VIF
	Estimate			Low	High	
Intercept	7.95	1	0.0392	7.86	8.03	
A- Polymer	0.0145	1	0.0360	-0.0658	0.0947	1.0000
B-SS	-0.2909	1	0.0360	-0.3711	-0.2106	1.0000
C-SA	0.3873	1	0.0360	0.3070	0.4675	1.0000
AB	-0.0870	1	0.0403	-0.1767	0.0027	1.0000
AC	-0.0705	1	0.0403	-0.1603	0.0192	1.0000
BC	0.2329	1	0.0403	0.1431	0.3226	1.0000
A ²	-0.6554	1	0.0687	-0.8084	-0.5024	1.82
B ²	0.1847	1	0.0687	0.0317	0.3378	1.82
C ²	1.02	1	0.0687	0.8656	1.17	1.82

Table 5.4: Coefficients in terms of coded factors for the generation of drug release % in 8 hr

Factor	Coefficient	df	Standard Error	95% CI		VIF
	Estimate			Low	High	
Intercept	7.88	1	0.0435	7.78	7.98	
A- Polymer	0.4933	1	0.0400	0.4041	0.5826	1.0000
B-SS	0.3051	1	0.0400	0.2158	0.3943	1.0000
C-SA	0.0199	1	0.0400	-0.0694	0.1091	1.0000
AB	-0.0051	1	0.0448	-0.1049	0.0946	1.0000
AC	-0.2767	1	0.0448	-0.3765	-0.1770	1.0000

BC	-0.0660	1	0.0448	-0.1657	0.0338	1.0000
A ²	1.13	1	0.0764	0.9572	1.30	1.82
B ²	-0.1903	1	0.0764	-0.3604	-0.0201	1.82
C ²	-0.6606	1	0.0764	-0.8308	-0.4905	1.82

Thus when compared with the other model statistics represented in Table 5.1 -5.4, it suggests that the quadratic model is in the comparison spectrum of the other model statistics like PRESS (Predicted Residual Error Sum of Squares), -2 log-likelihood, BIC (alternate to AICc) and AICc (Akaike's Information Criterion).

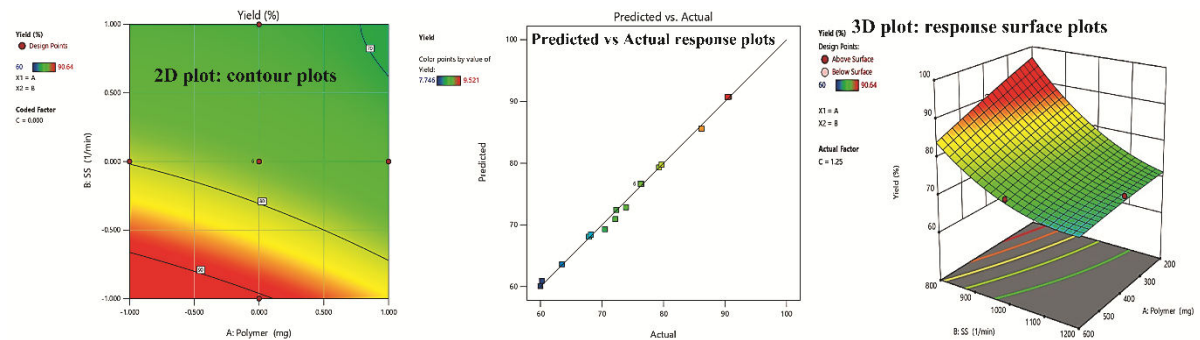


Figure 1.a. 2-D contour plots, 3-D response surface plots and Predicted against Actual response plots for the responses, Yield%

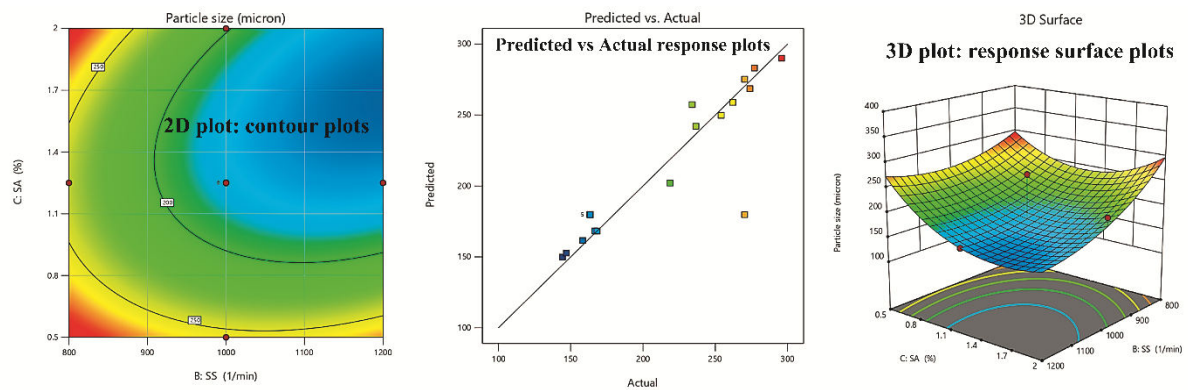


Figure 1.b. 2-D contour plots, 3-D response surface plots, and Predicted against Actual response plots for particle size (micron) responses.

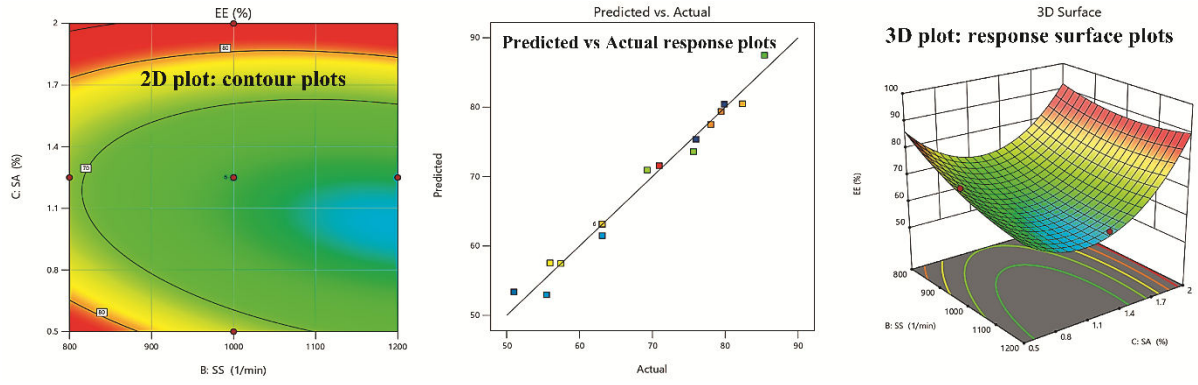


Figure 1.c. 2-D contour plots, 3-D response surface plots, and Predicted against Actual response plots, EE%.

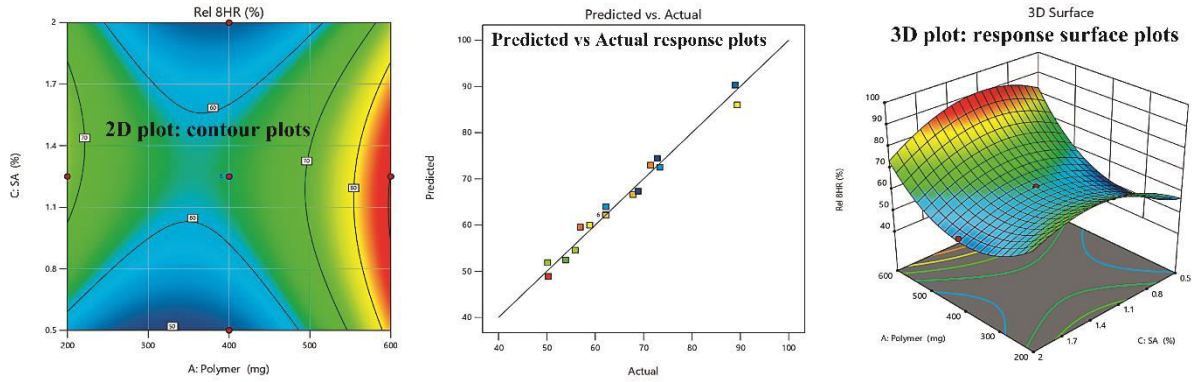


Figure 1.d. 2-D contour plots, 3-D response surface plots and Predicted against Actual response plots for the responses, drug release % in 8 hr.

$$\begin{aligned} \text{Sqrt}(\text{Yield}\%) = & 17.59783 - 0.005525 * \text{Polymer} - 0.014677 * \text{SS} + 1.91479 * \text{SA} + \\ & 3.55860E-06 * \text{Polymer} * \text{SS} + 0.00114 * \text{Polymer} * \text{SA} \\ & - 0.000374 * \text{SS} * \text{SA} - 5.20489E-07 * \text{Polymer}^2 \\ & + 5.48336E-06 * \text{SS}^2 - 0.808657 * \text{SA}^2 \end{aligned} \quad (4)$$

$$\begin{aligned} \text{Sqrt}(\text{average particle size}) = & 39.20616 + 0.007669 * \text{Polymer} - 0.0422651 * \text{SA} \\ & - 3.05997 * \text{SA} + 2.23143E-06 * \text{Polymer} * \text{SS} \\ & + 0.000675 * \text{Polymer} * \text{SA} - 0.006100 * \text{SS} * \text{SA} \\ & - 0.000014 * \text{Polymer}^2 + 0.000021 * \text{SS}^2 \\ & + 3.06748 * \text{SA}^2 \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Sqrt}(\text{EE}\%) = & 14.38846 + 0.015943 * \text{Polymer} - 0.011762 * \text{SS} - 5.37515 * \text{SA} \\ & - 2.17482E-06 * \text{Polymer} * \text{SS} - 0.000470 * \text{Polymer} * \text{SA} \\ & + 0.001552 * \text{SS} * \text{SA} - 0.000016 * \text{Polymer}^2 \\ & + 4.61844E-06 * \text{SS}^2 + 1.81092 * \text{SA}^2 \end{aligned} \quad (6)$$

$$\begin{aligned}
Sqrt(Rel\ 8\ hr\%) = & 1.73034 - 0.017647 * Polymer + 0.011639 * SS + 4.14018 * SA \\
& - 1.27718E-07 * Polymer * SS - 0.001845 * Polymer * SA \\
& - 0.000440 * SS * SA + 0.000028 * Polymer^2 \\
& - 4.75655E-06 * SS^2 - 1.17444 * SA^2
\end{aligned} \tag{7}$$

3.1.1. Numerical optimisation for yield %, average particle size, EE % and drug release % in 8 hr and post-analysis.

Figure 2 suggest Polymer, SS, SA as a function of yield %, average particle size, Entrapment efficiency % and drug release % in 8 hrs in optimised condition. Similarly, like the other figures, it can be inferred from the model that the desirability objective function D(X) can be expressed as follows;

$$D = (d_1^{r_1} \square d_1^{r_2} \square \square \square d_1^{r_n})^{\frac{1}{\sum r_i}} = \left(\prod_{i=1}^n d_i^{r_i} \right)^{\frac{1}{\sum r_i}} \tag{8}$$

Where n is the number of responses in the measure and desirable ranges for each response as d_i , the desirability function D(X) is based on each response assigned importance with the other responses. Importance (r_i) fluctuates from the least importance (+) a value of 1, to the most important (+++++) a value of 5. If all the important values are similar, then the objective function reduces to normal form. Constraints were set as per the following table. Various criteria of factors and responses were set for numerical optimisation, as shown in Table 6. The desirability is 0.737 for all the cases represented in figure 2. A high value of desirability coefficient ($0 < y < 1$) indicates that the operating points can produce an acceptable formulation. Based on input conditions of factors (Table 6), the design expert software generated a list of solutions.

Table 6. Criteria for numerical optimisation

Parameter	Goal	Lower limit	Upper limit
A: Polymer (mg)	In range	200	600
B: SS (min^{-1})	In range	800	1200
C: SA (%)	In range	0.5	2
R ₁ - Yield (%)	In range	60	90.64

R ₂ - Particle size (micron)	Minimized	141.84	291.88
R ₃ - EE (%)	Maximized	51	85.38
R ₄ - Rel 8h (%)	Maximized	50.14	89.35

The factor combinations (CPF1, CPF2 and CPF3) were chosen from the listed solutions to check the validity of the models by comparing experimental values and predicted values obtained from model equations.

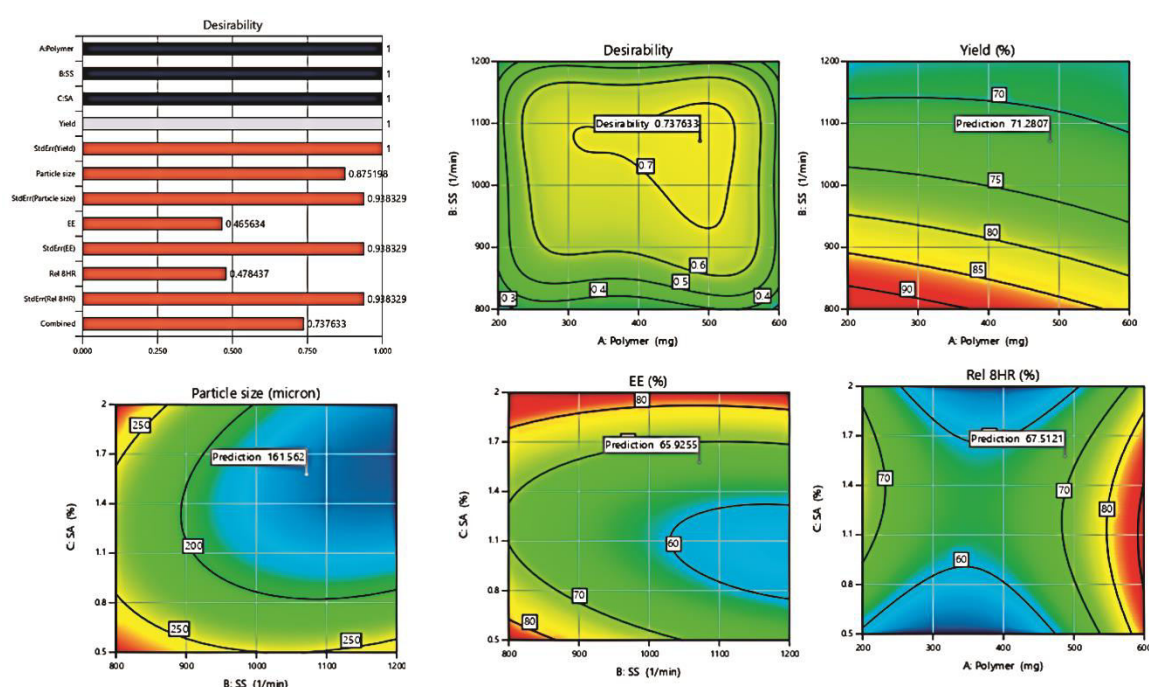


Figure 2: The schematic representation of desirability and prediction of yield%, average particle size (micron), EE%, drug release % in 8 hr as a function of polymer and SS

Table 7. Checkpoint formulations and responses.

	Factors	Responses	Experimental value	Predicted value	% Error
CPF1	Polymer-600 mg	Yield (%)	63.6257	61.5362	3.28405
	SS- 1198 min ⁻¹	Particle size (μm)	151.563	148.1948	2.22231
	SA- 2 %	EE (%)	75.3199	73.5508	2.348782

		Rel8hr (%)	74.4607	76.0979	-2.19874
CPF2	Polymer-200 mg	Yield (%)	64.0279	62.0668	3.062884
	SS- 1144 min ⁻¹	Particle size (µm)	149.203	153.3379	-2.77132
	SA- 1.9 %	EE (%)	72.8128	69.9038	3.995177
		Rel8hr (%)	69.8736	67.1058	3.961153
CPF3	Polymer-593 mg	Yield (%)	65.614	63.6514	2.99113
	SS-1199min ⁻¹	Particle size (µm)	151.137	145.4456	3.765722
	SA- 2 %	EE (%)	69.9459	67.8036	3.062796
		Rel8hr (%)	76.9556	74.9653	2.586297

From the result (Table 7), it was observed that % error is within $\pm 4\%$. Experimental values are very close to the predicted values, suggesting that the optimised formulation was reasonable and reliable. The models are thus validated. The combination of factors, polymer-600 mg, SS-1197.54 min⁻¹, SA-2 % (CPF1), is considered as optimum one as it showed maximum desirability factor (δ) of 0.737 and the product microsphere is optimised, which showed experimental yield 63.625 %, particle size 151.563 micron, entrapment efficiency 75.319 %, drug release from microsphere –gel 74.46%. A release study was conducted with the checkpoint formulations using an eluting fluid of pH7.4 and 5.5 phosphate buffer to mimic conditions in the oral and dermal systems.

3.2. Scale-up for more significant batch production of 5-FU microsphere gel

Reproducibility of a method can be checked for a larger batch size of the product by scale-up technique. To convert the formulation prepared in small scale to higher scale production, geometric similarities were maintained as much as possible with the power-law approach. The same stirring speed had been maintained on a larger scale. Specifications maintained in scale-up were displayed in Table 8. System geometry of scale-up was beakers diameter (6.8, 9.6 and 13.4 cm), impellers diameter (3.7, 5.2 and 7.3 cm) and clearance (1.13, 1.586 and 2.226 cm). Volumes of continuous phase were taken as per scale up. Shape factors or system geometry should be identical on each batch. The batch of products produced at each scale showed similar characteristics as that of optimised batches, such as yield, particle size, entrapment efficiency and Rel8hr (Table 9).

Table 8. Specifications maintained in the scale-up process

Parameters	Optimized batch	Scale- 1 batch	Scale- 2 batch
5-FU (mg)	50	100	400
Polymer (mg)	600	1200	4800
Volume of dispersion phase (mL)	15	30	120
Volume of continuous phase (mL)	100	200	800
Impeller dia.(Da-cm)	3.7	5.2	7.3
Beaker diameter (T-cm)	6.8	9.6	13.4
S1 (Da/T)	0.544	0.542	0.544
Impellor clearance from bottom (E-cm)	1.13	1.586	2.226
S2 (E/ Da)	0.305	0.305	0.305
Stirring rate (sec ⁻¹),N	20 sec ⁻¹	20 sec ⁻¹	20 sec ⁻¹
Density of dispersion phase ρ (gm/mL)	1.06	1.06	1.06
Viscosity of dispersion phase η (cp)	1.55	1.55	1.55
Reynolds no. ($N_{Re} = nDa^2\rho/\eta$)	18724	36983	72886

Table 9. Characterisations of batches and optimised 5-FU microsponges

Scale-up	Yield (%)	Particle size(μ m)	EE (%)	Rel. 8hr. (%)	
				pH 7.4	pH 5.5
Optimised batch	64.17	152.63	75.05	75.75	77.93
Scale- 1 batch	64.03	151.69	75.24	74.58	79.42
Scale- 2 batch	63.91	152.18	74.87	76.08	80.67

To verify the method's effectiveness, an approach was made to apply it to a higher scale. Table 9 shows the input data and response variables for three batches with increasing volume. The optimised formulation was produced in 100 mL of the continuous phase, and the scale was increased to 100mL:200mL:800 mL to check the reproducibility of quality and yield % of the product. The same system geometry (S_1 , S_2) was maintained at a larger scale. Reynolds

number is doubled on the larger scale. Similar rpm (1200 /min =20/sec) was kept on a higher scale as per power law ($N_2/N_1 = (D1/D2)^n$) by putting n equal to zero. Table 9 gives the evidence that products formed in scale-up volumes were similar concerning yield%, average particle size, entrapment efficiency and cumulative release% in 8 hr. Like any other chemical industry ,pharmaceutical manufacturing units too require to adopt scale up from the laboratory to the pilot plant to total production. The transition from one scale to another may cause alterations in macroscopic and microscopic properties of formulation components and products at different production scales [42]. The right Power-law approach in conjunction with geometric similarity [43] to the optimised formulation developed by a design expert is helpful in the scale-up method. Effectiveness of the scale-up procedure had been reported by L. Sánchez-Silva et al. [33] and K. Mitri et al. [34] who attempted to prepare microcapsules polystyrene and nanoemulsions (NEs) by emulsification and solvent diffusion process. In 2020, M. Rakicka –Pustułka et al. (Rakicka-Pustułka et al., 2020) reported the scale-up of microbial erythritol production from glycerol using the *Yarrowia lipolytica* strain MK1. Studying the effect of process variables on the product quality and the scale-up analysis of these processes would facilitate giving an idea about what will happen at a commercial scale. Thus it saves money and time in unproductive trial tests.

3.3. Drug release study of optimised and scaled up batches:

The study of drug diffusion is crucial to assure its release from microsp sponge products obtained from the optimised and scaled up batches. Release study was conducted in Franz diffusion cell containing receptor medium as aqueous phosphate buffer of pH 7.4 and pH 5.5 to mimic diffusion in the oral and dermal systems respectively. Drug load is ~625 µg / 10 gm microsp sponge per 1 gm of gel formulation. The content of the drug in each microsp sponge sample used for the release study is nearly the same. Therefore, the release pattern shown by the profiles were observed as identical (vide Figure 3.). Drug release in the buffer of pH 5.5 was observed ~4% higher than that of pH 7.4 as the drug is weakly basic.

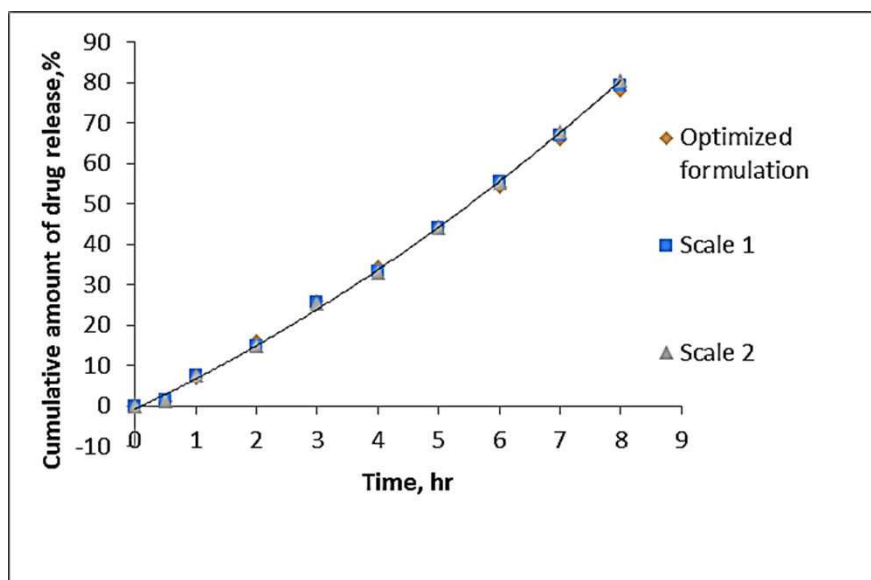


Figure 3. Plot of drug release study (in pH 5.5) profiles for the Optimised formulation, Scale 1 and Scale 2.

Micrometric properties such as bulk densities, tapped densities, Carr's index were found as 0.11-0.34 gm/mL, 0.11-0.39 gm/mL, 5-18.46% respectively for the products in 20 runs. No significant difference between bulk and tapped densities was found, suggesting uniform particle size and more sphericity. Optimized formulation showed bulk density, tapped density and Carr's index as 0.25 gm/mL, 0.2857 gm/mL and 12.49 % respectively. In consideration of Carr's index, microsphere particles appeared to have good flowability, which is desired parameter in tablet/capsule filling.

Release mechanisms were established by plotting the cumulative percentage of drug release (CPR) against time (zero-order, $R^2 = 0.992$), \ln (remaining drug%) against time (first order, $R^2 = 0.9964$), cubic function (Hixon Crowel model, $R^2 = 0.9979$), against time. From regression coefficients, it seems drug release from gel formulation fits with the above mentioned kinetic models.

3.4. Drug-polymer solid-state characteristics using FTIR (Fourier Transform Infrared Spectroscopy) and Differential scanning calorimetry (DSC)

The FTIR spectra of drug, polymer, drug-polymer physical mixture and optimised microsphere formulation are given in the figures below. Figure 4 shows the FTIR of 5-FU (drug), ethylcellulose, Eudragit RS 100, the physical mixture of drug-polymer (1:1) and microsphere formulation. Each spectrum was a plot as Wavenumber (cm^{-1}) against %Transmittance.

The spectrum of pure 5-FU (Fig. 4a) showed characteristics peaks of 1648.5 cm^{-1} and 3067.8 cm^{-1} owing to N-H bending and stretching. Characteristics peaks were seen at 1241 cm^{-1} and 1349.7 cm^{-1} owing to C-F stretching. The peak at 1720.2 cm^{-1} indicated C=O stretching, 1427.2 cm^{-1} indicated C=C stretching of carboxylate groups. Characteristics peaks exhibited at 2929.5 cm^{-1} and 743.8 cm^{-1} were due to the C-H stretch of alkane and aromatics (out of plane bend).

The FTIR spectra of ethyl cellulose (Figure 4b) showed peaks at 3648.8 and 2976.5 cm^{-1} owing to OH and CH groups. Peaks at 1454.8 and 1374.6 cm^{-1} indicated the stretching of CH_2 and CH_3 groups. C-O-C stretching at 1109 cm^{-1} and the peak at 1056.4 cm^{-1} was attributed to the OH bond. Peak was shown at 882.13 cm^{-1} owing to N-H bending.

The FTIR spectra of Eudragit RS 100 (Figure 4c) showed peaks at 2343 and 2376 cm^{-1} , representing the CH group's stretching. The peak at 3568.6 cm^{-1} showed N-H stretching, 1720.4 cm^{-1} showed stretching of C=O groups and a peak at 1457.6 cm^{-1} representing CH_3 stretch. In addition, peaks were established at 1145 and 992.8 cm^{-1} owing to C-H bending and C-O stretching vibrations.

The FTIR spectra of the physical mixture (Figure 4d) showed some characteristics peaks (3067.8, 1656.8, 1720.4 and 1429.9 cm^{-1}) of the drug and characteristics peaks (871.06, 3648.5, 1429.9, 2340.2 and 992.8 cm^{-1}) of the polymers. Therefore, it does not confirm any chemical incompatibility between the drug and the polymer.

The FTIR spectra of microsphere formulation (Figure 4e) showed resemblance with the FTIR spectra of the physical mixture. Some of the peaks of the drug are not visible in the FTIR spectrum of microsphere formulation. It suggests that some functional groups of drugs may form weak Vander Waals force with that of polymers. It showed characteristics peaks with slight shifts as found in drug spectra. It is suggested from the FTIR study that there is no chemical incompatibility of drugs with the polymers.

Differential scanning calorimetry or DSC is a technique in which the difference in the amount of heat required to increase temperature (30-500°C) of a sample and reference is measured as a function of temperature. Thus, both the sample and reference are maintained at nearly the same temperature throughout the experiment under a nitrogen purge of 25mL/min. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time.

The DSC thermogram of pure 5-FU is shown in Figure 5(a). It displays a sharp endothermic peak at 282°C, which corresponds to the melting point of 5-FU. Melting points of eudragit RS 100 (Figure 5, b) as 398°C and ethyl cellulose (Figure 5, c) as 220°C were much different from the melting point of the drug alone or in the formulation. DSC thermogram of the physical mixture (Figure 5,d) showed melting points of the drug, ethylcellulose and eudragit RS 100 with slight shifting. The absence of a 5-FU crystalline peak, which should have appeared at ~ 282°C, proved that the drug was in an amorphous state in drug-loaded microsphere particles (e). The above studies may confirm that the drug has no chemical incompatibility with the polymer. The second profile in each graph plot stands for TGA

(Thermogravimetric Analysis).

3.5. Surface Morphology of microsponges (SEM)

Figure 6 depicts the morphology of microsphere particles with a rough and fine porous surface. The rough surface of microspheres is due to high polymer content, shearing effect and solidification of large-sized globules in the emulsion at a high solvent evaporation rate. The particle's surface appears more to be more porous after 1hr of drug release as drug elutes from porous structure; pores were non-uniform and irregular and more extensive in shape due to erosion of the drug.

3.6. Rheology of Gel

Rheology is the study of the flow of matter, primarily in a liquid state, soft solids. It is a branch of physics that deals with the deformation and the flow of materials. Many of the materials we use each day are structured fluids. Several soft semisolid materials also fall under structured fluids since they have a multiphase structure and exhibit complex flow behavior. Many factors affect the stability of structured fluids. The structured fluid does not obey a simple linear relationship between applied stresses and flow (Newtonian fluid behavior). The rheological property of gel loaded with optimised microsphere was investigated in three test methods: flow behavior, amplitude/strain sweep and frequency sweep. In Figure 7, the flow curve of the gel is shown. The viscosity of gel was high at a meagre shear rate (953 Pa.s at 0.0018 sec^{-1}). Viscosity is dropped at higher rates of shear rate (9.93 Pa.s at 100 sec^{-1}). This is the ideal phenomenon of gel (shear thinning), which becomes more extensive as the shear rate increases. It showed pseudoplastic properties because viscosity decreased after increasing shear rate, which causes better drug release.

Figure 7 showed amplitude sweep analysis. It was performed to assess the linear viscoelastic range and viscoelastic properties of the polymer. The applied strain range within which G' and G'' remain constant represents the linear viscoelastic range (LVE). The strength of the gel was so high that's why its linear part is longer. At specific strain (50.1%), G' was declined. It was suggested that from this amount, strain% breakdown of the structure started. G'' is more significant than G' which indicated that gel was highly structured with elastic characteristics. Usually, the rheological properties of a visco-elastic material are independent of strain up. Beyond this critical strain level, the material's behavior is non-linear, and the storage modulus declines. So, measuring the strain amplitude, the dependence of the storage and loss moduli (G' , G'') is an excellent first step taken in characterising viscoelastic behavior: A strain sweep will establish the extent of the material's linearity. In this graph, G'' is more significant than G'

indicating the gel becomes progressively more fluid-like and the module decline.

Frequency sweep analysis within the LVE range obtained from the amplitude sweep test indicates the structural integrity and mechanical strength of material more precisely and accurately [44] (Figure 7). The structural integrity of the sample was determined by the structural response to deformation at longer and shorter oscillatory stress (100-0.1 rad/sec). Higher values of storage modulus (G') over the loss modulus indicate a solid elastic gel. Higher yield stress due to the sample was unable to show any crossover point. Moreover, the absence of any crossover region stated a lack of gel to solid transformation. In a frequency sweep, measurements are made over a range of oscillation frequencies at a constant oscillation amplitude and temperature. Below the critical strain, the elastic modulus G' is often nearly independent of frequency, as would be expected from a structured or solid-like material.

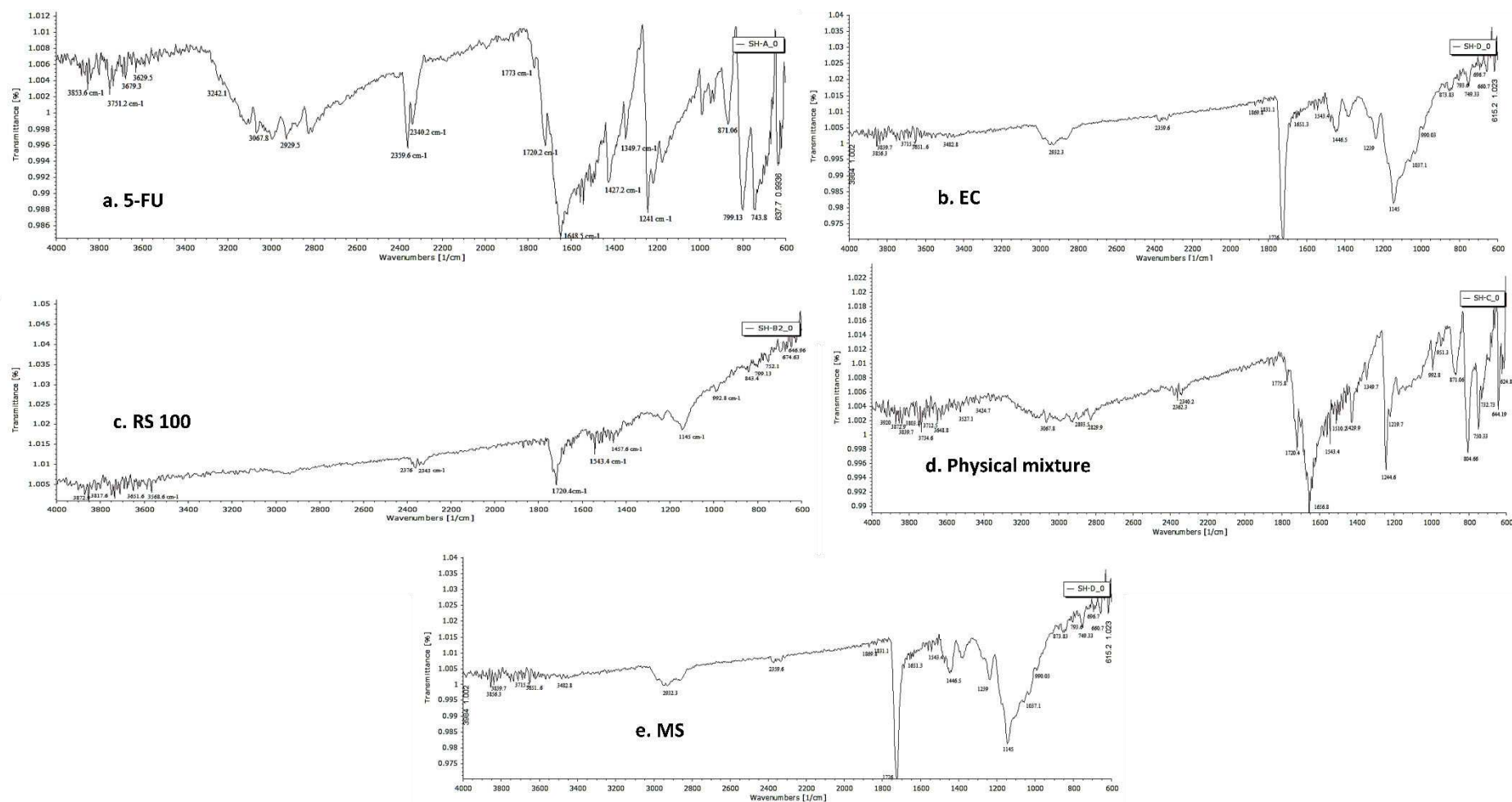


Figure 4. FTIR spectra of (a.) 5-FU, (b.) Ethyl Cellulose, (c.) Eudragit RS100, (d.) Physical mixture (1:1) and (e.) MS Formulation.

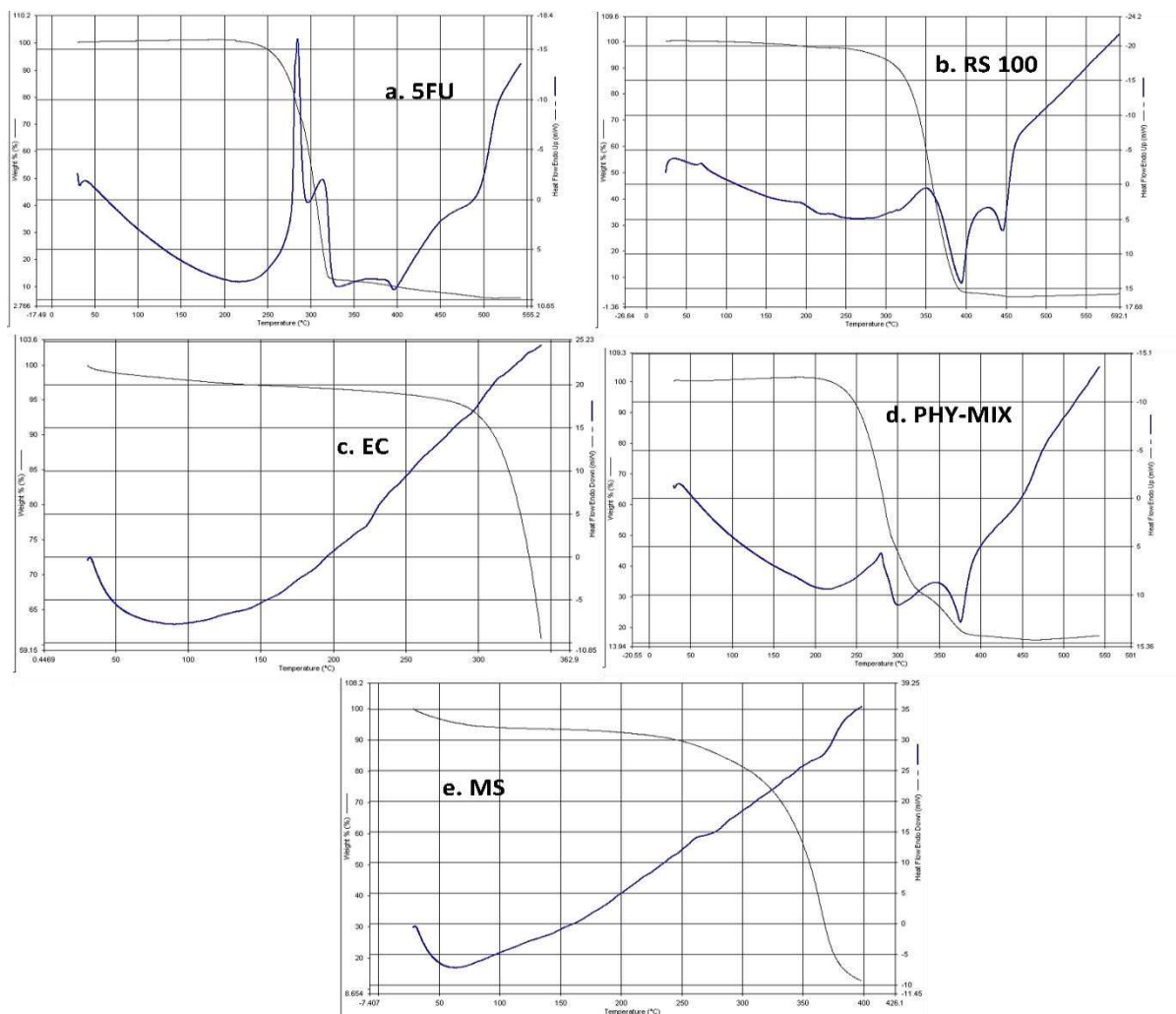
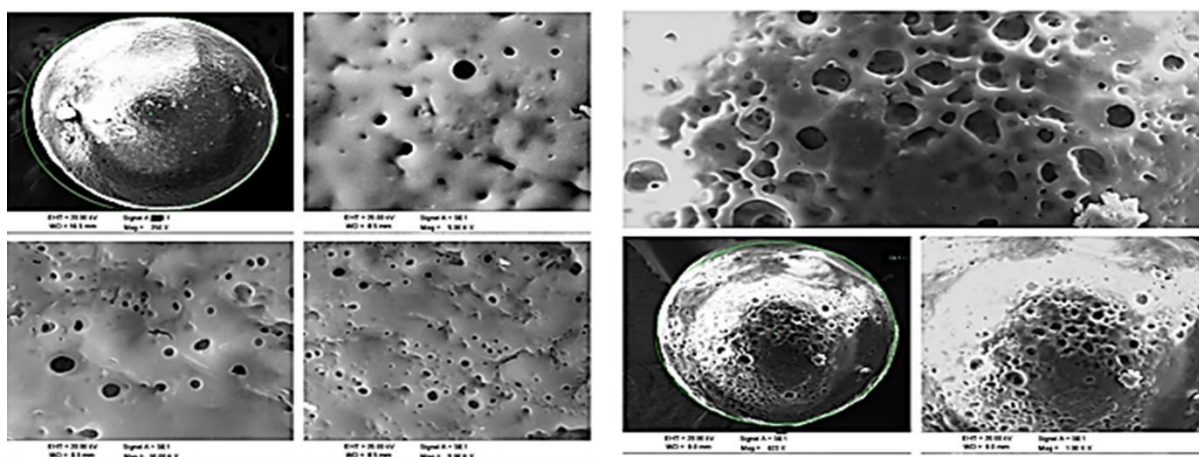


Figure 5. DSC of (a) pure 5-FU, (b) Eudragit RS-100, (c) Ethyl Cellulose, (d) Physical mixture of drug-polymer (1:1), (e) Microsponges.



Before Release of drug from particle

After Release of drug from particle

Figure 6. SEM images of microsphere particle (optimised) showing shape and surface before release and after 1hr release.

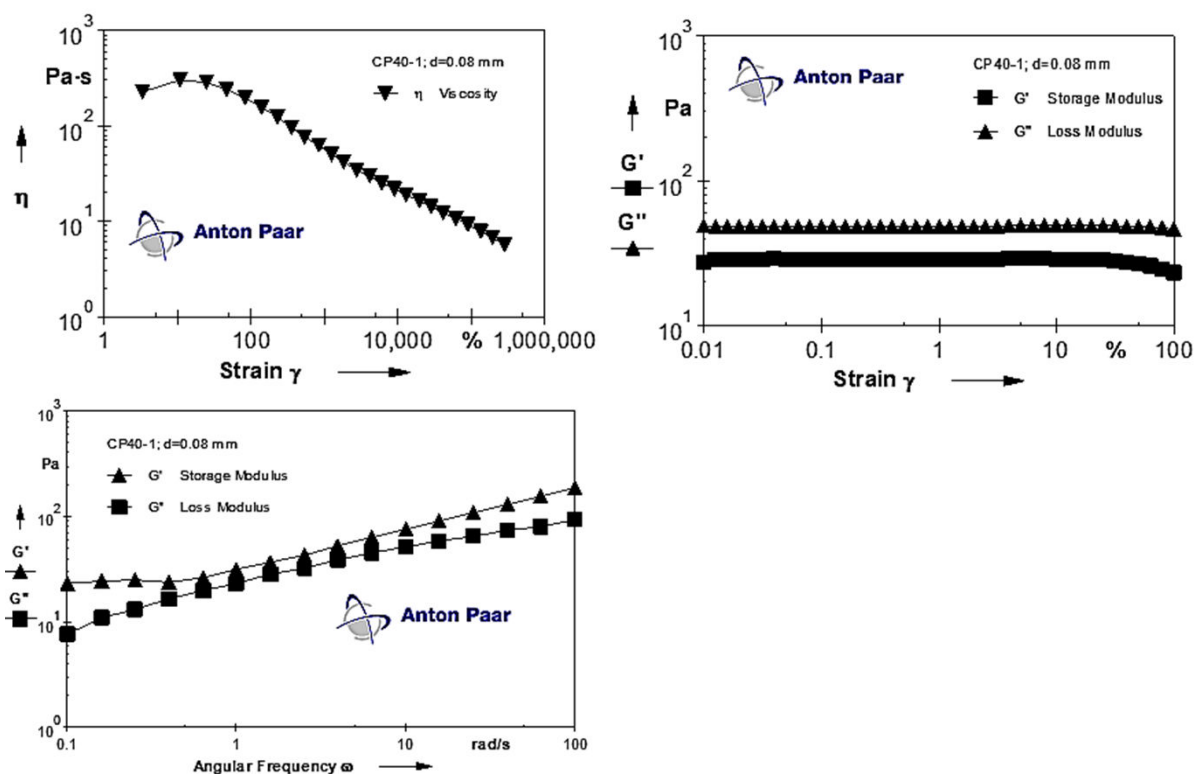


Figure 7. Effect in viscosity of gel after applying strain (i)(Flow Curve, η against strain %), (ii) Strain% against Storage Modulus (G') and Loss Modulus (G'') (Amplitude sweep curves), (iii) Angular Frequency (ω) against Storage (G') and Loss Modulus (G'') (Frequency sweep).

The more frequency-dependent the elastic modulus is, the more fluid-like is the material. In Figure 7 (strain vs G'/G''), high strain amplitudes showed better fluid-like behaviour ($G'' > G'$). Rheological study indicates that the gel behavior of the present microsp sponge formulation of 5-FU in gel form is suitable for dermal drug delivery.

4. Conclusion:

In the microencapsulation approach, it is challenging to encapsulate 5-Fluorouracil (water-soluble drug) with a single emulsion process. This active ingredient is mainly used as an oral dosage form (tablet, capsule and injections) to treat colon cancer, oesophageal cancer, stomach cancer, pancreatic cancer, breast cancer, and cervical cancer. The main objective of the present study is to prepare a microsp sponge of (5-FU) by w/o/w double emulsion method. Finally, its gel form is developed for dermal delivery of drugs used to treat actinic keratoses on the skin. The procedure is optimised by Response surface methodology. The study encompasses various preformulation studies to ensure drug compatibility with other

ingredients used, optimisation of the method and scale-up. The response-surface optimisation was carried out to optimise levels of the independent factors (polymer ratio, stirring speed and surfactant concentration) to achieve the desired responses. The ANOVA results showed that builder polymers, mixing rate and amount of surfactant (tween 80) had the most substantial effects on the percentage yield, particle size, entrapment efficiency and release in 8 hr. The combination of independent variables levels polymer (600 mg), stirring speed (1198 rpm) and surfactant (2% w/v) were found to give a desirability value of 0.737 (by design generated statistical method), showing yield (63.6257%), average particle size (151.563 μm), entrapment efficiency (75.319 %) and release in 8hr (76.097 at pH 7.4, 74.460 at pH 5.5). The final formulation was a gel, so rheological characteristics were also studied, confirming its shear-thinning property facilitating dermal applicability. The highlight of this study is the scale-up approach using the composition of optimised formulation. It was done by the 'power law approach' coupled with fixed shape factors (system geometry). The properties of product microsphere obtained from higher scale are found similar to that of lower scale.

The development of scale-up technology of this type is complex under the laboratory facility as the cost of drug and set up is high for a more significant scale. However, there is still plenty of scope for up-gradation of this method to a larger scale by the pharmaceutical industry.

Declarations

Ethics approval and consent to participate: This article does not involve human participants, so it is not applicable. All the experimental procedures were performed according to the guidelines of the Jadavpur University, West Bengal, India. The article also follows the National Institute of Technology guidelines, Tiruchirappalli, Tamil Nadu, India.

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Authors' contributions:

Shahjaman Halder - Conceptualization, Data curation, Formal analysis, Investigation.
Sourav Poddar - Conceptualization, Data curation, Formal analysis, Investigation, Software utilization, Validation, Visualization, Writing, review, and editing – original and final manuscript., **Jasmina Khanam** - Conceptualization, Data curation, Formal analysis, Investigation, Software utilization, Validation, Visualization, Writing, review, and editing – original and final manuscript

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