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Research Article

FORMULATION AND CHARACTERISTICS OF 5-FLUROURACIL MICROSPHERES BY SOLVENT EVAPORATION METHOD

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ABSTRACT

Microspheres of 5-Fluro uracil were prepared by solvent evaporation technique using dichloromethane and acetonitrile for the preparation of microspheres. Polyvinyl alcohol was used as processing medium to solidify the microspheres. Scanning electron microscopy of microspheres had shown their spherical shape with smooth surface. FT-IR spectra of 5-Fluro uracil and formulation indicated that that no any chemical transition has occurred during formulation. The effect of different concentrations of ethyl cellulose has shown no any significant difference in the percent yield, entrapment efficiency and mean particle size of 5-Fluro uracil microspheres. As concentration of ethyl cellulose increased drug release was decreased. No significant difference was observed in the percentage drug release for same concentration of ethyl cellulose with different rpm. It showed that all the formulations follows Higuchi model indicated that drug release form homogenous matrix was through diffusion.

Keywords: Microencapsulation, Solvent evaporation, Plasticizers.

INTRODUCTION

Numerous microspheres preparation methods and materials, which can be incorporated in microspheres, enable precise optimization of sustained release in different physiological conditions. Due to microscopic size, microspheres can be used alone or incorporated in other drug delivery systems and are suitable for various routes of application. Microencapsulation by the solvent evaporation method is a complex process, which can be influenced by many process parameters, e.g. solvent evaporation rate,[1] temperature,[2,3] solubility of polymer, drug and excipients in both emulsion phases,[4,5] dispersion stirring rate,[6] viscosity, solubility, volume and volume ratio between the inner and outer phases,[7] the quantity of polymer and drug,[8] and the physico-chemical properties and concentration of the stabilizers. In this present study 5-Fluro uracil microspheres were prepared by solvent evaporation technique using ethyl cellulose. Dichloromethane and acetonitrile were used for the preparation of microspheres. Polyvinyl alcohol was used as processing medium to solidify the microspheres. The effect of different concentrations of ethyl cellulose on the percent yield, entrapment efficiency, mean particle size and the drug release of microspheres was investigated.

MATERIALS AND METHODS

Ethyl cellulose (EC) was obtained as gift sample from Rohm Pharma, GmbH, Darmstadt, Germany. 5-Fluro uracil (FU) was obtained as gift sample from Alkem laboratories, Mumbai (India). All other chemicals used of analytical grade were purchased from Loba Chemicals Pvt. Ltd., Mumbai (India).

Preparation of 5-Fluro uracil microspheres

Required amount of Ethyl cellulose was dissolved into a 7 ml mixture of dichloromethane (DCM): acetonitrile (ACN) in a ratio of 1:1. Then, required amount of 5-Fluro uracil (previously passed through 120-mesh sieve) was added to the (EC) solution by stirring with a magnetic stirrer. The resultant solution was poured into 100 ml 2% PVA solution, in a 250 ml beaker. The resulting microspheres were filtered through a Whatman no. 1 filter paper. The residue was washed 4 to 5 times in distilled water each. Microspheres were dried at room temperature for 24 hrs. The various formulations with their processing variables were as shown in table 1.

Table 1: Formulation codes with quantities

Drug : EC	Formulation codes		
	For 250rpm	For 500rpm	For 750 rpm
1:1	F1	F2	F3
1:2	F4	F5	F6
1:3	F7	F8	F9

Drug: 5-Fluro uracil, EC: ethyl cellulose.

Evaluation of microspheres

Percent yield and entrapment efficiency

Prepared microspheres were weighed after drying, and percent yield was calculated using following formula

Percentage Yield = (Actual weight x 100)/ Theoretical Weight (1)

Microspheres of known weights were stopper tightly in a flask containing 50 ml of 6.8 pH phosphate buffer. The flasks were shaken using orbital shaker for 48 hours to break the beads completely. After 48 hours the solution was filtered using whatman's No. 1 filter paper and the filtrate was centrifuged using a tabletop centrifuge to remove the polymeric debris. Then the polymeric debris was washed twice with fresh solvent (water) to extract any adhered drug. The clear supernatant solution was then analyzed for 5-Fluro uracil content by a UV spectrophotometer (JASCO-V500, Japan) at the λ max value of 265 nm. The complete extraction of drug was confirmed by repeating the extraction process on the already extracted polymeric debris. The % entrapment efficiency of the matrix was then calculated as

% Entrapment efficiency = (Drug loading / Theoretical drug loading) x 100. (2)

Micrometric properties

Diameters of the dried microspheres were measured by optical microscopy.

Bulk density and tap density was determined according to following method:

A 50 ml glass cylinder was weighed and filled with 30 ml of sample and reweighed. The opening was secured with parafilm. The cylinder was gently reversed once and the powder was carefully leveled without compacting. Bulk volume was determined after one mechanical tap on a tap density tester (Dolphin™). Tap volume was measured after 2000 taps. Each analysis was repeated twice. [9] Values of bulk density and tap density used to calculate Carr's index.[10] The flow behavior of 5-Fluro uracil loaded microspheres were determined by Angle of repose. Fixed funnel method was used for determination of angle of repose. [11]

Scanning electron microscopy (SEM)

Microspheres were coated with a thin gold-palladium layer by sputter coater unit (VG- Microtech, United Kingdom), and the

surface topography was analyzed with a Cambridge Stereoscan S120 scanning electron microscope (SEM; Cambridge, United Kingdom) operated at an acceleration voltage of 10 kV.

Fourier transforms infrared spectroscopy (FT-IR)

Fourier transforms Infrared spectroscopy of 5-Fluro uracil and formulation F1, F3 and F5 were recorded using Jasco V5300 (Jasco, Japan) FT-IR system using potassium bromide (KBr) pellet method. Each spectrum was derived from single average scans collected in the region 4000 to 600 cm^{-1}.

In vitro dissolution study

Preparation of calibration curve: Different concentrations of 5-Fluro uracil (2 to 20 µg/ml) were prepared in 7.4 pH phosphate buffer and absorbance is measured by UV- visible spectrophotometer (JASCO-V500, Japan) at the λ max value of 265 nm. Calibration curve was plotted to determine R^2 and the equation of straight line which is used to calculate drug release.

The dissolution studies were performed by using USP 26 type II dissolution test apparatus (Electrolab TDT-06P, Mumbai, India). Dissolution medium used were phosphate buffer (pH 7.4), each 900 ml, temperature was maintained at 37 ± 2°C and 100 rpm stirring was provided for each dissolution study. 5-Fluro uracil microspheres equivalent to 100 mg of pure drug were used for each dissolution study. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through Whatman no. 1 filter paper, concentration of 5-Fluro uracil was determined spectrophotometrically at 265 nm. Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of drug release from beads. The kinetic models used were zero order, second order, Higuchi and Hixon-Crowell model. For zero order release kinetics equation is, $Q = K_0 t$, Where, Q = amount of drug release per unit surface area, K_0 = Zero order release rate constant and t = time.

For first order release kinetics equation is, $\ln q_0/q = -K_1 t$, Where, q = Amount of drug released per unit surface area, K_1 = First order release rate constant, q_0 = Initial amount, C_s = Saturation Solubility and C_t = Concentration and t = time.

For Hixon Crowell release kinetics equation is, $W_0^{1/3} - W^{1/3} = KHC\ t$, Where, W_0 = Initial weight of the particles, W = Weight of the particles, K_{HC} = Hixon Crowell release rate constant and t = time. For Higuchi release kinetics equation is, $Q = K_{HG}\ t^{0.5}$, Where, Q = Amount of drug released per unit surface area of the dosage form, D = Diffusion co-efficient of the drug, ε = Porosity of the matrix, ζ = tortuosity of the matrix, Cs = Saturation solubility of the drug in the surrounding liquid, K_{HG} = Higuchi release rate constant, A = Conc. of the drug in the matrix, and t = time.

RESULTS AND DISCUSSION

Preparation of 5-Flurouracil microspheres

5-Fluro uracil microspheres were prepared by solvent evaporation method. During optimization of the process various parameters were tried. It was found that for less than 1:1ratio of drug: ethyl cellulose concentration respectively there were no proper formation of microspheres. For more than 1:4 ratio of drug: ethyl cellulose concentration respectively it was observed that drug particles were precipitated and aggregated masses of particles were formed.

Evaluation of microspheres

Percent yield and entrapment efficiency

The percentage yield and percentage entrapment efficiency of eudragit coated 5-Fluro uracil microspheres were as given in Table 2. It was observed that there was no significant difference in the percent yield for all formulations. Entrapment efficiency for formulation F9 (72.9 %) was maximum and minimum for formulation F1 (40.3 %). It was observed that as concentration of ethyl cellulose increases entrapment efficiency of 5-Fluro uracil increases may be with increase in ethyl cellulose concentration increases crosslinking which leads to increases in drug holding capacity but no significant difference was for rpm.

Micrometric properties

Micromeritic properties of all formulations were given in table no. 2. Mean particle diameter (MPD) was in the range of 262 to 354 µm. Values of Carr's index represents its good flowability and compressibility.[10]

Scanning Electron Microscopy (SEM)

SEM of microspheres was given in Fig. 1. It showed that the microspheres were spherical in shape. The surface is smooth and showed cross linking by physical observation.

Table 2: Percentage yield and percentage entrapment efficiency (EE), mean particle diameter (MPD), Carr's index and angle of repose (AOR) of 5-Fluro uracil loaded eudragit microspheres.

Formulation Code	Yield (%)	EE (%)	MPD (µm)	Carr's Index (%)	AOR (°)
F1	76.3 ± 2.8	40.3 ± 1.8	262 ± 2	12 ± 1	22 ± 1
F2	77.4 ± 1.6	40.7 ± 0.9	274 ± 3	13 ± 1	21 ± 1
F3	78.2 ± 248	42.2 ± 1.2	280 ± 1	14 ± 1	19 ± 1
F4	75.1 ± 1.3	59.4 ± 1.6	308 ± 3	13 ± 1	23 ± 1
F5	78.7 ± 2.1	60.2 ± 2.2	311 ± 2	12 ± 1	21 ± 1
F6	79.4 ± 2.3	62.6 ± 1.4	319 ± 3	14 ± 1	23 ± 1
F7	76.2 ± 1.8	71.4 ± 1.9	354 ± 1	12 ± 1	21 ± 1
F8	78.6 ± 1.6	72.3 ± 1.4	262 ± 3	13 ± 1	20 ± 1
F9	80.1 ± 1.9	72.9 ± 1.5	278 ± 2	14 ± 1	19 ± 1

Fourier transforms infrared spectroscopy (FT-IR)

FT-IR spectra of 5-Fluro uracil and formulation F1, F4 and F7 given in figure 2 exhibited identical IR spectra which indicated that microspheres were not associated with changes at the molecular level. It revealed that no any chemical transition has occurred during formulation.

In vitro dissolution study

The calibration curve generated using standard solution gives R^2 value 0.999, the linearity was observed in the range of 2 to 20 µg/ml and the equation of the line is y = 0.034 X + 0.012. The percentage drug release for all formulations was as given in Fig. 3. As concentration of ethyl cellulose increases drug release was decreased. For formulations with drug: ethyl cellulose ratio 1:3 drug release was sustained. No significant difference was observed in the percentage drug release for same concentration of ethyl cellulose with different rpm. Model fitting data for dissolution profile was as given in table no. 3. It showed that all the formulations follows Higuchi model.[12] It has indicated that drug release form homogenous matrix was through diffusion. It revealed that increase in concentration of ethyl cellulose decreases the drug diffusion from microsphere.

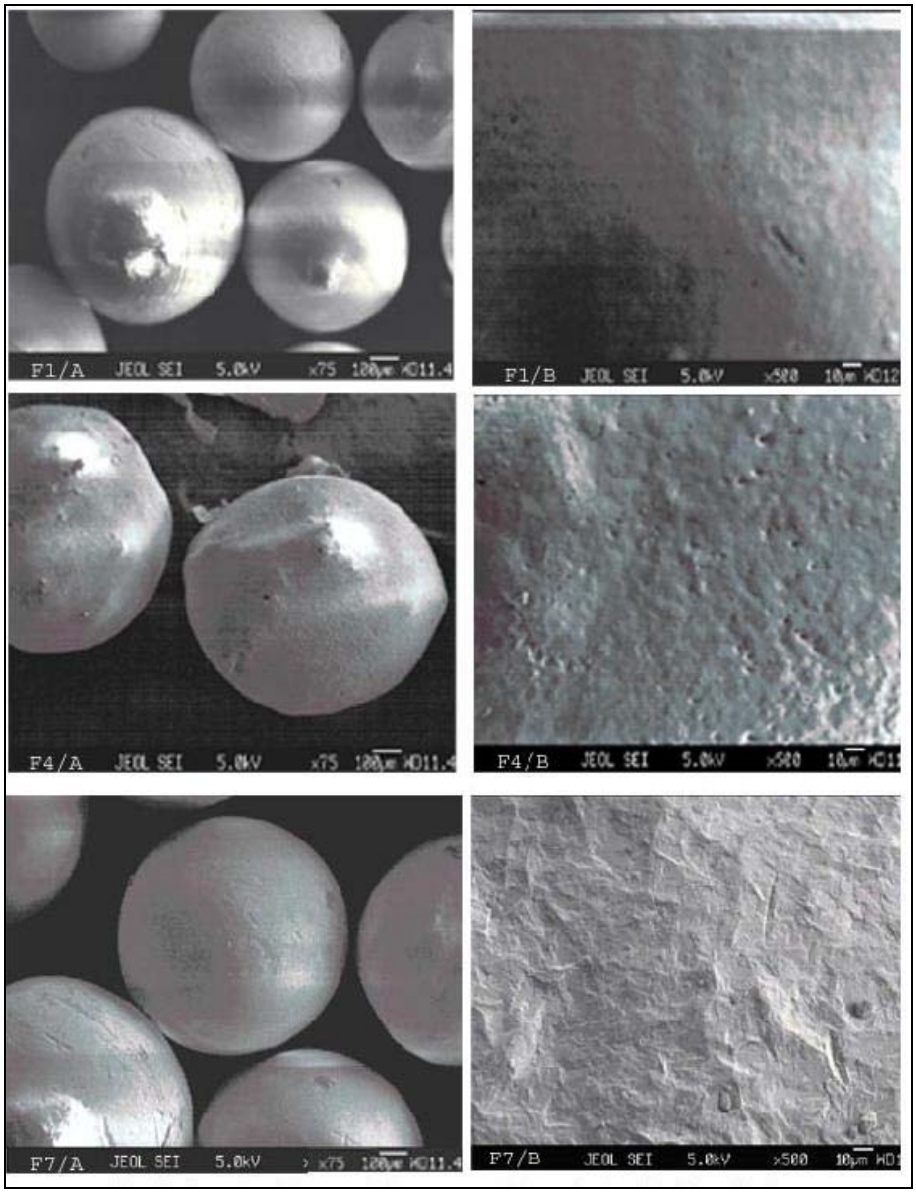

Fig. 1: Scanning elecron microscopy of formulation F1, F4 and F7. (A: 75X,B: 500 X)

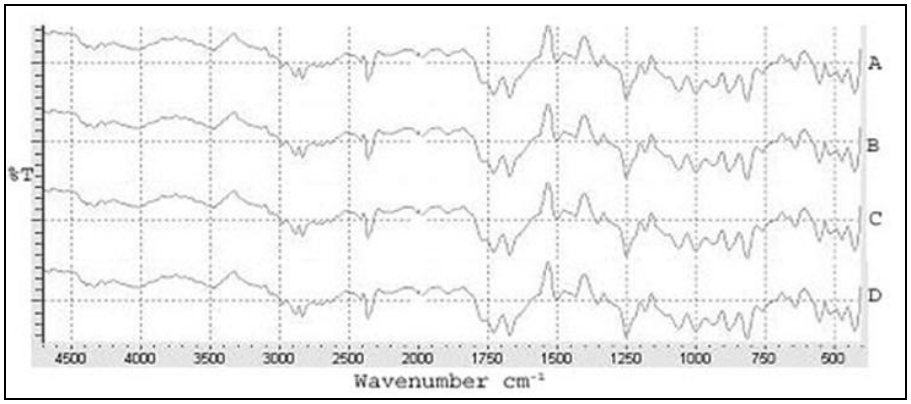

Fig. 2: Spectra of A: 5-Fluro uracil, B: formulation F1, C: formulation F4 and D: formulation F7

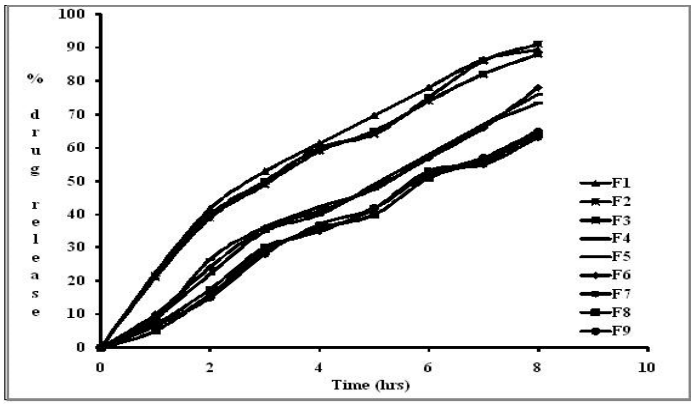

Fig. 3: Percentage drug release of 5-Fluro uracil microsphere

Table 3: R^2 values of mathematical models for dissolution profiles of 5-Fluro uracil microspheres

Formulation code	R^2 values of mathematical models for dissolution profiles			
	Zero order	First order	Higuchi	Hixson-Crowell
F1	0.971	0.977	0.996	0.992
F2	0.976	0.979	0.989	0.986
F3	0.980	0.988	0.991	0.989
F4	0.993	0.996	0.998	0.996
F5	0.984	0.996	0.998	0.997
F6	0.980	0.997	0.998	0.994
F7	0.952	0.973	0.992	0.967
F8	0.947	0.970	0.984	0.964
F9	0.973	0.994	0.998	0.988

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