Acta Pharmaceutica Sciencia
52: 391-399 (2010)

Preparation and characterization of 5-FU loaded microspheres of Eudragit and ethylcellulose

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Abstract

In the present investigation 5-fluorouracil loaded microspheres of Eudragit (RS 100, RL 100 and RSPO) and ethylcellulose were prepared. "O/O solvent evaporation" technique was used for preparation of microspheres using (methanol + acetone)/liquid paraffin system. Magnesium stearate was used as the droplet stabilizer and *n*-hexane was added to harden the microspheres. The prepared microspheres were characterized for their micromeritic properties and entrapment efficiency; as well by Fourier transform infrared spectroscopy (FTIR) and thin layer chromatography (TLC). Photomicrographs were taken to study the shape of microspheres. The best fit release kinetics was achieved with Higuchi plot. Mean particle size, entrapment efficiency and production yields were highly influenced by the type of polymer and polymer concentration. It is concluded from the present investigation that various Eudragit and ethylcellulose are promising controlled release carriers for 5-FU.

Keywords: 5-fluorouracil, eudragit RS 100, eudragit RL 100, eudragit RSPO, ethylcellulose, microspheres, solvent evaporation.

Introduction

5-Fluorouracil (5-FU) is an antimetabolite of the pyrimidine analog class which is widely used alone or in combination chemotherapy regimens. It interferes with nucleic acid synthesis, inhibits DNA synthesis, and eventually inhibits cell growth (Rahman et al. 2006). It has been the only agent with clinical activity against colorectal cancer. It is also used for malignancies, such as those of the breast, head and neck (Rahman et al. 2006). 5-FU is poorly absorbed after oral administration with extremely variable bioavailability (Zinnuti et al. 1998). These disadvantages make it an appropriate candidate for microencapsulation. Microspheres are one of the multiparticulate delivery system and are prepared to obtain prolonged or controlled drug delivery to improve bioavailability or stability and to target drug to specific sites. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance (Haznedar and Dortunç 2004). Eudragit polymers are series of acrylate and methacrylate polymers available in different

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ionic forms. Eudragit RL 100, Eudragit RS 100 and Eudragit RSPO are insoluble in aqueous media but they are permeable and have pH-independent release profiles. The permeability of all the three polymers in aqueous media is due to the presence of quarternary ammonium groups in their structure; Eudragit RL 100 has a greater proportion of these groups and as such is more permeable than Eudragit RS 100 and Eudragit RSPO, while Eudragit RS 100 and Eudragit RSPO have same permeability due to their structural similarity. They differ in the physical forms where the previous has granular form and later has powder form. Ethylcellulose is an ethyl ether of cellulose, a long chain polymer consisting of anhydroglucose units joined together by acetal linkages. It is not metabolized following oral consumption and is therefore a noncaloric substance. It is generally regarded as a nontoxic, nonallergenic and nonirritant material. The main use of it in oral formulations is as a hydrophobic coating agent for tablets and granules. Release of a drug from its microcapsule is a function of capsule wall thickness. The aim of this study was to prepare Eudragit and ethylcellulose microspheres containing 5-FU to achieve a controlled drug release profile suitable for peroral administration. The microspheres were prepared by solvent evaporation technique using Eudragit and ethylcellulose as a matrix polymer. (Methanol + acetone)/Liquid paraffin system was used for the preparation of microspheres. Magnesium stearate was used as a droplet stabilizer to prevent droplet coalescence in the oil medium and *n*-hexane was added as a non-solvent to the processing medium to solidify the microspheres (Sahoo et al. 2005). Firstly, we investigated formulation variables (polymer type and drug:polymer ratio) to obtain spherical particles. The effects of various Eudragit and ethylcellulose on the yield of production, particle size distribution, encapsulation efficiency and 5-FU release rate from microspheres were investigated. The influences of formulation variables on the microsphere properties were examined. The prepared spherical microspheres were evaluated for micromeritic properties and drug content, and also by FTIR, TLC as well as for *in vitro* drug release studies (Sahoo et al. 2005).

Materials and Methods

Materials

Eudragit RS 100, Eudragit RL 100 and Eudragit RSPO, Röhm GmbH&Co.,Darmstadt, Germany; Ethylcellulose, S.D. Fine Chem. Ltd, Mumbai, India; 5-FU, Biochem; Magnesium stearate, Ottokemi, Mumbai, India; *n*-hexane, Spectrochem PVT Ltd., Mumbai, India; Liquid paraffin Light, Methanol and Acetone, Central Drug House Ltd., New Delhi, India; Petroleum ether, Labort Fine Chem Pvt. Ltd. Gujarat, India; Toluene, Merck, NJ, USA; Other substances used were all of pharmaceutical grade.

Preparation of microspheres

The technique used in preparation of microspheres was "O/O emulsion" solvent evaporation. As shown in table 1, three different formulations of each polymer (Eudragit RS 100, Eudragit RL 100, Eudragit RSPO and ethylcellulose) with drug (5-FU) in different drug:polymer ratios 1:1, 1:2, and 1:3 were prepared. The polymers were dissolved in 10 mL of acetone separately. Pure 5-FU was dissolved in 13 mL of methanol. Both the solutions were mixed and 10 mg of Mg-stearate was dispersed in solution containing polymer and 5-FU. The dispersion was then stirred for 15 min. using magnetic stirrer. The resultant dispersion was then poured into 500 mL beaker containing the external phase (135 mL liquid paraffin light + 15 mL *n*-hexane) with stirring. Three-blade mechanical stirrer was used. Stirring (at 750 rpm) was continued for 4 hrs until acetone and methanol had evaporated completely. After evaporation of solvents, the microspheres formed were filtered using Whatman no.41 filter paper. The residue was washed 4-5 times in 25 mL *n*-hexane followed by 4-5 times in 50 mL petroleum ether (40-60°C). Thereafter, the

microspheres were dried in a desiccator for 24 h at room temperature. The microspheres were then stored in the desiccator (Sahoo et al. 2005).

Production yield

The yield was calculated by dividing the weight of the collected microspheres by the weight of all the non-volatile components used for the preparation of microspheres and expressed in the terms of percentage (Chun et al. 2005).

Yield (%) = (the amount of microspheres obtained / the theoretical amount) x 100

Table 1. Formulations of 5-FU loaded microspheres.

Formulation	Drug (mg)	Polymers (mg)				Mg-stearate (mg)	Curing time (h)
		RS100	RL100	RSPO	EC		
AS1	100	100	-	-	-	10	4
AS2	100	200	-	-	-	10	4
AS3	100	300	-	-	-	10	4
AL1	100	-	100	-	-	10	4
AL2	100	-	200	-	-	10	4
AL3	100	-	300	-	-	10	4
AP1	100	-	-	100	-	10	4
AP2	100	-	-	200	-	10	4
AP3	100	-	-	300	-	10	4
AE1	100	-	-	-	100	10	4
AE2	100	-	-	-	200	10	4
AE3	100	-	-	-	300	10	4

Particle size distribution analysis

Formulations of the microspheres were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 7.5 µm. 300 microspheres' sizes were calculated under 10x magnification (Polk et al. 1994).

Drug entrapment efficiency (DEE)

Ten mg 5-FU loaded microparticles were dissolved in 100 mL of PBS (pH 7.4) by shaking with magnetic stirrer for 24 h. The solution was filtered through Whatman no. 41 filter paper. An aliquot was assayed spectrophotometrically (UV-1601 Shimadzu Corporation, Japan) for 5-FU at 266 nm. Drug entrapment efficiency was determined by using the following relationship.

% Entrapment = (Actual content / Theoretical content) x 100

In vitro drug release study

The dissolution rate of 5-FU from the microspheres were studied using phosphate buffer solution (PBS) pH 7.4 by paddle method (USP XXIII). Accurately weighed microspheres (equivalent to 10 mg of 5-FU) were taken for dissolution studies. The dissolution medium was kept at 37±0.5°C. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 266 nm. The volume withdrawn at each time intervals replaced with the same amount of fresh dissolution medium.

Release kinetics

Data obtained from *in vitro* release studies were fitted to various kinetics equations to find out the mechanism of drug release from microspheres. The kinetic models used were Zero order, First order,

Higuchi and Korsemeyer-Peppas models. The rate constants were also calculated for the respective models (Sahoo et al. 2005).

FTIR study

Drug-polymer interactions were studied by FTIR spectroscopy. IR spectra for drug and drug loaded microspheres were recorded in a Fourier transform infrared (FTIR) spectrophotometer (FTIR-8400 S, Shimadzu, Japan) with KBr pellets. The scanning range was 400-4000 cm^{-1}.

Thin layer chromatography (TLC)

Pure 5-FU and drug loaded microspheres were dissolved in methanol separately and about 10 µg samples were spotted on pre-coated silica gel G plate. The solvent used was methanol. The plates were developed for at least 10 cm and then air dried. The R_f values were calculated and compared with the monographs (Baomi and Al-badar 2005).

Photomicrographs of microspheres

To study the shape of microspheres, photograph were taken using trinocular microscope (labomed, Olympus, CX_{Rii}) attached with camera.

Results

Mean particle size

The effects of parameters like the type of polymer and polymer concentration on the production yield, entrapment efficiency, particle size distribution, *in vitro* drug release and drug polymer interaction were studied.

In the preparation methanol was used to dissolve the drug. As shown in Table 2, the mean particle size for the formulations of Eudragit RS 100 was obtained in the range of 42.5 ± 3.387 µm to 44.3 ± 4.405 µm, for Eudragit RL 100 it was 58.1 ± 1.345 µm to 80.1 ± 3.345 µm, for Eudragit RSPO, it was 99.5 ± 3.245 µm to 123.0 ± 8.479 µm and for ethylcellulose it showed the range between 226.9 ± 5.214 µm to 267.1 ± 3.857 µm.

Table 2. Percentage production yield, mean particle size and percentage entrapment efficiency of Formulations AS1-AE3

Formulations	% yield *	Mean Particle Size* (µm)	% Entrapment Efficiency*
AS1	8.38 ± 0.652	42.5 ± 3.387	28.80 ± 2.405
AS2	14.47 ± 1.063	43.5 ± 1.100	33.09 ± 3.779
AS3	21.45 ± 0.661	44.3 ± 4.045	39.18 ± 2.660
AL1	13.78 ± 0.833	58.1 ± 1.345	22.36 ± 3.887
AL2	19.37 ± 0.682	72.6 ± 4.943	29.49 ± 2.842
AL3	29.48 ± 0.883	80.1 ± 3.345	35.63 ± 2.792
AP1	12.50 ± 1.176	99.5 ± 3.245	28.45 ± 1.463
AP2	17.15 ± 2.038	118.8 ± 2.179	34.98 ± 1.637
AP3	22.85 ± 2.553	123.0 ± 8.479	40.63 ± 1.802
AE1	12.78 ± 0.937	226.9 ± 5.214	25.71 ± 2.785
AE2	16.64 ± 1.678	247.7 ± 6.736	34.71 ± 2.979
AE3	17.42 ± 1.889	267.1 ± 3.857	44.53 ± 3.181

* indicates average of three readings ± SD

Production yield

Production yields of the preparation for all the polymers and polymer concentrations were found to be very less. As shown in Table 2, for Eudragit RS 100 the % yield was obtained in the range of 8.38 ± 0.652 to 21.45 ± 0.661, for Eudragit RL 100 it was 13.78 ± 0.833 % to 29.48 ± 0.883 %, for Eudragit RSPO it was 12.50 ± 1.176 % to 22.85 ± 2.553 % and for ethylcellulose it showed the range 12.78 ± 0.937 % to 17.42 ± 1.889 %.

Entrapment efficiency

As shown in Table 2, the entrapment efficiency was less for all formulations. As shown in Table 2, for Eudragit RS 100 the entrapment efficiency was obtained in the range of 28.80 ± 2.405 % to 39.18 ± 2.660 %, for Eudragit RL 100 it was 22.36 ± 3.887 % to 35.63 ± 2.792 %, for Eudragit RSPO it was 28.45 ± 1.463 % to 40.63 ± 1.802 % and for ethylcellulose and it showed in the range of 25.71 ± 2.785 % to 44.53 ± 3.181 %. The data revealed that particle size, entrapment efficiency, was highly influenced by type of polymer, polymer concentration and solvent used to dissolve the drug and polymer (Bhalerao et al. 2001, Haznedar and Dortunc 2004, Lamprecht et al. 2004, Sengel et al. 2006, Paharia et al. 2007). Methanol was used to dissolve the drug for all the formulations and it was found to be important factor to affect the production yield. The polymers were sticking to the vessel and the stirrer while evaporation of methanol, resulted in less production yield.

In vitro release study

In vitro release studies of the formulations were carried out in the PBS (pH 7.4) at 37 ± 0.5°C. As shown in Fig. 1, 2, 3 and 4 the initial higher release of 5-FU from all the formulations was might have resulted from the dissolution of the drug crystals presented on the surface of the microspheres (Paharia et al. 2007).

The formulations of Eudragit RS, AS1, AS2 and AS3 showed the complete drug release after 8, 9 and 11 h respectively as shown in Fig. 1. The formulations of Eudragit RL, AL1, AL2 and AL3 as shown in Fig. 2 were not able to sustain the drug release for 12 h and completely released after 5, 5, 8 h respectively. Release rates of 5-FU from Eudragit RL were faster than from Eudragit RS due to the fact, that the amount of quaternary ammonium groups of Eudragit RS is lower than that of Eudragit RL, therefore, Eudragit RL is more permeable to water, so that release was less retarded (Haznedar and Dortunc 2004). The formulations of Eudragit RSPO, AP1, AP2 and AP3 were also not able to sustain the drug release for 12 h and completely released after 9, 9 and 10 h respectively as shown in Fig. 3. The release of Eudragit RSPO microspheres was nearly same as that of Eudragit RS due to the same characteristics of both the polymers.

As shown in Fig. 4, formulations E1 and E2 were failed to sustain the drug release up to 12 h and showed complete release after 9 h and 10 h respectively. Formulation E3 was the only formulation showing about 97 % release after 12 h, hence it was chosen as the optimized formulation. The dissolution data revealed that for all the formulations as the polymer concentration was increased, the drug release rate decreased, depending on the drug-polymer ratio (Sengel et al. 2006).

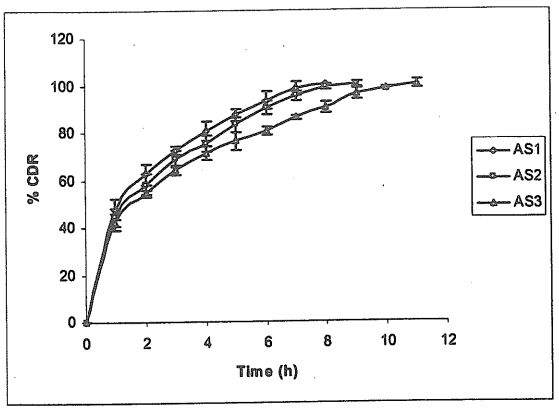

Figure 1. *In vitro* release profile of 5-FU (n=3) from AS1, AS2 and AS3 formulations

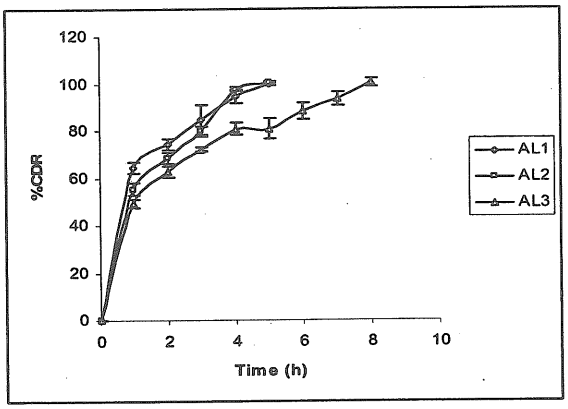

Figure 2. *In vitro* release profile of 5-FU (n=3) from AL1, AL2 and AL3 formulations

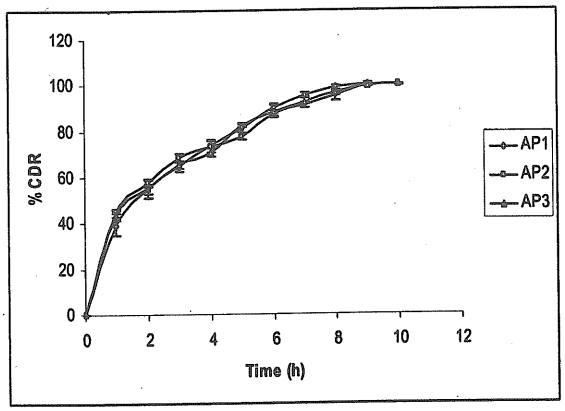

Figure 3. *In vitro* release profile of 5-FU (n=3) from AP1, AP2 and AP3 formulations

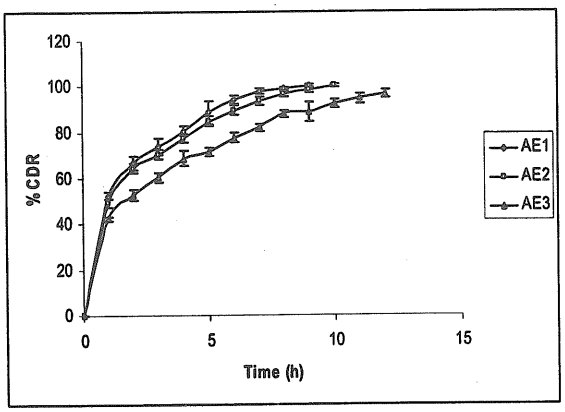

Figure 4. *In vitro* release profile of 5-FU (n=3) from AE1, AE2 and AE3 formulations

Release Kinetics

The release kinetics of all the formulation was checked by fitting the release data to various kinetic models, and the release was best fitted to the Higuchi model. It was further confirmed by fitting the data to Korsmeyer-Peppas equation and the n value for all the formulations obtained between 0.2769 and 0.4399 revealed that the release was followed square root of time mechanism (Rahman et al. 2006). The R2 values for all the models are shown in Table 3.

Table 3. Correlation coefficients of different mathematical models for 5-FU microspheres

Sl.	Formulations	Zero order	First order	Higuchi	Korsmeyer-Peppas	
		R^2	R^2	R^2	n	R^2
1	AS1	0.8471	0.9342	0.9913	0.3634	0.9961
2	AS2	0.8755	0.9124	0.9941	0.3813	0.9968
3	AS3	0.8147	0.8867	0.9937	0.3642	0.9976
4	AL1	0.9823	0.9564	0.9931	0.2769	0.9886
5	AL2	0.9692	0.8714	0.9901	0.3893	0.9817
6	AL3	0.9472	0.9486	0.9910	0.3267	0.9912
7	AP1	0.8728	0.9291	0.9904	0.4399	0.9962
8	AP2	0.8364	0.8341	0.9868	0.3540	0.9951
9	AP3	0.8354	0.8352	0.9912	0.3826	0.9905
10	AE1	0.7620	0.9611	0.9882	0.3048	0.9943
11	AE2	0.7967	0.9768	0.9876	0.3161	0.9973
12	AE3	0.8186	0.9789	0.9933	0.3400	0.9971

FTIR spectroscopy

Drug polymer interaction was checked by the IR spectrum of the optimized formulations with the IR spectrum of pure drug. The IR spectrum of pure drug shows the characteristic peaks at 3124 cm^{-1} for NH stretching, 1716 cm^{-1} and 1657 cm^{-1} for C=O stretching, 1245 cm^{-1} for CH in plane deformation and 813 cm^{-1} for CH out of plane deformation (Baomi and Al-badar 2005). They were checked in the IR spectrum of optimized formulations. As shown in Fig. 5, Fig. 6 and Fig. 7, there were no significant difference in the IR spectra of pure 5-FU and drug loaded formulations AS3 and AE3.

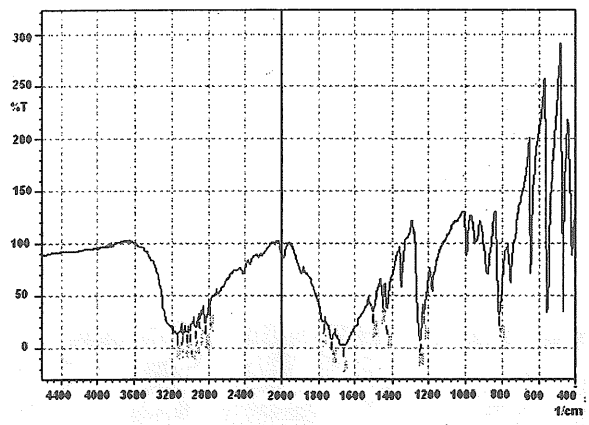

Figure 5. FTIR Spectra of pure 5-FU

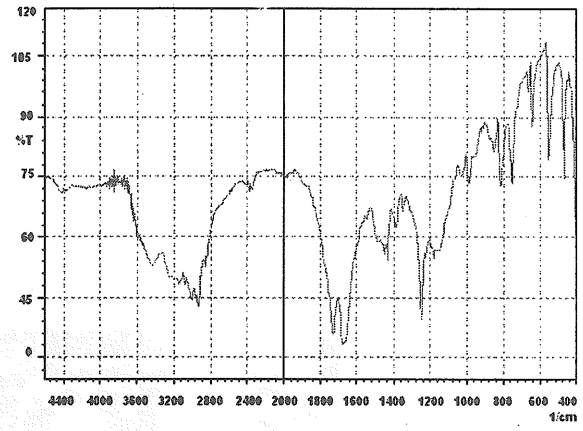

Figure 6. FTIR Spectra of Formulation AS3

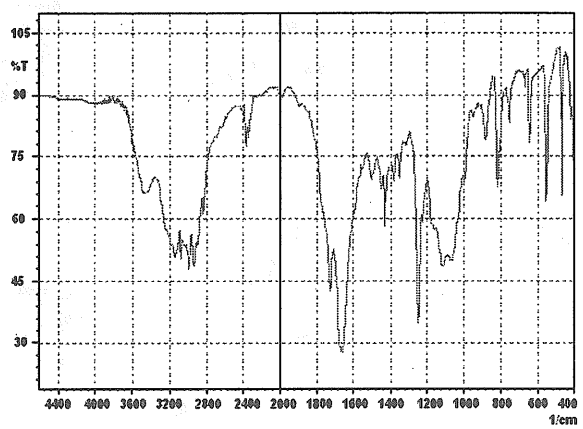

Figure 7. FTIR Spectra of Formulation AE3

TLC study

TLC of pure drug and that of formulations were carried out using methanol as solvent system on precoated silica gel plate. Iodine vapor was used for detection of spots. The R_f values for pure drug and the formulations are reported in Table 4.

Table 4. Thin layer chromatography of 5-FU, formulations S5, L6, P5 and E3

Sample	R_f Values				
	5-FU	AS3	AL3	AP3	AE3
1	0.8	0.79	0.78	0.79	0.79
2	0.8	0.78	0.79	0.77	0.79
3	0.79	0.8	0.81	0.8	0.8
Mean	0.7967	0.79	0.7933	0.7867	0.7933
SD	0.0058	0.01	0.0153	0.0153	0.0058

FTIR and TLC study suggested drug stability and no drug-polymer interaction was occurred during the encapsulation process. Photomicrograph study revealed the sphere shape of microspheres.

Photomicrographs of microspheres

To study the shape of microspheres photograph were taken using trinocular microscope (Labomed, CXRii, Olympus) attached with camera. Study revealed the spherical shape of the microspheres as shown in Figure8.

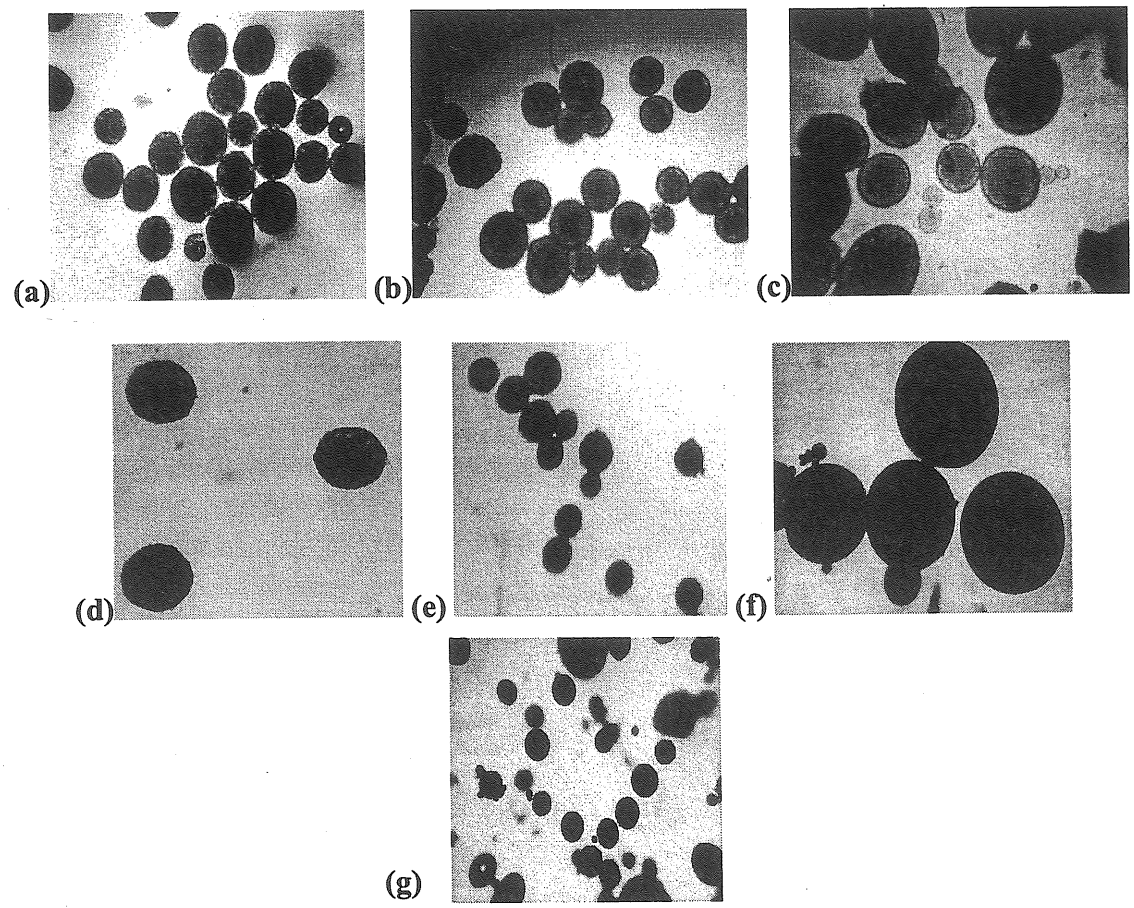

Figure 8. Photomicrographs of formulations (a) AS1, (b) AS3, (c) AL1, (d) AL2, (e) AP1, (f) AE2, (g) AE3

Conclusion

5-FU microspheres were prepared easily and successfully using the solvent evaporation technique. The yield and entrapment efficiency were found to be very less for all the formulations prepared. Particle size obtained for the microspheres was less for all the formulations. Particle size, entrapment efficiency and production yield were found to be highly influenced by the type of polymer and polymer concentration. It was found that the release of drug from the formulations followed diffusion mechanism. The release kinetics of all the formulations was best fitted to the Higuchi model which revealed that the release followed square root of time mechanism. According to the results of FTIR and TLC no drug interaction was occurred with polymer and 5-FU was found to be in crystal form in the prepared microspheres.

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Received: 10.02.2010
Accepted: 19.04.2010