ISSN: 0975-766X
CODEN: IJPTFI
Available Online through *Research Article*
www.ijptonline.com

BLEND MICROSPHERES FOR THE CONTROLLED DELIVERY OF FLUTAMIDE, AN ANTICANCER DRUG

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Received on: 12-02-2017 *Accepted on: 28-03-2017*

Abstract

Poly (vinyl pyrrolidone) (PVP)/ Carbopol 934 (CPL) blend microspheres were prepared through W/O emulsion-solvent diffusion method using ethanol as solvent. Flutamide (FLT), an anti Cancer drug, was encapsulated in PVP/CPL microspheres for drug release studies. Morphology, size, encapsulation efficiency and drug release from these microspheres were evaluated. Drug loaded microspheres were analyzed by Fourier transform infrared spectroscopy (FTIR), to undrstand the chemical interactions and formation of IPN blend structures. Differential Scanning Calorimetry (DSC) measurements on drug-loaded microspheres confirmed the molecular level dispersion of Flutamide in the microspheres. Scanning electron microscopy (SEM) confirmed the spherical nature and smooth surface of the microspheres produced. X-ray diffraction studies (X-RD) was performed to understand the crystalline nature of drug after encapsulation into these microspheres. *In-vitro* release studies indicated a dependence of release rate on the extent of cross linking, amount of drug loading and the amount of PVP, but slow release rates was extended up to 14 h.

Keywords: Poly (vinyl pyrrolidone), Carbopol 934, microspheres, SEM, X-RD.

1. Introduction

Oral controlled release dosage forms developed over the past three decades due to their considerable therapeutic advantages, such as ease of administration, patient compliance, and flexibility in formulation. Incorporation of the drug in controlled dosage forms can remain in the gastric region for several hours which would significantly prolong the

gastric residence time of drugs and improve their bioavailability, reduce drug wastage, and enhance the solubility of drugs [1]. Microsphere carrier systems made from the naturally occurring biodegradable polymers [2-4] have attracted considerable attention for several years in sustained release drug delivery. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes [5-7]. This can be achieved by blending with two polymers for developing novel IPN microspheres. IPN microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the specific targeting of drugs to the absorption site [8-10]. Techniques for controlled release of medicine must be the most basic and very important factor for the drug delivery system. The solid dispersion method is one pharmaceutical idea for controlling medicine release. The structure of the solid dispersion is monolithic where drug molecules homogeneously disperse, and it has a great advantage for avoiding the risk of a burst release feared concerning the reservoir type preparations. The solid dispersion method has been generally use to improve the dissolution properties and bioavailability of slightly water soluble medicines by dispersing them into water soluble carriers [11-16]. The authors have studied a series of the application of the solid dispersion method to the controlled release of medicines. We have applied the polymer blending technique to the solid dispersion method, and reported that it is feasible to precisely control the release rate of low water soluble medicine (Flutamide) by formation and retention of the swollen phase of water soluble PVP/CPL system. Poly (vinylpyrrolidone) (PVP) is a class of crystalline and water soluble linear polymer. It has been used in agricultural engineering, food and pharmaceutical fields due to its aqueous solubility, high gelation and low toxicity [17-18]. PVP consists of repeat units of CH_2-CH-Pyrrol group- (Fig 1.) , and it is known that PVP forms a complex with poly (carboxylic acid) [19-21]. Carbopol 934 is a kind of poly (carboxylic acid) and has been studied as bioadhesive and controlled release matrix [22-27]. It consists of chains of poly (acrylic acid) having cross linking agent, allyl ethers of sucrose (allylsucrose) [Fig 1.] [28-29]. It has recently attracted considerable interest in the field of drug delivery as a means of providing an on-off release by shrinking and swelling in response to change in pH [30-31]. Carbopol-934P (acrylic acid homopolymer) is an anionic polymer that has been used in mucoadhesive systems by several researchers [32-35]. Carbopol-934P has been selected as a polymer in the preparation of microspheres because of its good adhesive properties and is not absorbed by body tissues and being totally safe for human oral consumption.

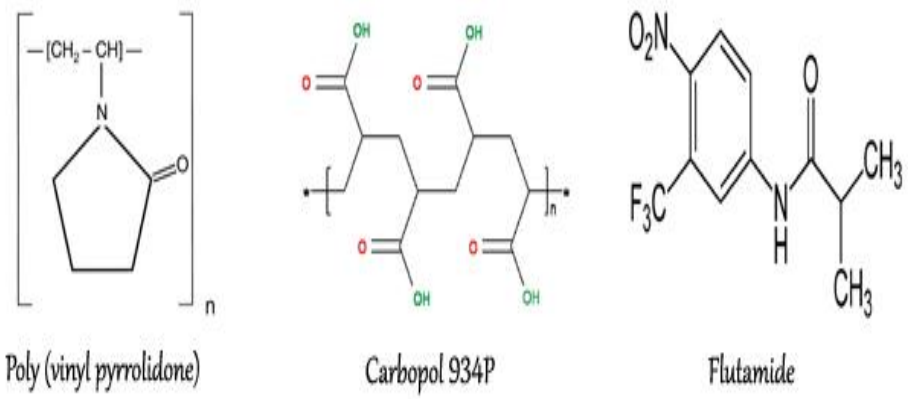

Fig. 1. Chemical structures of Poly (vinyl pyrrolidone), Carbopol 934P and Flutamide.

Prostate cancer has the highest incidence of all kinds of cancers and is the second most deadly cancer in men, after lung cancer [36]. Flutamide (FLT) (Fig. 1) is an antiandrogenic agent presently used for monotherapy of androgen dependent prostate cancer [37]. It acts by inhibiting the uptake and/or binding of dihydro testosterone to the target cell receptor, thus interfering with androgen action, which requires stability in blood for a sufficient amount of time [37]. However, the low bioavailability of FLT after oral administration could be attributed to its poor wettability, low aqueous solubility, poor permeability, or low concentration at the absorption surface [38]. Moreover, FLT undergoes a rapid first pass hepatic metabolism after oral administration, resulting in a relatively short halflife of 5–6 hours. Reported FLT toxicity includes hepatotoxicity, nausea, and diarrhea. Thus, the pharmacokinetics and dosage characteristics (250 mg three times daily) of FLT make it a suitable candidate for the design of controlled release delivery systems in order to enhance patients' compliance and to reduce the incidence of side effects [39]. Thus, the development of controlled release dosage forms would clearly be advantageous.

We have noticed some interaction between the polymer carriers of solid dispersion, and actively using the interaction, have attempted to control the medicine release from the solid dispersion composed of the PVP/CPL inter polymer complex. In the present study, we selected Carbopol 934 (CPL) and attempted to control the medicine release from the solid dispersion composed of the PVP/CPL interpolymer complex by varying the drug loading, CPL percentage and crosslinking and to clarify the control mechanism. Thus, an attempt was made in the present investigation to use Carbopol-934 as a mucoadhesive polymer and Poly (vinyl pyrrolidone) as carrier polymer, in order to prepare mucoadhesive FLT microspheres. The microspheres were characterized by different characterization techniques such as FTIR, DSC, X-RD, SEM and in-vitro release tests used to optimize the variables.

2. Experimental Methods

2.1 Materials

Carbopol 934 (0.2% viscosity about 3000cps) from Molychem, Mumbai, Poly (vinyl pyrrolidone), liquid paraffin oil(light) and Tween 80 from SD Fine Chem Ltd, Mumbai, Flutamide (anticancer drug), dichloromethane were purchased from Sigma Aldrich Chemicals (St. Louis), USA.

An analytical reagent grade glutaraldehyde (GA) solution (25% v/v), petroleum ether, and liquid paraffin oil were all purchased from Loba chemicals, Mumbai, India. The water used was a high-purity graded one collected after double distillation and deionization.

2.2 Preparation of Poly (vinyl pyrrolidone)/Carbopol 934 blend microspheres

Flutamide loaded blend microspheres of PVP and CPL were prepared by emulsion solvent evaporation technique as reported before with slight modifications [7]. Poly (vinyl pyrrolidone) was dissolved in 20 ml of ethanol. Flutamide and Carbopol-934P were dispersed in the ethyl cellulose solution, under stirring. The resultant mixture was extruded through a syringe (No. 20) in 500 ml of liquid paraffin (light,1:1 ratio) containing 2.0 % v/v Span 80 and stirring was carried out using a propeller stirrer (Remi, Mumbai, India) at 800 rpm and stirring was continued for 30 min. To this, GA as a crosslinking agent containing 0.1 HCl was added slowly, and the mixture was stirred for 3 h. All other variables were similar to the preliminary trial batches. Microspheres thus obtained were filtered and washed several times with petroleum ether (80:20) to remove traces of oil. The microspheres were then dried at room temperature (25^{o}C and 60 % RH) for 24 h. The effect of formulation variables and encapsulation efficiency of the microspheres has been summarized in Table 1.

Table 1: Formulation parameters and % of Encapsulation of the investigated Flutamide loaded PVP/CPL blend microspheres

Formulation code	PVP (% Wt)	CPL (% Wt)	% of drug	% of crosslinker (reduce gap between '%' and 'of')	% of encapsulation efficiency (reduce gap between '%' and 'of')
CPF - 1	80	**20**	**10**	**1**	68.54
CPF – 2	80	20	**15**	1	74.65
CPF – 3	80	20	**20**	1	78.96
CPF – 4	90	**10**	10	1	72.08

CFL – 5	70	**30**	10	1	64.25
CPF - 6	80	20	10	**1.5**	62.17
CPF - 7	80	20	10	**2**	59.76
CPF – 0	80	20	-	1	--

2.3 Estimation of drug and encapsulation efficiency

Estimation of drug concentration from the microspheres was done as per the method described earlier [40]. A specific amount of (~10mg) of drug loaded dry microspheres was grounded to get the powder using an agate mortar, extracted with 20ml of 7.4ph buffer solution and sonicated (Optics technology, India) for 60 min. The solution was centrifuged (Remi, India) to remove the polymer particles and washed twice to extract the drug completely. The clear solution was analyzed using UV Spectrophotometer (Labindia, Hyderabad) at λ_{max} = 228 nm. These results of drug loading and encapsulation efficiencies were calculated using equation (1) and (2) respectively. The results were based on triplicate and the average values and complied in Table 1.

$$\% \text{ of drug loading} = \frac{\text{Amount of drug in microspheres}}{\text{Theoretical loading}} \times 100 \quad (1)$$

$$\% \text{ of Encapsulation efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100 \quad (2)$$

2.4 In vitro release studies

In vitro release studies were carried out using the Tablet dissolution tester (Lab India DS 8000, Mumbai, India) equipped with eight baskets (glass jars). Dissolution rates were measured at 37 ± 0.5^{o}C at constant speed of 100 rpm. Drug release from the microspheres was studied in 7.4 pH phosphate buffer solution. At regular intervals of time, sample aliquots were withdrawn and analyzed using UV spectrophotometer (Lab India, Mumbai, India) at the fixed λ_{max} value of 228 nm. After each sample collection, the same amount of fresh medium at the same temperature was added to the release medium to maintain the sink condition. All measurements were carried out in triplicate, and values were plotted with standard deviation errors.

2.5 Fourier transforms infrared (FTIR) spectroscopy studies

The FTIR spectra of pure drug, placebo microspheres and drug loaded microspheres were recorded with Nicolet, Model Impact 410 (USA) instrument to confirm the cross-linking reaction between Carbopol 934 and Poly (vinyl pyrrolidone)

microspheres. Polymeric microspheres finely grounded with KBr to prepare pellets under a hydraulic pressure of 600 kg/cm^2 and spectra scanned in the range of 4,000-500 cm^{-1} at ambient temperature.

2.6 Differential Scanning Calorimetry (DSC) studies

Differential scanning calorimetry (DSC) thermograms of Pure drug, placebo microspheres and drug loaded microspheres were recorded by Differential scanning calorimeter (TA instruments - Model: STA, Q600 USA). Samples (10-12 mg) were placed in aluminum pan and heated at 10^{o}C/min under a nitrogen atmosphere (flow rate of 100 mL/min) in the 30^o- 600^{o}C range.

2.7 X-ray diffraction (X-RD) studies

X-RD measurement of plain drug, placebo microspheres, drug-loaded microspheres were carried out using a Rigaku Geiger flex diffractometer (Tokyo, Japan) equipped with Ni-filtered Cu-Kα radiation (k = 1.548 A°). The scanning rate was 2^o/min over a diffraction angle of 2Θ (2 Theta) and in the range of 5^o–60^o. The analysis was carried out at room temperature under ambient conditions.

2.8 Scanning Electron Microscopy (SEM) studies

The surface morphology of placebo microspheres and drug loaded microspheres was examined by means of a JSM 840 scanning electron microscope. To determine the particle size and size distribution, ~100–200 microspheres were taken on a glace slide and their sizes were measured using an optical microscope under regular polarized light.

3. Results and Discussions

3.1 FTIR Studies

FT-IR spectra of drug (Flutamide) (a), PVP/CPL placebo microspheres (b) and drug loaded PVP/CPL microspheres (c) are presented in Fig. 2. FTIR studies were carried out to confirm the chemical interaction of the FLT in the IPN microspheres.

From Fig. 2 (b) shows the FTIR spectra of PVP/CPL pure blend microspheres crosslinked with glutaraldehide. From this, it is noticed that a characteristic peak between 3500cm^{-1} and 3400 cm^{-1} indicates the presence of –OH group in CPL. The prominent band between 1750 and 1700 cm-1 was assigned to carbonyl C=O stretching band and 1590 cm^{-1}and 1354 cm^{-1} indicates the presence of symmetric and asymmetric stretching of vibrations of acetyl groups of PVP/CPL in blend microspheres. Again the characteristics peak at 1616 cm^{-1}and 1425cm^{-1}noticed due to carboxylate group of the

CPL. In addition to this a peak at 1020cm^{-1} is observed which may be due to the presence of acetal group formed by reaction of GA between PVP/CPL.

In case of drug loaded microspheres Fig. 2(c), it can be observed that the FLT drug is completely molecularly dispersed in the blend matrix. In case of FLT, a broad band at 3390 cm^{-1} is due to N-H stretching vibrations. Bands between 2919 and 3127 cm^{-1} corresponds to symmetric and asymmetric stretching vibrations of $–CH_3$ groups. The carbonyl stretching vibration band of FLT is observed at 1720 cm^{-1}. The symmetric and asymmetric stretching vibrations $–NO_2$ group are observed at 1344 and 1541 cm^{-1} respectively. The trifluoromethyl group stretching vibration are observed in the range of 1244 and 1345 cm^{-1}.

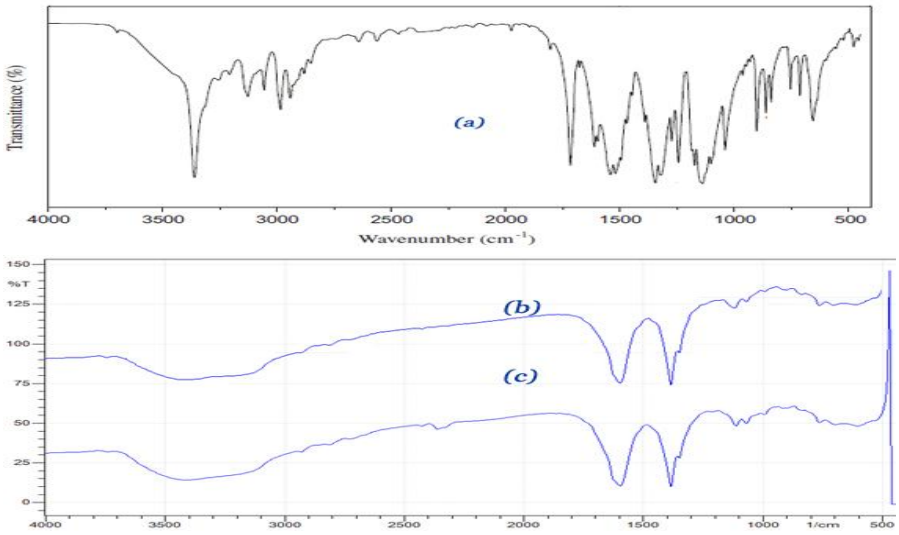

Fig. 2. FTIR spectrums of (a) Pure drug (Flutamide) (b) Placebo microspheres and (c) drug loaded microspheres.

3.2 Differential Scanning Calorimetry studies

DSC thermograms of pure FLT [Fig. 3(a)], drug loaded microspheres [Fig. 3(b)] and placebo microspheres [Fig. 3(c)] are shown in Fig. 3. Pure drug FLT in its natural state exists as crystals, which are characterized by the high melting peak around 112.4^{o}C [33, 41]. However, no characteristic peak of FLT was observed in DSC curves of the drug loaded microspheres and placebo microspheres. This may be explained by the total incorporation of the drug into the microspheres, suggesting a molecular dispersion of drug inside the polymer matrix. Similar results were observed by Elgindy *et al*. [41] for FLT loaded chitosan microspheres. In the PVP/CPL pure blend matrix, the broad endothermic peak of PVP/CPL blend was detectable at 123^{o}C. The endothermic peak of the polymeric matrix at 123°C [3(c)] after loading the drug decreased to 115°C [3(b)] due to the possible formation of a loose network as the result of the creation of extra

free space after drug loading which also indicates the decreasing crystalline nature of drug in a polymer matrix. Further which indicates the increase of an amorphous nature of the polymer matrix.

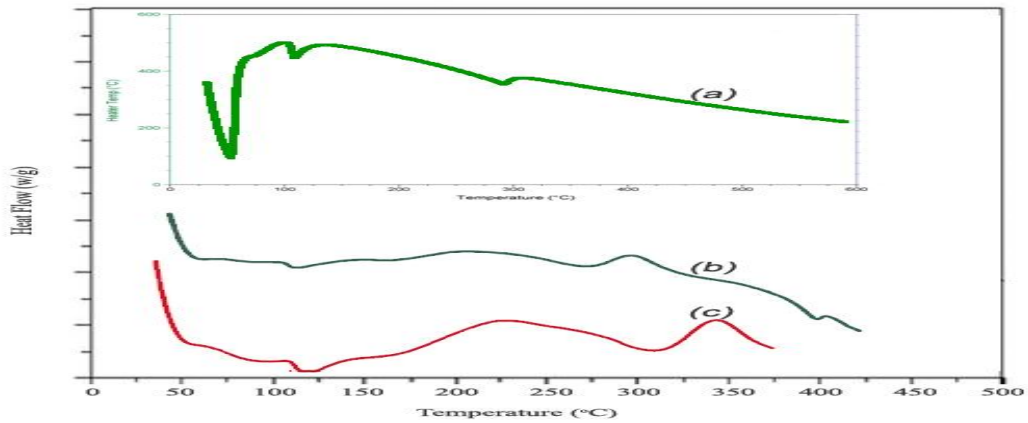

Fig. 3. DSC thermograms of Pure FLT (drug) (a), drug loaded PVP-CPL Microspheres (b), without drug loaded PVP-CPL microspheres (c).

3.3 SEM Studies

The SEM micrographs of Placebo blend microspheres (a), FLT loaded blend microspheres (b) are shown in Fig. 4. As seen in Fig. 4, they were spherical in shape and exhibited smooth surfaces due to the presence of drug in the microspheres. The mean particle size of PVP-CPL-6 formulated microspheres is around 10 - 15µm. The size distribution is normal distribution showing 12µm. The particle size analysis also supports the formation of smooth microspheres.

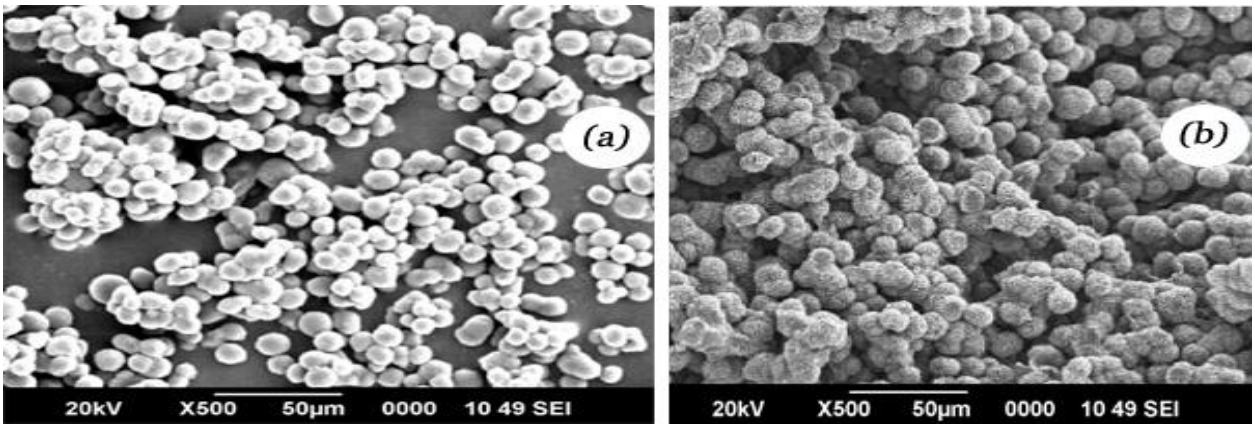

Fig. 4. SEM micrographs of placebo blend microspheres (a) and FLT loaded blend microspheres (b).

3.4 X-ray diffraction studies

X-RD study is an important characterization technique in case of drug delivery applications, to study the crystallinity of drug present in the polymer matrix. X-RD patterns of pure drug (FLT) (a), drug loaded microspheres (b) and placebo blend microspheres (c) are shown in Fig. 5. X-RD pattern of pure FLT provides the clues about the crystallinity of drug

in the microspheres. Here, the FLT drug peaks are observed at 2θ of 15.6° and 32.5° which are due to crystalline nature of FLT, while in the case of drug loaded microspheres the drug peaks are slightly observed which indicate that the drug particles are dispersed at molecular level in the polymer matrix.

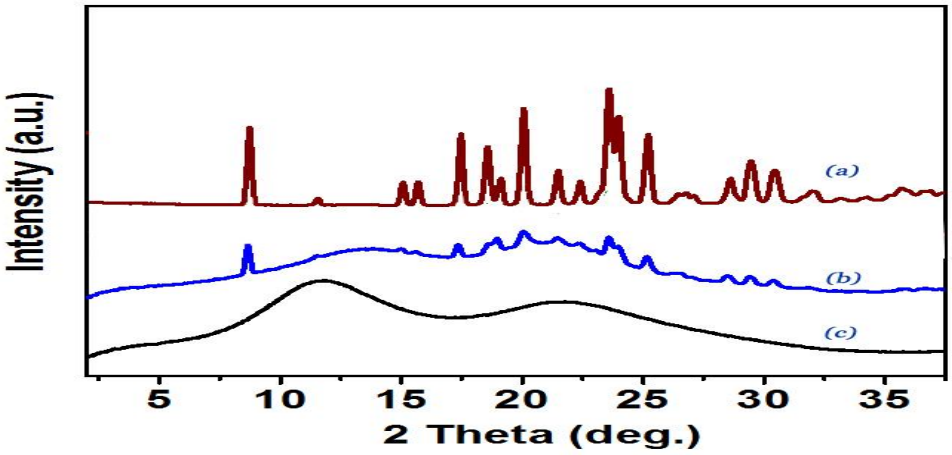

Fig. 5. XRD patterns of (a) pure FLT, (b) drug loaded microsphere, and (c) pure blend microspheres.

3.5. Encapsulation efficiency

To develop successful formulations containing FLT in polymeric matrices, it is important to achieve high encapsulation efficiency (EE). As per the literature information, generally the EE values are dependent on process variables like drug-polymer ratio, blend composition and extent of crosslinking.

Results presented in Table 1, clearly show the dependence of encapsulation efficiency on different parameters. For example, in case of CPF1, CPF2 and CPF3 formulations containing 80 wt. % of PVP, 20 wt.% of CPL and 1 ml GA, EE values increased systematically from 68.54 to 78.96% with increasing FLT loading. For decreasing blend compositions of 90 to 70wt.% of PVP (CPF4, CPF1 & CPF5), keeping FLT loading (10%) and amount of GA (1 ml) as constant, the EE values decreases from 72.08 to 64.25% because of decrease of CPL in blends composition. On the contrary, for the blend of 80 wt.% of PVP (i.e CPF1, CPF6 & CPF7) with a constant FLT loading of (10wt.%) and varying amounts of GA from 1, 1.5 and 2 ml, we observed a systematic decrease of EE values from 68.54 to 59.76% because of increased rigidity of the network. The above results clearly show that encapsulation efficiency values depended on the variables of the process.

3.6. *In-vitro* release studies:

In-vitro release data are discussed in terms of drug loading, effect of crosslinking and IPN blend composition.

3.6.1. Effect of blend composition:

The release study of Flutamide (FLT) from microspheres, through *in-vitro* release experiments were carried out in intestinal pH 7.4 phosphate buffer solution. Release of FLT from microspheres with variation of CPL content for formulations CPF-4, CPF-1, CPF-5 at pH 7.4 is displayed in Fig. 6. The % of cumulative release is lower in case of high amount of CPL with slow release rate for a prolong period up to 14hr. This can be explained on the basis of dissolution behavior of FLT in PVP/CPL matrix, a dense polymer network formed when microspheres could intact with alkaline condition, FLT is not easily leaching out from the microspheres giving dense network structure. Dense network microspheres are produced after the dissolution, suggesting that the incorporation of CPL in to PVP indicates drug release is influenced by the content of CPL in the polymer matrix. Based on these results we can conclude that the release of FLT restricts from microspheres at pH 7.4, due to the formation of rigid network structure.

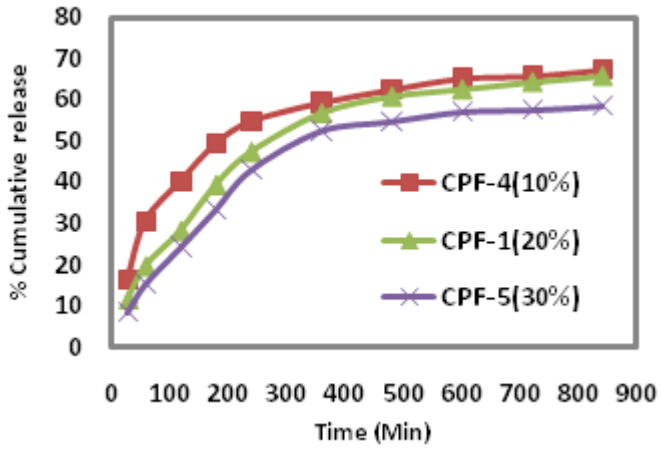

Fig. 6. % Cumulative release of FLT loaded microspheres for different ratios of polymer composition CPF-4 (10% CPL), CPF-1(20% CPL), and CPF-5 (30% CPL) containing 10% FLT at pH 7.4.

3.6.2. Effect of Drug content:

Fig. 7. shows the release profile of FLT loaded PVP/CPL blend microspheres at

different amounts of drug (10%, 20% and 30%) loading and at pH 7.4. The release data showed that formulations containing highest amount of FLT (30 wt %) displayed higher release rates than those containing lower of amount of FLT (10%). Formulation containing highest amount of FLT released 71.8 % of the total encapsulated drug. On the other hand, formulations containing lower amount of FLT have released only 62.3 % of FLT. Thus, sustained release was observed for the formulation containing lower amount of FLT. Thus the release rates are slower for lower amount FLT in the matrix, probably due to the availability of more free void spaces through which a lesser number of drug molecule will

transport.

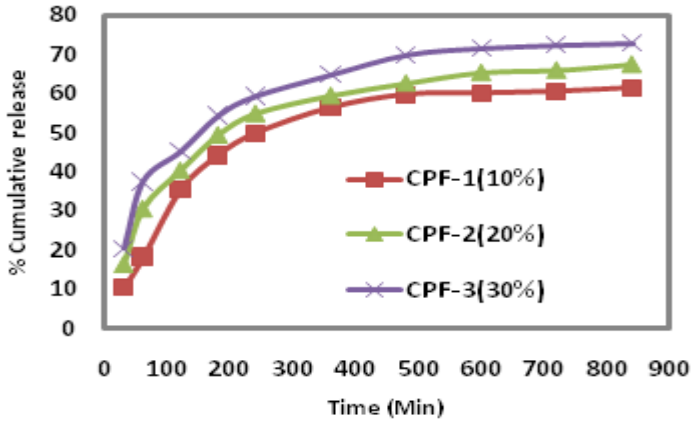

Fig. 7. % Cumulative release of FLT loaded microspheres containing different amounts of drug CPF-1 (10%), CPF-2 (20%), and CPF-3 (30%) at pH 7.4.

3.6.3. Effect of crosslinker content:

Fig. 8. shows the release profile of FLT loaded PVP/CPL blend microspheres at different amounts of crosslinking agent (GA) (1 ml, 1.5 ml and 2 ml) loading and at pH 7.4. The release data showed that formulations containing 2 ml of GA displayed lower release rates than those containing 1 ml of GA amount. Formulation containing highest amount of GA released 54.3 % of the total encapsulated drug. On the other hand, formulations containing lower amount of FLT have released only 64.5 % of FLT. In other words, the release is slower for a formulation containing higher amount of GA compared to that with the lower amount of cross-linker. This may be due to the fact that at higher cross-linking, free volume of the matrix will be reduced, thereby hindering easy transport of drug molecules through the matrix.

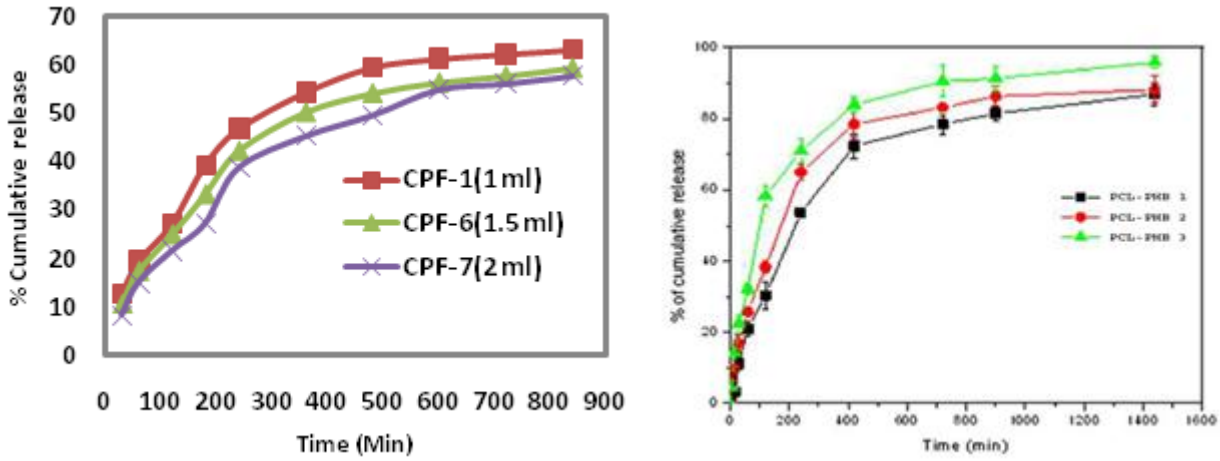

Fig. 8. % Cumulative release of FLT through microspheres containing different amounts of crosslinker formulations CPF -1 (1 ml), CPF-6 (1.5 ml), and CPF-7 (2 ml) at pH 7.4.

3.7. Kinetics of In vitro release studies:

Drug-release kinetics was analyzed by plotting the cumulative release data versus time by fitting the data to a simple exponential equation. 3 [42].

$$M_t / M_\infty = Kt^n \quad \text{---- (3)}$$

Where M_t and M_∞ represent the fractional drug release at time t, k is a constant characteristic of the drug-polymer system and 'n' is an empirical parameter characterizing the release mechanism. Using the least square procedure, we have calculated the values of 'n' and 'k' for all the formulations and these values are given in **Table 2.** The values of correlation coefficient (r) was calculated by using the above equation and the values are included in **Table 2.** If n= 0.5, the drug diffuses and release from the polymer matrix following a Fickian diffusion. If n > 0.5, anomalous or non-Fickian drug diffusion occurs. If n = 1, a completely non-Fickian or case-II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to an anomalous type diffusive transport [43].

The values of 'k' and 'n' show a dependence on the extent of crosslinking, the percentage of drug loading and CPL content in the matrix. Values of 'n' for microspheres are calculated by varying the amount of CPL (10%, 20%, 30%), keeping flutamide (10%) and GA (1 ml) constant, and range from 0.486 to 0.627, leading to the shift transport of anomalous type. The flutamide loaded microspheres containing different amounts of flutamide (10, 20 and 30%) and GA exhibited 'n' values ranging from 0.502 to 0.681, and the values for these formulations are presented table 2, indicating the anomalous type release. This may be due to the reduction in the reasons of low micro viscosity and closure micro cavities in swollen state of the polymer. Similar findings were also observed by Lyu *et al*. [43] from their drug release studies where they have reported the effect of monomer, drug and crosslinker content on dissolution kinetics.

Table 2. Results of % of release kinetics parameters (k, n and r) of drug in different blend microsphere formulations.

Formulation codes	k	n	Correlation coefficient (r)
CPF - 1	0.152	0.681	0.921
CPF – 2	0.126	0.539	0.950
CPF – 3	0.092	0.502	0.916
CPF – 4	0.145	0.486	0.987

CFL – 5	0.197	0.627	0.979
CPF - 6	0.162	0.542	0.962
CPF - 7	0.129	0.592	0.994

3.8. Conclusions

Poly (vinyl pyrrolidone)/Carbopol 934 blend microspheres were developed by emulsion solvent evaporation method to study the controlled release of Flutamide (FLT), an anticancer drug. SEM, particle size analysis gave surface morphology and particle size of microspheres. DSC and XRD analysis of FLT loaded microspheres have shown molecularly dispersed drug in the microspheres. Based on *In vitro* release studies the FLT was released in a controlled manner by influencing the variation of blend composition, drug and crosslinker for more than 14 h.

References

1. R. Garg, G. D. Gupta. Progress in controlled gastroretentive delivery systems. *Trop J Pharm Res,* 2008;7:1055-1066.
2. M. C. Gohel, A. F. Amin. Formulation optimization of controlled release diclofenac sodium microspheres using factorial design. *J Contr Rel,* 1998;51:115–122.
3. B. H. Woo, G. Jiang, Y. W. Jo, P. P. DeLuca. Preparation and characterization of a composite PLGA and poly (acryloyl hydroxymethyl starch) microsphere system for protein delivery. *Pharm Res,* 2001;18:1600–1606.
4. Y. Capan, G. Jiang, S. Giovagnoli, P. P. DeLuca. Preparation and characterization of poly (D,L-lactide-co-glycolide) microsphere for controlled release of human growth hormone. *AAPS Pharm Sci Tech,* 2003;4: Article 28.
5. M. J. Schaefer, J. Singh. Effect of isopropyl myristic acid ester on the physical characteristics and in-vitro release of etoposide from PLGA microspheres. *AAPS Pharm Sci Tech,* 2000;1:E32.
6. S. Govender, V. Pillay, D. J. Chetty, S. Y. Essack, C. M. Dangor, T. Govender. Optimisation and characterisation of bioadhesive controlled release tetracycline microspheres. *Int J Pharm,* 2005;306:24–40.
7. J. K. Patel, R. P. Patel, A. F. Amin, M. M. Patel. Formulation and evaluation of mucoadhesive glipizide microspheres. *AAPS Pharm Sci Tec,* 2005;6:E49–55.
8. C. M. Lehr, J. A. Bouwstra, E. H. Schacht, H. E. Junginger. In vitro evaluation of Mucoadhesive properties of chitosan and some other natural polymers. *Int J Pharm,* 1992;78:43–48.

9. L. Henriksen, K. L. Green, J. D. Smart, G. Smistad, J. Karlsen. Bioadhesion of hydrated chitosans: An in vitro and in vivo study. *Int J Pharm*, 1996;145:231–240.
10. K. P. R. Chowdary, Y. S. Rao. Design and in vitro and in vivo evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: A technical note. *AAPS Pharm Sci Tech*, 2003;4:article 39.
11. K. Sekiguchi, N. Obi. Studies on absorption of eutectic mixtures. I. A comparison of the behavior of eutectic mixtures of sulfathiazole and that of ordinary sulfathiazole in man. *Chem. Pharm. Bull.* 1961;9:866–872.
12. W. L. Chiou, S. Riegelman. Oral absorption of griseofulvin in dogs. Increased absorption via solid dispersion in poly-ethylene glycol 6000. *J. Pharm. Sci*, 1970;59:937–942.
13. M. Moriyama, A. Inoue, M. Isoya, M. Tanaka, M. Hanano. Dissolution properties and gastrointestinal absorption of chloramphenicol from hydrophilic high molecular compound coprecipitates. *Yakugaku Zasshi*, 1978;98:1012–1018.
14. I. Sugimoto, A. Kuchiki, H. Nakagawa, K. Tohgo, S. Kondo, I. Iwane, K. Takahashi. Dissolution and absorption of nifedipine from nifedipine–polyvinylpyrrolidone coprecipi-Tate. *Drug Dev Ind Pharm.* 1980;6:137–160.
15. H. Takeuchi, T. Handa, Y. Kawashima. Spherical solid dispersion containing amorphous tolbutamide embedded in enteric coating polymers or colloidal silica prepared by spray-drying technique. *Chem Pharm Bull*, 1987;35:3800–3806.
16. H. Suzuki, H. Sunada. Influence of water-soluble polymers on the dissolution of nifedipine solid dispersions with combined carriers. *Chem Pharm Bull*, 1998;46:482– 487.
17. M. Sairam, V. Ramesh Babu, K. S. V. Krishna Rao, T. M. Aminabhavi. Poly(methylmethacrylate)-poly (vinyl pyrrolidone) microspheres as drug delivery systems: Indomethacin/cefadroxil loading and invitro release study. *J Appl Polym Sci*, 2007;104: 1860-1865.
18. M. K. Chun, C. S. Cho, H. K. Choi. Mucoadhesive drug carrier based on interpolymer complex of poly(vinyl pyrrolidone) and poly(acrylic acid) prepared by template polymerization. *J Control Release*, 2002a;81:327–334.
19. K. L. Smith, A.E. Winslow, D.E. Peterson. Poly(alkylene oxides) and polymeric poly(carboxylic acids). *Ind Eng Chem*, 1959;51:1361–1364.
20. F. E. Bailey Jr, R. D. Lundberg, R. W. Callard. Some factors affecting the molecular association of poly(ethylene oxide) and poly(acrylic acid) in aqueous solution. *J Polym Sci., Part A*, 1964;2:397–406.

21. I. Iliopoulos, R. Audebert. Influence of concentration, molec-ular weight and degree of neutralization of polyacrylic acid on interpolymer complexes with polyoxyethylene. *Polym Bull*, 1985;13:171–178.
22. Y. Machida, H. Masuda, N. Fujiyama, M. Iwata, T. Nagai. Preparation and phase II clinical examination of topical dosage forms for the treatment of *carcinoma colli* containing bleomycin, carboquone, or 5-fluorouracil with hydroxypropyl cellulose. *Chem. Pharm.Bull,* 1980;28:1125–1130.
23. K. Morimoto, H. Akatsuchi, R. Akikawa, M. Morishita, K. Morisaka. Enhanced rectal absorption of [Asu1,7]-eel cal-citonin in rat using polyacrylic acid aqueous gel base. *J Pharm Sci*, 1984;73:1366–1368.
24. K. Takayama, T. Nagai. Application of interpolymer com-plexation of polyvinylpyrrolidone / carboxyvinyl polymer to control of drug release. *Chem Pharm Bull,* 1987;35:4921–4927.
25. G. Ponchel, F. Touchard, D. Wouessidjewe, D. Douchene, N.A. Peppas. Bioadhesive analysis of controlled-release systems. III. Bioadhesive and release behavior of met-ronidazole-containing poly(acrylic acid)–hydroxypropyl methylcellulose systems. *Int J Pharm*, 1987;38:65–70.
26. A. Deshpande, N. Shah, C. Rhodes, W. Malick. Development of a novel controlled-release system for gastric retention. *Pharm Res*, 1997;14:815–819.
27. V. R. Goskonda, I. K. Reddy, M. J. Durrani, W. Wilber, M.A. Khan. Solid-state stability assessment of controlled release tablets containing Carbopol 971P. *J Control Release*, 1998;54:87–93.
28. A. H. Hosmani. Carbopol and its Pharmaceutical Significance: A Review; Available from: http://www.pharmainfo.net/reviews/carbopol- and-its-pharmaceutical significance- review, [Last accessed on 2011 Feb 15].
29. A. P. Cruz, P. O. Rodrigues, T. M. Cardoso, M. A. Silva. Mechanical and imaging studies of hydrophilic matrices formed by Polymeric Blends of HPMC and carbopol. *Am J Pharm*, 2007;26:171-178.
30. I. Y. Galaev, B. Mattiasso. 'Smart' polymers and what they could do in biotechnology and medicine. *Trends Biotechnol*, 1999;17:335-340.
31. B. Jeong, A. Gutowska. Stimuli-responsive polymers and their biomedical applications.*Trends Biotechnol*, 2001;20:305-311.

32. M. K. Chun, C. S. Cho, H. K. Choi. Mucoadhesive drug carrier based on interpolymer complex of poly(vinyl pyrrolidone) and poly(acrylic acid) prepared by template polymerization. *J Control Release,* 2002;81:327-334.
33. M. K. Chun, C. S. Cho, H. K. Choi. Mucoadhesive microspheres prepared by interpolymer complexation and solvent diffusion method. *Int J Pharm*, 2005;20:288-295.
34. H. Blanco-Fuente, S. Anguiamo-Igea, F. J. Otero-Espinar, J. Blanco-M'endez. *In vitro* bioadhesion of carbopol hydrogel. *Int J Pharm*, 2004;276:51-58.
35. D. Chickering, J. Jacob, E. Mathiowitz. Bioadhesive microspheres II. Characterization and evaluation of bioadhesion involving hard, bioerodiable polymers and soft tissue. *Reactive Polymers,* 1995; 25:198-206.
36. C. L. Martel, P. H. Gumerlock, F. J. Meyers, P. N. Lara. Current strategies in the management of hormone refractory prostate cancer. *Cancer Treat Rev,* 2003;29:171–187.
37. B. R. Goldspiel, D. R. Kohler. Flutamide: an antiandrogen for advanced prostate cancer. *DICP*. 1990;24:616–623.
38. Z. Zuo, G. kwon, B. Stevenson, J. Diakur, L. I. Wiebe. Hydroxypropylβ-cyclo Dextrinflutamide Inclusion complex. I. Formulation, physical characterization and absorption studies using the Caco2 in vitro model. *J Pharm Pharmaceut Sci,* 2000;3:220–227.
39. Z. Zuo, Y. K. Tam, J. Diakur, L. I. Wiebe. Hydroxypropyl-β-cyclodextrin flutamide Inclusion complex. II. Oral and intravenous pharmacokinetics of flutamide in rat. *J Pharm Pharmaceut Sci,* 2002;5:292–298.
40. K. Sudhakar, P. Kumara Babu, M. N. Prabhakar, A. Chandra Babu, K. Madhusudhana Rao, M. C. S. Subha, K. Chowdoji Rao. Development of pH sensitive poly (caprolactone) based microspheres for in vitro release studies of Triprolidine Hydrochloride. *Des monomers and Polym.* 2014;17:617-623.
41. N. Elgindy, K. Elkhodairy, A. Molokhia, A. O. Elzoghby. Lyophilized flutamide dispersions with polyols and amino acids: preparation and in vitro evaluation. *Drug Dev Ind Pharm,* 2011;37:446-455.
42. P. L. Ritger, N. A. Peppas. A simple equation for description of solute release ii. Fickian and anomalous release from swellable devices. *J Control Release*, 1987;5:37-42.
43. S. P. Lyu, R. Sparer, C. Hobot, K. Dang. Adjusting drug diffusivity using miscible polymer blends. *J Control Release*, 2005;102:679-687.