

# **Original Research Article**



# Development of Pectin-Poly (Vinyl Pyrrolidone) blend micro beads for controlled release of 5-FU

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#### Abstract

In the present work we developed Pectin-poly (vinyl pyrrolidone) (PC-PVP) based micro beads using oil in water emulsion cross linking method employing Ca+2 as an ionic crosslinker. 5-fluorouracil drug was loaded into these beads via blending method. Various formulations were prepared by varying ratios of PC-PVP, crosslinker and % of 5-FU loading. Micro beads were characterized by Fourier transforms infrared spectroscopy (FTIR), differential scanning calorimetric (DSC), X-Ray diffraction(X-RD) and Scanning electron microscopy (SEM). DSC and XRD studies were performed to understand the crystalline nature of drug after encapsulation into semi IPN micro beads. SEM images gave the beads with smooth surface. FT-IR spectroscopy of micro beads shows to confirm the formation of co-polymerization between the polymers. The encapsulation efficiency was found up to 82% as measured using UV-Visible spectrophotometer. Drug release profiles of the semi IPN micro beads at gastro intestinal tract (GIT) fluids (pH 1.2 and 7.4) confirmed that beads formed are pH-sensitive, resulting controlled release of drug during in vitro dissolution experiments. It has been analyzed with an empirical equation to understand the diffusion nature of drug through the micro beads. Both encapsulation efficiency and release patterns are found to depend on the nature of the cross-linking agent as well as amount of drug loading and % of pectin-PVP. In-vitro release studies indicated that the beads have potentially used for site specific drug delivery for colon cancer. Keywords: Pectin, PVP, Micro beads, 5-Fluorouracil, Drug delivery and Colon cancer.

# Introduction

5-Fluorouracil is an anticancer agent and the most widely used drug in the treatment of malignancies arising from breast, gastrointestinal tract, head, and neck regions of the body for several decades [1]. It is considered the major chemotherapeutic agent with clinical activity against colorectal cancer [2, 3]. Localizing 5-FU directly to the colon is expected to reduce systemic side effects allowing more effective and safe therapy with high tumor diffusivity [4]. Because of its incomplete and erratic oral bioavailability, 5-FU is commonly administered intravenously. However, patients prefer oral rather than intravenous therapy, with oral treatment potentially more convenient and easy [5]. Site-specific delivery of 5-FU may reduce the systemic side effects and provide effective and safe therapy of colorectal cancer that may reduce the dose and duration of therapy when compared with the conventional treatment [6].

Development of anti cancer drugs for specific colon delivery, pHsensitive carriers have attracted increasing attention. Swelling of such carriers in the stomach should be minimal instead water absorption and drug release should increase as the drug carriers pass down the intestinal tract due to an increase in pH. Several synthetic or natural polymers with acidic or basic groups have been employed to prepare pH-sensitive IPN systems. The coating of pH- sensitive polymers to the tablets, capsules or pellets provides sustained release along with protection of the active drug from gastric fluid [7, 8]. Polysaccharides and its derivatives, retain their integrity in the upper gastrointestinal tract because they are resistant to the digestive action of gastrointestinal enzymes. A combination of judicially selected natural and synthetic polymers based networks has been found to be useful in enhancing the release of short halflived drugs under physiological conditions. In order to achieve this, the properties of natural and synthetic polymers have been modified by grafting, blending and other means. Blending of synthetic polymers into cellulose and modified celluloses are widely accepted. Pectin consists mainly of linearly connected a-(1-4)-D-galacturonic acid residues partially esterifies with methanol. The degree of methoxylation (DM) is used to classify the pectins as high methoxyl pectins (DM > 50) and low methoxyl pectins (DM < 50) [9, 10].Pectin, a structural plant polysaccharide, remains as aggregated of macromolecules in acid environments. At natural solution pH pectin aggregates tends to dissociate and expand. Also, pectin is resistant to protease and amylase which are active in the upper gastrointestinal tract, whereas they are digested by a large number of micro floras of the colon (11, 12, and 13). Due to these properties it is highly possible that pectin could function as a delivery vehicle to escort protein, polypeptide, and drugs from the mouth to colon.

Although research on pectin based oral delivery system is proceeding at a rapid pace, most of the studies focused on the construction of the vehicles by using pectin as shield material. Pectin is a natural, non-toxic and anionic polysaccharide extracted from cell walls of most plants. The most attractive property of the pectin for industrial applications is its gelling activity.

Polyvinyl pyrrolidone (PVP) is a polymeric lactam and synthetic water soluble linear polymer used in a large number of pharmaceutical formulations. It is used for major additives to gastrointestinal pharmaceutical preparations. Its inclusion dry form pharmaceuticals offer enhancement of drug release and bioavailability of drugs with low solubility. Enhancement of drug release is caused by the inhibition of crystallization of drugs. Many researchers have been developed pectin based formulations for colon specific drug delivery. Recently Wei et al developed a pectin/ethyl cellulose film coated 5-Fluorouracil pellets and were used for colon cancer drug delivery to improve anti tumor activity with low systematic toxicity [14]. Gaur gum based carriers were developed for site specific delivery of 5-fluorouracil to the colon without the drug being released in the stomach or small intestine [2]. A macro molecular carrier was developed via conjugation of hydroxyethyl starch with 5-Fluorouracil through ester bonds and were relatively stable in acidic buffer solution. These macromolecular carriers are also used for sustained release of anti tumour drugs [15]. Recently Madhu et al; also developed a pH sensitive IPN microspheres for controlled release of anti cancer drug [16]. In the present study we developed pectin/PVP micro beads and were encapsulated with 5-FU in via in-situ method. The developed micro beads were potentially used for controlled release of 5-FU in GIT fluids, because these blends have the ability to enhance and improving their dissolution rates at intestinal tract minimizing at gastric medium.

# **Materials and Methods**

#### **Materials**

Pectin (PC) and 5-fluorouracil (5-FU) were purchased from Himedia, Mumbai., India. Poly (vinyl pyrrolidone), methanol, and calcium chloride were purchased from S.D. fine chemicals, Mumbai, India. All the chemicals were used without further purification. Throughout the experiment double distilled water was used.

# Preparation of Pectin-poly (Vinyl Pyrrolidone) Micro Beads

Pectin (1.8 gm) and PVP (0.2 gm) were dissolved separately in 25 mL of distilled water until clear solution is formed. The two solution were mixes to get homogenous solution and taken in hypodermic syringe and added drop wise into aqueous methanol solution (80:20 methanol: water) containing  $CaCl_2$  for 10 sec under constant stirring. The beads were formed and then separated from the solution and washed with water for several times. The resulting beads were dried at 40 °C until to attain constant weight was reached. The 5-FU drug loaded beads were prepared by adding to blend solution before

crosslinking. Various formulations were prepared by different amounts of PVP, varying % of drug, and crosslinker variation (see table 1).

#### **Swelling Studies**

Dynamic swellings of beads were performed in water and different pH conditions. In swelling experiments, beads were soaked in solvent, and were removed from the swelling bottles after 24 hours and blotted carefully with tissue papers (without pressing hard) to remove the surface-adhered buffer solution. The beads were then weighed (w<sub>1</sub>) on an electronic microbalance (ADAM AFP-210L England accurate to  $\pm$  0.0001 g). The beads were dried to a constant weight (w<sub>2</sub>) in an oven maintained at 40 °C for 5 hrs. Swelling experiments were repeated thrice for each sample and average values were used in data analysis. The standard deviations (S.D.) in all cases were < 5 %. The weight % of water uptake was calculated as:

% Water uptake  
= 
$$\left(\frac{\text{wt of swollen beads } (W_1) - \text{wt of dry beads } (W_2)}{\text{wt of dry beads } (W_2)}\right) \times 100$$
 (1)

#### Estimation of Drug loading and Encapsulation Efficiency

The loading efficiency of 5-FU in the beads was determined spectrophotometrically. About ~10 mg of the drug-loaded beads were placed in 10 mL of buffer solution and stirred vigorously for 48 hours to extract drug from the beads. The solution was filtered and assayed by UV spectrophotometer (Lab India, Mumbai, India) at fixed  $\lambda_{max}$  value of 270 nm. The results of % drug loading and encapsulation efficiency were calculated, using by following Eqs. (2), (3), respectively. These data are compiled in Table 1,

% Drug loading  
= 
$$\left(\frac{\text{Amount of drug in micro beads}}{\text{Amount of micro beads}}\right) \times 100$$
 (2)

% of Encapsulation efficiency =

$$\left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) 100 \tag{3}$$

#### In vitro Release Studies

In vitro release studies were carried out using Tablet dissolution tester (LAB INDIA, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at  $37\pm 0.5$  °C at constant speed of 100 rpm. Drug releases from the micro beads were carried out in pH 1.2 and 7.4 phosphate buffer solution at 37 °C. At regular intervals of time, sample aliquots were withdrawn and analyzed using UV spectrophotometer (Lab India, Mumbai, India) at the fixed  $\lambda_{max}$  value of 270 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.

#### **FT-IR** analysis

Infrared spectroscopy is one of the most powerful analytical tools, which provides the possibility of chemical identification. It provides information regarding the structure of a molecule. FT-IR is based upon the simple principle that a chemical substance shows selective absorption in the infrared region giving rise to absorption bands called an IR absorption spectrum, over a wide wavelength range. The PC-PVP semi-IPN micro beads were finely ground with spectroscopic grade KBr to prepare pellets using a hydraulic pressure of 600 kg/cm<sup>2.</sup> Different bands will be present in the IR spectrum, which will correspond to the characteristic functional groups and bonds present in a chemical substance. The infrared absorption spectra of the PC-PVP micro beads were obtained using a FT-IR spectrophotometer (PerkinElmer Spectrum Two, UK).

#### Differential Scanning Calorimetric (DSC) analysis

DSC curves of pure beads, 5-Fluorouracil, and drug loaded beads were recorded using TA instruments sequential thermal analyzer (Model-SDT  $Q_{600}$ , UK). The sample was weighed between 10 to 12mg. The samples were heated from 50° to 400 °C Analysis of the samples was performed at heating rate of 10°C/min under N<sub>2</sub> atmosphere at a purging rate of 100 mL/min.

#### X-ray Diffraction studies (XRD)

The X-ray diffraction patterns of PC-PVP micro beads, pure 5-FU and PC-PVP drug loaded micro beads were carried on a Shimadzu Lab-XRD-6000X diffractometer (Japan), using Nickel-filtered Cu K radiation ( $\lambda$ =1.548A°) at 40 kV and 50 mA in the 2 $\theta$  range of 0-50°C.

#### Scanning Electron Microscopy (SEM)

To determine the particle size and size distribution, drug loaded micro beads were taken on a glass slide and their sizes were measured using an optical microscope under regular polarized light. SEM images of micro beads were recorded using a JSM 6400 SEM (JEOL Ltd., Akishima, Tokyo, Japan) at X50 and X500 magnifications. Working distance of 8.5-9.5 mm was maintained and the acceleration voltage used was 10 kV, with the secondary electron image (SEI) as a detector.

### **Results and Discussions**

#### **FT-IR Analysis**

The objective of FT-IR analysis was to investigate the molecular interaction between PC-PVP micro beads, and drug loaded micro beads. The FT-IR analysis of pectin-pvp micro beads were as shown in figure (1) here a broad peaks at 3418, 2934 cm<sup>-1</sup> due to – OH group in polymer and –CH<sub>2</sub> functional group in polymer as well as in vinyl pyrrolidone. A peak at 1747, 1651, 1325, 1440 to 1492 cm<sup>-1</sup> due to carbonyl group, bending vibration of imides group , –CH and –CH<sub>2</sub> bending vibration . The peak at 1102 cm<sup>-1</sup> is attributed to COO stretching mode in Polymer, while the peak at 832 cm<sup>-1</sup> is due to the -OH out of plane motion of the carboxylic acid group in pectin. The peaks at 1145, 1233 cm<sup>-1</sup> O-H bending and C-O stretching vibrations. The peak at 1326cm<sup>-1</sup> in pure 5-fluorouracil is due to –C-F stretching vibrations after drug loading the peak intensity is very low this is due to uniformly molecular dispersion was observed.

#### Differential Scanning Calorimetric (DSC) studies

DSC thermograms of pure 5-FU (a), PC-PVP micro beads (b), and drug loaded micro beads (c) is shown in figure (2). DSC curve of pure beads which indicates an endothermic peak appeared at 221 °C is corresponds crosslinking of Ca<sup>+2</sup> between two –COO·Na<sup>+</sup> of PC. The pure 5-FU exhibits a sharp peak at 285 °C is due to polymorphism and melting. However, this peak is not appeared in the case of drug loaded beads which conforms that the drug is molecularly dispersed in the polymeric matrices.

#### X-ray Diffraction Studies

X-ray diffraction technique is useful for the drug crystallinity in the polymeric matrices. Figure 2 shows the X-ray diffractograms of pure 5-FU (a), 5-FU loaded micro beads (b), and plain micro beads(c). 5-FU has shown characteristic intense peaks at  $2\theta$  of 17°, 29°, and 32° suggesting its crystallinity. In the case of 5-FU loaded micro beads these peaks are not found indicating the 5- FU is dispersed at a molecular level within polymeric matrices. In the case of plain and drug loaded beads semi crystalline peaks at 21° is due to the formation of hydrogen bonding between pectin and PVP. A strong crystalline peak was observed at 45.5° C in the hydrogel beads due to the ionic crosslinking of pectin.

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Figure 1. FTIR Spectra of PC-PVP micro beads and PC-PVP Drug loaded micro beads.

Figure 2. DSC thermo grams of (a) pure 5-FU, (b) pristine PC-PVP micro beads and (c) 5-FU loaded PC-PVP micro beads.



#### Scanning Electron Microscopy Studies

Figure 4 (a-c) shows SEM micrographs of 5-FU loaded PC-PVP micro beads. Figure: 4(a) and 4(b) displays the average size of the beads is approximately 500  $\mu$ m and figure: 4(c) shows average size of the beads is approximately 50  $\mu$ m measured as different

magnifications from SEM images. Beads of this study were almost spherical with smooth surfaces Figure 4(c) showed no phase separation was also observed. Optical microscopy gave particle size of the micro beads for all formulations the same results and is presented in Table1.



Figure 3. X-RD Spectra of (a) 5-FU Drug, (b) drug loaded PC-PVP micro Beads and (c) pristine PC-PVP micro beads.



Figure 4. Scanning Electron Micrographs of PC-PVP micro beads for different magnifications (a-c).



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| Formulation codes | %of Pectin in beads | % of PVP in beads | %(5-FU) loaded | % of<br>CaClo | % Encapsulation | % Water |
|-------------------|---------------------|-------------------|----------------|---------------|-----------------|---------|
| PC pure           | 100                 | 00                | 20             | 1             | $69.3 \pm 1.5$  | 189     |
| PC-PVP-1          | 80                  | 20                | 20             | 1             | $72.5 \pm 0.9$  | 193     |
| PC-PVP -2         | 70                  | 30                | 20             | 1             | 76.4 ± 0.6      | 206     |
| PC-PVP -3         | 60                  | 40                | 20             | 1             | 81.2 ± 1.4      | 238     |
| PC-PVP -4         | 70                  | 30                | 10             | 1             | 70.6 ± 1.2      | 195     |
| PC-PVP -5         | 70                  | 30                | 30             | 1             | 79.1 ± 0.8      | 214     |
| PC-PVP -6         | 70                  | 30                | 10             | 0.5           | 82.9 ± 1.2      | 261     |
| PC-PVP -7         | 70                  | 30                | 10             | 2             | 66.8 ± 1.6      | 186     |

Table 1: Results of % of encapsulation efficiencies and % water uptake of different Formulations

#### **Encapsulation Efficiency**

The encapsulation efficiencies were calculated for all formulations as shown in table1. The % of encapsulation efficiency increased with increasing amount of PVP in the micro beads. For beads containing 100/0, 80/20, 70/30 and 60/40 and 20 wt. % 5-FU with 1 wt. % of CaCl<sub>2</sub>, encapsulation efficiencies were 69.3, 72.5, 76.4 and 81.2 %, respectively. For beads crosslinked with 0.5, 1 and 2 wt. % of CaCl<sub>2</sub>, encapsulation efficiencies are, 82.9, 70.6 and 66.8 % respectively. Such a decreasing trend is due to an increase in crosslink density, because the beads will become rigid, thereby reducing the free volume spaces within the polymer matrix and hence, a reduction in encapsulation efficiency is observed.

#### **Swelling Studies**

The % of equilibrium swelling decreased from 261 to 186 with increasing amount of CaCl<sub>2</sub> from 0.5 to 2 wt. %. This is due to increased crosslink density and decreased pore volume of the polymer matrix [17] with increasing amount of CaCl<sub>2</sub> in the matrix. By increasing the drug loading of the matrix, % water uptake also increased i.e., as the drug wt. % loading increased from 10 to 30 %, the % equilibrium swelling were 195, 206 and 214, respectively. Such an increase in swelling of the matrix is due to the incorporation of hydrophilic PC along with PVP chains to form network matrix. However, % water uptake (or) % dynamic swelling of the matrix containing 100/0, 80/20, 70/30 and 60/40 of PC and PVP are 189, 193, 206 and 238, respectively. The % water uptake has increased with an increasing amount of PVP in the polymeric matrix. This is due to the fact that as the amount of PVP increases in the matrix, hydrophilicity of the matrix could increase slightly.

#### In-vitro release studies

#### Effect of Cross-linking agent

The % cumulative release data against time plots for varying amounts of CaCl<sub>2</sub> such as 0.5, 1.0 and 2 % at the fixed amount of the drug (10 %) are displayed in figure 5. The % cumulative release is quite fast and large at the lower amount of Ca<sup>+2</sup> (i.e., 0.5 %), whereas the release is quite slower at higher amount of Ca<sup>+2</sup> (i.e., 2 %). The % cumulative release of formulation containing

lower amount of crosslinker (0.5 %) a 100% was observed at 600 min but those formulations containing higher amount of crosslinker the % cumulative release was observed even more than 740 min. The cumulative release is somewhat smaller when lower amount of Ca<sup>+2</sup> was used probably because at higher concentration of Ca<sup>+2</sup>, polymeric chains become rigid due to the contraction of micro voids, thus decreasing % cumulative release of 5-FU through the polymeric matrices. As expected, the release becomes slower at higher amount of Ca<sup>+2</sup>.

Figure 5. Percentage cumulative release of 5-FU (10%) through PC-PVP micro beads containing different amounts of crosslinker PC-PVP -6 (0.5%), PC-PVP -4 (1%), and PC-PVP -7 (2%).



#### Effect of drug percent loading

Figure 6. shows the release profiles of 5-FU loaded beads at different amount of drug loadings. The % cumulative release of formulations PC-PVP-4, PC-PVP-2, and PC-PVP-5 beads are 62, 73, and 94% respectively. Release data showed that formulations containing the highest amount of drug (30 %) displayed fast and

higher release rates than those formulations containing a small amount of 5-FU (10 and 20%). A prolonged release was observed for the formulation containing lower amount of 5-FU. Due to the availability of more free void spaces through which lesser number of drug molecules will transport.





#### Effect of PVP content

In vitro release profiles of 5-FU from formulations of PVP and it is prepared by taking different amounts of PVP (20, 30, 40%) with 20% of 5-FU are shown in figure 7. Faster release rates were observed from formulations prepared with higher amount of PVP (i.e., 96%) observed than those formulations prepared using a lower amount of PVP (i.e., 65%). In the case of pure PC beads the release of 5-FU was observed 56%. Faster drug release observed from formulations prepared with higher amount of PVP is due to the coating of higher amount of PVP on PC particles. Since the

increasing the hydrophilic character of beads in increasing of PVP. These results are shown in table 1. Increase in the amount of PVP in the beads, increases the % water uptake of PC-PVP beads. When the swelling degree increases, amorphous regions produces free volumes that are suitable for penetration of liquid molecules to beads and then diffusion of drug to external media. Therefore, cumulative release of 5-FU increases with the PVP content of beads [18].







# Effect of pH

The in vitro release experiments were carried out 5-FU loaded beads in gastric and intestinal pH conditions. Releases of 5-FU from beads with 1.2 pH and 7.4 pH solutions at 37  $^{\circ}$ C are shown in

figure 8. The release of 5-FU is higher at pH 7.4 than pH 1.2 This can be explained on the basis of higher degree of swelling due to ionization of carboxylic groups in the networks at pH 7.4. This indicates the pH sensitivity of beads.





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#### **Drug release kinetics**

Drug release kinetics was analyzed by plotting the cumulative release *versus* time and by fitting these data to the exponential equation [19].

$$M_{\star}/M = kt^n$$
 ------ (4)

Here, M/M represents 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. We have estimated the values of n and k for all t formulations by using the least square procedure and these values are given in table 1. If n = 0.5, then drug diffuses and releases from the polymer matrix following a Fickian diffusion. For n > 0.5, anomalous or non-Fickian

type drug diffusion occurs. If n=1, a completely non-Fickian or Case II release kinetics is operative. The intermediate values ranging between 0.5 and 1.0 are attributed to the anomalous type transport [20]. The values of *k* increased with increasing % loading of 5-FU into the polymeric beads, but the *n* values decreased with decreasing % loading of 5-FU. This indicates the interaction between the polymer beads and drug as studied from the release kinetics represented by Eq. (4) proposed by Peppas *et.al*, [20]. The values of exponent *n* are found to range between 0.328 and 0.472 as calculated from the empirical equation, which indicated that drug release slightly deviated from the Fickian trend, while 0.5 < *n* <1.0 indicates non-Fickian or anomalous transport. The values of the correlation coefficient, 'r' is calculated and included in tables 2 and indicating a good fit of the experimental data.

Table 2: Release kinetics parameters for different formulations at pH-7.4

| Formulation codes | k     | п     | Correlation coefficient( r) |
|-------------------|-------|-------|-----------------------------|
| PC pure           | 1.174 | 0.354 | 0.992                       |
| PC-PVP-1          | 1.217 | 0.373 | 0.979                       |
| PC-PVP-2          | 1.187 | 0.472 | 0.956                       |
| PC-PVP-3          | 0.821 | 0.328 | 0.991                       |
| PC-PVP-4          | 0.867 | 0.337 | 0.982                       |
| PC-PVP-5          | 0.826 | 0.421 | 0.992                       |
| PC-PVP-6          | 0.938 | 0.364 | 0.938                       |
| PC-PVP-7          | 0.857 | 0.382 | 0.994                       |

# Conclusions

pH sensitive 5-FU loaded PC-PVP micro beads prepared using Ca<sup>+2</sup> as a crosslinker, DSC and X-RD studies confirmed the molecular level dispersion of drug in the beads. SEM pictures have shown the good compatibility of PC and PVP compositions present in the beads with smooth surface. The encapsulation efficiency was found to vary between 69 and 82% depending upon the blend composition, cross-linking and the amount of drug loading. Drug

release studies indicated controlled release of 5-FU for more than 10 h and potentially used for colon cancer drug delivery.

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