Supporting Information File

Therapeutically effective controlled release formulation of pirfenidone from non-toxic biocompatible carboxymethyl pullulan-poly(vinyl alcohol) interpenetrating polymer networks

Saundray Raj Soni†, Bibhas K. Bhunia§, Nimmy Kumari†, Subhashis Dan§, Sudipta Mukherjee‡, Biman B. Mandal§* and Animesh Ghosh†*

†Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi – 835215, Jharkhand, India.
§Biomaterial and Tissue Engineering Laboratory, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India.
‡Division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India.
§Bioequivalence Study Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India.

Corresponding Authors:

1. Animesh Ghosh, PhD
   Assistant Professor
   Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra
   Ranchi – 835215, Jharkhand, India
   E-mail: aghosh@bitmesra.ac.in, anim_1607@yahoo.co.in

2. Dr. Biman B. Mandal
   Associate Professor
   Department of Bioscience and Bioengineering, Indian Institute of Technology Guwahati
   E-mail: biman.mandal@iitg.ac.in, mandal.biman@gmail.com
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S.4.2. Synthesis of sodium carboxymethyl pullulan (CMP)

CMP was synthesized by adopting the similar method as reported earlier by our group with slight modification. Briefly, 5 g of pullulan was kneaded using varying quantities of 10 M ice cold sodium hydroxide for approximately 45 min. to which different quantities of monochloroacetic acid (MCA) was added slowly up to 60 min. at 15 °C. Then the mixture was stirred continuously for next 60 min. at a constant temperature of 65 °C. The final pH was adjusted using glacial acetic acid to pH 7.0, washed with 80 % (v/v) methanol/water and dialyzed against water for 48 h, then after dried to constant weight and grinded to fine powder.

S.4.2.1. Determination of degree of substitution (DS)

The simplest method of DS determination is acid-base titration method. The method utilizes the concept of back titration of the excess acid present in the system. An accurately weighed quantity of carboxymethyl pullulan (CMP) was taken and treated with HCl, which converts the sodium salt of polymer to free acid form. Then sodium hydroxide is added to it in known excess quantity leading to formation of sodium carboxylate. The excess amount of sodium hydroxide is back titrated with HCl and degree of substitution is calculated using the equation as given below.

\[ DS = \frac{0.162A}{(1 - 0.058A)} \]

Where, A is the milliequivalents of NaOH required per gram of sample.

S.4.3. Preparation of placebo PVA-CMP IPN microspheres

PVA-CMP IPN microsphere was prepared by adopting the similar method as reported earlier by our group with slight modification. Briefly, the aqueous phase consisted of 10 % (v/v) polymeric dispersion of PVA and CMP in the ratio of 3:1, while oil phase was light liquid paraffin. Tween 80 was used as emulsifying agent. Initially oil phase and Tween 80 was mixed at 800 rpm for 15 minutes to which aqueous phase was added slowly and after
formation of stable emulsion 1M HCl (0.5 mL) glutaraldehyde (crosslinker) was added and reaction was continued for 3 h to get microspheres. These microspheres were subsequently washed with petroleum ether, glycine and water. Finally, microspheres were dried at 40 °C in vacuum oven for 24 h till constant weight was achieved and stored in desiccator for further use.

**S.4.4. Physicochemical characterization**

Viscosity of pullulan and different batches of carboxymethyl pullulan was measured at concentration of 1 % w/v, using Brookfield viscometer (LVTDV-II, Brookfield Engineering Labs, Inc., Stoughton, MA), at 25 °C, with spindle rotation at 3 rpm (Spindle no. CPE 41).

The molecular weight of pullulan and different batches of CMP were determined using gel permeation chromatography technique (Waters Corporation, USA) using refractive index detector. A hydrogel-1000 column (7.5 mm × 300 mm; 8 µm; Waters, USA) was used with mobile phase 0.1% (w/w) sodium nitrate (Acros Organics, USA) in Milli-Q water at flow rate of 0.5 mL/min. The standard molecular weights used were of 150,000, 410,000, 670,000, 1,400,000 and 2,000,000 Da, purchased from Dextran (Sigma Aldrich, Germany).

The elemental analysis (C, H, O) for pullulan and different batches of CMP was performed using elemental analyzer (Model Vario EL III, Elementer, Hanau, Germany).

Optical contact angle was performed to measure the wetting ability of pullulan and CMP. Sample for analysis was prepared by casting polymeric dispersion on glass slide and measurement was performed by video based contact angle metre OCAH 20 (Data Physics, Germany). Surface energy was calculated using equation of state, Schultz Method-2, by means of Data Physics SCA20 software (Version 2.01).

FTIR analysis was performed for pure drug, PVA, pullulan, CMP, placebo microsphere and drug loaded microsphere. The FTIR spectra was recorded using KBr pellet
method (1wt % of sample content) by scanning in the range of 600-4000 cm\(^{-1}\) using FTIR-8400S (Shimadzu, Japan).

Raman spectroscopy was performed to characterize PVA, pullulan, CMP and PVA-CMP IPN placebo microsphere. The spectroscopy was performed using Renishaw confocal microscopy. Raman spectroscopy system consisting of microscope with magnification of 50X used 785 nm, He-Ne laser beam as the excitation source and focused on the sample with a spot of size of about 1 µm diameter. Analysis was performed using a charged coupled device (CCD) detector in the scan range of 3300 cm\(^{-1}\) to 300 cm\(^{-1}\).

Solid state \(^{13}\)C NMR spectroscopy was performed for pullulan, CMP and PVA-CMP placebo IPN microspheres. The study was conducted to assure the modification of pullulan and formation of GA assisted crosslinking between PVA and CMP for IPN formation. The analysis was performed by inserting about 300 mg of the sample in ceramic rotor on a JEOL ECX 400 (Peabody, MA, USA) spectrometer operated at 75 MHz.

X-ray diffraction spectroscopy was carried out for pirfenidone, placebo microsphere and pirfenidone loaded microsphere, using X-ray diffractometer (Bruker AXS D8 advance, Germany; X-ray Cu, wavelength 1.5406 Å; detector: Si (Li) PSD) in the range of 10 to 60° at the scanning speed of 2°/minute and a chart speed of 2°/2 cm/20.

Differential scanning calorimetry was performed for PVA, CMP, pirfenidone, blank microspheres and pirfenidone loaded microsphere using DSC-4000 (Perkin Elmer, USA). Each sample approximately 3 to 5 mg was weighed accurately and sealed hermetically in aluminium pan using crimper press, which was heated in an inert atmosphere of nitrogen (flow rate 20 mL/min) from 30 °C to 250 °C at a rate of 10 °C/minute.

Field emission scanning electron microscopy (FESEM) was performed to study the shape, surface morphology of CMP and cross sectional networks of PVA-CMP IPN microsphere using (FESEM, Supra 55, Zeiss, Germany). Samples were prepared by mounting
on stubs and coated with gold-palladium to thickness of 2 nm under vacuum and then seen at suitable magnification.

**S.4.5. Thermal kinetic study**

Thermal degradation kinetics is studied using thermal analysis and all the kinetic studies utilizes the basic rate equation.

\[
\frac{d\alpha}{dt} = kf(\alpha) \tag{2}
\]

The above equation gives idea about the rate of conversion \( \frac{d\alpha}{dt} \) at a constant temperature \((T)\) as a function of reactant concentration \(f(\alpha)\) at rate constant \(k\).

In case of degradation kinetics of polymers it is assumed that the rate of conversion is proportional to the concentration of reactant remaining for the reaction, as described by the following equation:

\[
f(\alpha) = (1 - \alpha)^n \tag{3}
\]

Arrhenius equation correlates the temperature dependence of the rate constant, which can be explained by following equation:

\[
k(T) = A \exp \left( -\frac{E}{RT} \right) \tag{4}
\]

Where, \(A\) denotes the pre-exponential factor, \(E\) is the activation energy and \(R\) is the gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)).

Upon combining equations (2-4), yields equation (5)

\[
\frac{d\alpha}{dt} = A(1 - \alpha)^n \exp(-E/RT) \tag{5}
\]

The thermal degradation rate is mainly dependent on two parameters, temperature \((T)\) and degree of conversion \((\alpha)\) and the equation (5) is the most useful mathematical equation for the calculation of kinetic parameters from thermal degradation data.

The degree of conversion \((\alpha)\) is calculated using the equation (6)

\[
\alpha = \frac{w_0 - w_t}{w_0 - w_\infty} \tag{6}
\]
Where, \( w_0 \), \( w_t \) and \( w_\infty \) denotes initial weight, weight at given temperature \((t)\) and after complete degradation, respectively. The \((\alpha)\) value denotes overall transformation progress comprising of various steps, each of which has its specific extent of conversion and \( f(\alpha) \) denotes the kinetic reaction model.

At constant heating rate, \( \beta = \frac{dt}{dt} \), equation (2) can be arranged as:

\[
\beta \frac{d\alpha}{dT} = A \exp \left( \frac{-E}{RT} \right) f(\alpha) \quad (7)
\]

Activation energy \((E)\) and pre-exponential factor \((A)\) can be calculated using different mathematical models, without assuming any reaction model. Ozawa-Flynn-Wall\(^7\) and Kissinger-Akahira-Sunose\(^9\) are two widely used isoconversional model free methods for calculation of activation energy from thermal data.\(^11\)

**S.4.5.1. Ozawa-Flynn-Wall (OFW)**

This is one of the simplest isoconversional method for calculation of activation energy, directly from weight loss v/s temperature data obtained at various heating rates \((\beta)\). Activation energy is the function of conversion factor \((\alpha)\) and can be calculated from the slope value obtained from the linear relationship between \( \ln (\beta) \) and \( 1/T \) from equation (8)

\[
\ln(\beta_i) = \left[ \ln \left( \frac{A_\alpha R}{E_{\alpha i}} \right) - \ln f(\alpha) \right] - \left( \frac{E_{\alpha i}}{RT_{\alpha i}} \right) \quad (8)
\]

**S.4.5.2. Kissinger-Akahira-Sunose (KAS)**

In this method, activation energy is calculated from slope using the linear relationship between \( \ln \beta / T^2 \) and \( 1/T \) from equation (9), which is represented by \(-E/R\) multiplied with gas constant \(R\).

\[
\ln \left( \frac{\beta_i}{T_{\alpha i}^2} \right) = \left[ \ln \left( \frac{A_\alpha R}{E_{\alpha i}} \right) - \ln f(\alpha) \right] - \left( \frac{E_{\alpha i}}{RT_{\alpha i}} \right) \quad (9)
\]
Where, $E_a$ and $A_\alpha$ are the apparent activation energy and pre-exponential factor at a given degree of conversion, $\alpha_i$. $T_{a_i}$ is the temperature at which the conversion factor absolute temperature and degree of conversion $\alpha_i$ is reached at heating rate $\beta$.

S.4.6. In vitro enzymatic degradation study

Preparation of 3,5-dinitrosalicylic acid (DNS) reagent: The reagent was prepared according to the method as described earlier. Briefly, 1 g of DNS and 30 g of sodium potassium tartrate was added to 80 mL of 0.5 N of sodium hydroxide and heated at around 60 °C to dissolve the reagents. Finally volume was made up to 100 mL with Milli-Q water.

Preparation of calibration curve of maltotriose: Structurally pullulan is composed of maltotriose units linked by $\alpha$-(1→6) glycosidic bond and pullulanase specifically cleaves $\alpha$-(1→6) glycosidic bond, yielding maltotriose. So, calibration curve of standard maltotriose $>90\%$ (HPLC) (Sigma, St.Louis, USA), was prepared in the range of 0.1 mg/mL to 1 mg/mL and standard equation was generated by plotting a graph between absorbance (540 nm) v/s concentration (mg/mL).

Assay for determination of sugar content: The concentration of maltotriose with time was analyzed by method as described earlier. Briefly, 1 mL of sample aliquot was withdrawn at predetermined time interval and added to 4 mL of DNS, heated for five minutes and finally cooled in ice bath and kept aside to equilibrate at room temperature. Absorbance of the sample was taken at 510 nm using UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) and digestibility curve was plotted.

S.4.7.1. Cell culture and maintenance

HepG2 (human hepatocellular carcinoma) cells were procured from National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in a tissue culture flask (T25, Tarsons,
India) containing high glucose Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco, Life Technologies, USA). The flasks were then kept at 37 °C in a humidified atmosphere of 5% CO₂. On attaining 80-90% confluence, cells were trypsinized with 0.25% trypsin and 0.52mM EDTA (Gibco, Life Technologies, USA), resuspended in complete media and sub-divided into three flasks.

S.4.8. Acute oral toxicity study

Acute oral toxicity of CMP and PVA-CMP placebo IPN microspheres were conducted as per Organization of Economic Co-operation and Development (OECD guideline) for the test of chemicals 425, adopted on 17th December 2001. Prior to initiation of the study, protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology, Ranchi, India, with approval no.: 1972/PH/BIT/4/17/IAEC. Five female nulliparous, non-pregnant, five week old mice each for (control, CMP and IPN microsphere) were grouped together and then housed in polycarbonate cage with sufficient quantity of suitable food and water on a timely basis at 25-30 °C and 40-70% relative humidity in a 12 h light on/off cycle. To the treatment group, one of the mice was dosed at 2000mg/kg of CMP and microsphere respectively and continuously observed up to 4 h for their survival or mortality. After their survival, the remaining animals were also given same dose and observed for 14 days. The hematological and serum biochemical parameters of all the three groups were reported along with the mortality observed if any.

S.4.10.1. Drug release kinetics and mechanism modelling

Drug release kinetics and its release mechanism from various different formulations were studied using different kinetic models.
The drug release profile of various different modified release dosage forms like transdermal systems, osmotic systems, matrix tablets or biphasic hydrogels with low soluble drugs, etc. can be explained using zero order kinetic equation. The dosage form following zero order kinetics, releases the same amount of drug per unit time and is considered to be ideal formulation for prolonged action.

\[ Q_t = Q_0 - K_0 t \]  

Where, \( Q_t \) is the amount of drug released in time \( t \), \( Q_0 \) is the initial amount of drug present in the system, \( K_0 \) is the release rate constant and \( t \) is time.

First order kinetics is presented by equation (11), which is generally followed by the dosage forms containing water soluble drugs in porous matrices. The dosage unit releases drug in such a fashion that the drug released is proportional to the amount of drug remaining in the polymeric matrices, in such a manner that the amount of drug released by unit of time diminish.

\[ \log Q_t = \log Q_0 - Kt/2.303 \]  

Where, \( Q_t \) is the amount of drug released at time \( t \) and \( Q_0 \) is the initial amount of drug present in the delivery system, \( k \) is the first order rate constant and \( t \) represents the time.

Hixon-Crowell square root kinetic model is applicable to the dosage forms like tablet, where dissolution occurs in planes parallel to the drug surface and the tablet surface diminishes proportionally in such a manner that the initial geometrical form is constant all the time, which can be described by equation (12).

\[ W_0^{1/3} - W_t^{1/3} = Kt \]  

Where, \( W_0 \) and \( W_t \) represents the amount of drug present initially and amount of drug released after time \( t \) respectively. \( K \) is the Hixon-Crowell dissolution rate constant.

Higuchi model describes the drug release as a diffusion mechanism based on Flick’s law, dependent on square root of time, equation (13). This equation can be applied to describe the
drug release mechanism from different modified pharmaceutical dosage forms like transdermal system and matrix tablets containing water soluble drug.\(^{19}\)

\[
Q_t = Q_0 + K_H t^{1/2}
\]  

(13)

Where, \(Q_0\) and \(Q_t\) are the initial drug available in the system and drug released at time \(t\) respectively.

Korsmeyer-Peppas model is generally used to explain the release mechanism from polymeric dosage form, for which release mechanism is not well known or when more than one type of release mechanism may be involved.\(^{20}\) Korsmeyer-Peppas model is explained by equation (14).

\[
\frac{M_t}{M_\infty} = K t^n
\]

(14)

Where, \(M_t/M_\infty\) is the fraction of drug released at time \(t\) and the value of ‘\(n\)’ characterizes the release mechanism of drug. For the case of cylindrical tablets or spherical polymeric devices, \(n \leq 0.45\) corresponds to a Fickian diffusion mechanism, \(0.45 < n < 0.89\) corresponds to non-Fickian transport, \(n = 0.89\) to Case II (relaxational) transport, and \(n > 0.89\) refers to super case II transport.

S.4.10.2. Stability study

The stability study for the optimized formulation based on the highest entrapment and drug release profile (F5) was conducted as per International Conference on Harmonization (ICH) guidelines (Q1E, Step4) for oral finished dosage form under accelerated conditions. The microspheres were sealed in glass vial using rubber stopper and aluminium cap with the help of crimper and stored in stability chamber (Newtronics, Mumbai, India) at 40 °C ± 2 °C / 75 % RH ± 5 % RH for six months. The samples were withdrawn periodically after 1, 2, 3 and 6 months and evaluated for description, assay value, drug release profile and compared with
initial data. The analysis was conducted in triplicate and results were presented as average ± SD (n = 3).

S.4J.11.2. Animal housing and handling

The study was conducted on male albino rabbits (1.5 kg to 2 kg), three rabbits each group, which were housed individually in polycarbonate cages with sufficient food and water at 25-30 °C and 40-70% relative humidity in a 12 h light on/off cycle, in the animal house facility at the Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, India. The study was performed in accordance with the prior approved protocol (IAEC approval no.: 1972/PH/BIT/5/17/IAEC) by the Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology, Ranchi, India.
**Table S1.** Different values of digestibility rate constant and product concentration at the end of the reaction for CMP and PVA-CMP IPN microsphere.

| Test material                  | Rapid phase (Phase-1) | Slower phase (Phase-2) | Total |
|--------------------------------|-----------------------|------------------------|-------|
|                                | $k_1$ (min$^{-1}$)    | $C_{1\infty}$ (%)      | $K_2$ (min$^{-1}$) | $C_{2\infty}$ (%) | $C_{\infty}$ (%) |
| CMP                            | 0.0301                | 24.99                  | 0.0019 | 20.78              | 45.77           |
| PVA-CMP IPN miceosphere        | 0.0142                | 7.07                   | 0.0006 | 34.9               | 41.97           |

$k_1$: rate constant in rapid digestion phase; $k_2$: rate constant in slow digestion phase; $C_{1\infty}$: extent of digestion in rapid phase; $C_{2\infty}$: extent of digestion in slower phase; $C_{\infty}$: sum of both phases ($C_{1\infty}$ and $C_{2\infty}$)
Table S2. Hematological findings in control and treated group after 14 days of oral application of CMP and PVA-CMP IPN in Swiss albino mice.

| Group          | WBC (m/mm³) | RBC (m/mm³) | Hb (g/dL) | HCT (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) | PLT (m/mm³) | PCT (%) |
|----------------|-------------|-------------|-----------|---------|----------|----------|-------------|-------------|---------|
| Control (n=5)  | Mean 6.6    | 8.5         | 12.5      | 42.2    | 49.6     | 14.7     | 29.6        | 1127.0      | 0.92    |
|                | ± SD 1.4    | 2.2         | 3.3       | 3.4     | 2.4      | 3.2      | 3.8         | 6.3         | 0.06    |
| Treatment-1 (n=5) | Mean 6.2   | 8.9         | 12.9      | 45.7    | 51.6     | 14.6     | 28.2        | 1011.0      | 0.73    |
|                | ± SD 2.2    | 2.0         | 2.4       | 3.6     | 3.0      | 1.2      | 2.6         | 8.5         | 0.04    |
| Treatment-2 (n=5) | Mean 7.2   | 9.6         | 14.2      | 44.0    | 49.2     | 14.90    | 30.2        | 1251.0      | 0.88    |
|                | ± SD 2.4    | 2.3         | 2.5       | 2.1     | 1.2      | 0.96     | 3.3         | 7.6         | 0.07    |

**Treatment-1: Treatment with CMP**

**Treatment-2: Treated with PVA-CMP placebo microsphere**

WBC: white blood cells; RBC: red blood cells; Hb: haemoglobin; HCT: haematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: Platelet; PCT: plateletcrit.
Table S3. Serum biochemical findings in control and treated group after 14 days of oral application of CMP and PVA-CMP IPN in Swiss albino mice.

| Group         | GLU (mg/dL) | UREA (mg/dL) | CRE (mg/dL) | SGPT (U/L) | SGOT (U/L) | ALP (g/dL) | TP (g/dL) | CHOL (mg/dL) | LDL (mg/dL) | HDL (mg/dL) | VLDL (mg/dL) | TGL (mg/dL) | UA (mg/dL) |
|---------------|-------------|--------------|-------------|------------|------------|------------|-----------|--------------|-------------|-------------|---------------|-------------|-----------|
| Control       | Mean 116.0  | 19.0         | 0.71        | 58.0       | 153.0      | 138.0      | 4.8       | 139.0        | 30.0        | 75.0        | 24.0          | 92.0        | 1.26      |
| (n=5)         | ± SD 5.3    | 1.1          | 0.08        | 2.9        | 7.6        | 9.1        | 1.2       | 4.6          | 1.1         | 2.3         | 1.9           | 4.9         | 0.06      |
| Treatment-1   | Mean 109.0  | 21.0         | 0.78        | 51.0       | 124.0      | 132.0      | 5.5       | 117.0        | 22.0        | 64.0        | 21.0          | 105.0       | 1.54      |
| (n=5)         | ± SD 8.2    | 2.0          | 0.11        | 3.1        | 9.6        | 6.3        | 1.9       | 5.6          | 1.8         | 1.4         | 2.2           | 5.5         | 0.04      |
| Treatment-2   | Mean 120.0  | 20.0         | 0.65        | 53.0       | 143        | 122.0      | 5.6       | 105.0        | 20.0        | 63.0        | 22.0          | 110.0       | 1.63      |
| (n=5)         | ± SD 6.4    | 1.9          | 0.09        | 2.9        | 10         | 8.3        | 2.1       | 4.9          | 1.2         | 2.3         | 2.3           | 6.8         | 0.05      |

Treatment-1: Treatment with CMP

Treatment-2: Treated with PVA-CMP placebo microsphere

GLU: glucose; CRE: creatinine; SGPT: Serum glutamic pyruvic transaminase; SGOT: serum glutamic oxaloacetic transaminase; ALP: Alkaline phosphatase; TP: total protein; CHOL: cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; VLDL: very low-density lipoprotein; TGL: triglycerides; UA: uric acid.
Table S4. $3^2$ Factorial design lay out.

| Code | A: GA (mL) | B: PFD (% w/w) | Y1: (%) Yield | Y2: (%) DEE | Y3: Cumulative (%) drug release after 2 h | Y4: Cumulative (%) drug release after 8 h |
|------|------------|----------------|---------------|-------------|------------------------------------------|------------------------------------------|
| F1   | 3          | 45             | 66.76         | 39.62       | 61.13                                    | 90.72                                    |
| F2   | 1          | 30             | 62.77         | 25.84       | 99.87                                    | 102.77                                   |
| F3   | 3          | 30             | 74.54         | 29.35       | 56.14                                    | 88.98                                    |
| F4   | 5          | 30             | 83.81         | 36.43       | 49.62                                    | 67.28                                    |
| F5   | 5          | 45             | 72.24         | 46.72       | 54.09                                    | 76.37                                    |
| F6   | 3          | 15             | 86.78         | 27.82       | 49.38                                    | 85.04                                    |
| F7   | 1          | 45             | 54.9          | 32.86       | 102.69                                   | 104.54                                   |
| F8   | 5          | 15             | 92.96         | 31.45       | 34.82                                    | 63.83                                    |
| F9   | 1          | 15             | 75.3          | 22.56       | 90.98                                    | 99.82                                    |
Table S5. Kinetic modelling of drug release using different kinetic equations.

| Formulation code | Zero order | First order | Higuchi kinetic | Hixson Crowell | Korsmeyer-Peppas model |
|------------------|------------|-------------|-----------------|----------------|------------------------|
|                  |            |             |                 |                | n          | R²        |
| F1               | 0.797      | 0.971       | 0.956           | 0.928          | 0.516      | 0.990    |
| F2               | 0.629      | 0.920       | 0.882           | 0.861          | 0.330      | 0.834    |
| F3               | 0.838      | 0.978       | 0.972           | 0.944          | 0.579      | 0.973    |
| F4               | 0.755      | 0.869       | 0.927           | 0.834          | 0.504      | 0.914    |
| F5               | 0.775      | 0.900       | 0.943           | 0.864          | 0.547      | 0.990    |
| F6               | 0.883      | 0.979       | 0.986           | 0.963          | 0.551      | 0.949    |
| F7               | 0.664      | 0.987       | 0.971           | 0.949          | 0.324      | 0.944    |
| F8               | 0.906      | 0.968       | 0.995           | 0.961          | 0.557      | 0.996    |
| F9               | 0.620      | 0.851       | 0.783           | 0.778          | 0.346      | 0.903    |
**Table S6.** Stability data for optimized formulation (F5) at accelerated condition up to six months.

| Time interval | Description | Assay value | Percentage drug release in 0.1 N HCl and PB pH 6.8 |
|---------------|-------------|-------------|---------------------------------------------------|
|               |             | Q<sub>2h</sub> | Q<sub>8h</sub> |
| Initial       | Complies    | 99.9 ± 1.6  | 54.1 ± 1.8  | 76.4 ± 1.3  |
| 1 month       | Complies    | 99.6 ± 2.6  | 55.0 ± 2.6  | 77.9 ± 2.2  |
| 2 month       | Complies    | 98.7 ± 1.9  | 55.5 ± 2.5  | 78.1 ± 2.9  |
| 3 month       | Complies    | 98.3 ± 2.7  | 56.2 ± 3.0  | 79.2 ± 3.5  |
| 6 month       | Complies    | 97.1 ± 2.1  | 58.5 ± 2.2  | 81.5 ± 3.5  |
Figure S1. Diagrammatic presentation of reaction scheme for preparation of carboxymethyl pullulan using Williamson synthesis.
**Figure S2.** Graphical representation of effect of reaction parameter variables on (a) NaOH (10 M) concentration (b) MCA concentration and (c) Temperature on DS.
Figure S3. Proposed possible reaction pathway for the formation of PVA-CMP IPN hydrogel microspheres.
Figure S4. Optical contact angle of different batches of CMP and native pullulan in water.
Figure S5. XRD spectra of (a) pure drug, (b) placebo IPN and (c) drug loaded IPN.
Figure S6. DSC thermogram of (a) PVA, (b) carboxymethyl pullulan (c) pure drug, (d) placebo IPN and (e) drug loaded IPN.
Figure S7. Weight loss thermogram (TG) and derivative (DTG) for CMP at (a) 5 K/min, (b) 10 K/min, (c) 15 K/min and (d) 20 K/min.
Figure S8. Weight loss thermogram (TG) and derivative (DTG) for IPN microspheres prepared with 1 mL GA at (a) 5 K/min, (b) 10 K/min, (c) 15 K/min and (d) 20 K/min.
Figure S9. Weight loss thermogram (TG) and derivative (DTG) for IPN microspheres prepared with 3 mL GA at (a) 5 K/min, (b) 10 K/min, (c) 15 K/min and (d) 20 K/min.
Figure S10. Weight loss thermogram (TG) and derivative (DTG) for IPN microspheres prepared with 5 mL GA at (a) 5 K/min, (b) 10 K/min, (c) 15 K/min and (d) 20 K/min.
Figure S11. Weight loss thermogram (TG) and derivative (DTG) for raw drug pirfenidone (a) 5 K/min, (b) 10 K/min, (c) 15 K/min and (d) 20 K/min.
Figure S12. Weight loss thermogram (TG) and derivative (DTG) for optimized formulation of IPN microsphere (F5) (a) 5 K/min, (b) 10 K/min, (c) 15 K/min and (d) 20 K/min.
Figure S13. Graphical representation of the \( \ln \beta \) v/s inverse of temperature (1/T) calculated for different values of the degree of conversion (\( \alpha \)) by OFW method, (a) CMP, PVA-CMP placebo IPN microsphere prepared using (b) 1mL GA, (c) 3 mL GA and (d) 5mL GA, (e) raw drug pirfenidone and (f) optimized formulation (F5).
Figure S14. Graphical representation of the $\ln(\beta/T^2)$ v/s inverse of temperature ($1/T$) calculated for different values of the degree of conversion ($\alpha$) by KAS method, (a) CMP, PVA-CMP placebo IPN microsphere prepared using (b) 1mL GA, (c) 3 mL GA and (d) 5mL GA, (e) raw drug pirfenidone and (f) optimized formulation (F5).
Figure S15. Histopathological images of vital organs (a-c) heart, (d-f) liver, (g-i) kidney and (j-l) stomach.
**Design-Expert® Software**

Yield
- **Design Points**
  - 92.96
  - 54.9

X1 = A: Glutaraldehyde
X2 = B: Drug loading

(a)

**Design-Expert® Software**

DEE
- **Design Points**
  - 46.72
  - 22.56

X1 = A: Glutaraldehyde
X2 = B: Drug loading

(b)
Figure S16. Contour plot depicting the effect of the percentage drug loading and quantity of glutaraldehyde on (a) yield, (b) drug entrapment efficiency, (c) cumulative percentage release after 2 h and (d) cumulative percentage release after 8 h.
Figure S17. Histogram of the optimized formulation (F5).
Figure S18. Dissolution profile of initial sample (F5) and sample after 1, 2, 3 and 6 months (F5) at accelerated condition.
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