International Journal for Pharmaceutical Research Scholars (IJPRS)

V-9, I-4, 2020 ISSN: 2277 - 7873

RESEARCH ARTICLE

# *Formulation and Evaluation of Interpenetrating polymer network Microspheres containing ritonavir*

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Manuscript No: IJPRS/V9/I4/00008, Received On: 23/12/2020, Accepted On: 25/12/2020, Publish On: 17/01/2021

## ABSTRACT

The IPN microspheres were prepared by using emulsion crosslinking method. In that the chitosan is used as crosslinking polymer while Hydroxy propyl cellulose(HPC), Hydroxy propyl methyl cellulose (HPMC) and sodium carboxy methyl cellulose (Na CMC) were used as neutral polymer by using glutaraldehyde as crosslinking agent and span 80 as emulsifying agent. Drug was scanned under UV spectroscopy and maximum absorbance was found at 288.2 nm in both pH 1.2 and pH 6.8 medium. Both drug and polymer were investigated for interaction by FTIR spectroscopy. In the present work nine batches were prepared by varying the different ratio of polymer concentration in each batch. First three batches (P1-P3) contain chitosan:

## KEYWORDS

Interpenetrating polymer network

## INTRODUCTION

An ideal dosage regimen in the drug therapy of any diseases is the one which immediately attains the desired therapeutic concentration of drug plasma (or at site of action) and maintains it constant for entire duration of treatment.

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This is possible through administration of conventional dosage form in particular dose and at particular frequency. The frequency of administration or the dosing interval of any drug depends upon its half-life or mean residence time and its therapeutic index. In most cases, the dosing interval is much shorter than the half-life of the drug resulting in a number of limitations associated with such a conventional dosage form.

The oral sustained release formulation has been developed in an attempt to release the drug slowly into the GIT and maintain an effective drug concentration in serum for longer period of time.[1]

### 1.1 INTERPENETRATING POLYMER NETWORK

Over the past decades, blends have been investigated to satisfy the need of specific sectors of polymer industry. Such polymeric blends showed superior performances over the conventional individual polymers and consequently, the range of applications have grown rapidly for such class of materials. In the recent years, carbohydrate and biodegradable polymers have been extensively used to develop the controlled release formulations of drugs

having short plasma life. Among the various polymers employed, hydrophilic biopolymers are quite suitable in oral applications due to their inherent advantages over the synthetic polymers.[2] The importance of biocompatible and biodegradable polymers is continuously increasing in pharmaceutical applications because of their propensity to form crosslinked three-dimensional network hydrogels that tend to swell in water or biological fluids. Such systems have been considered as a the potential candidate to deliver bioactive molecules, particularly in controlled release applications[3]

Interpenetrating polymer network (IPN) is regarded as one of the most useful novel biomaterial. The excellent biocompatibility and safety due to its physical characteristics such as impart stability of the drug in the formulations, improves solubility of hydrophobic drugs, excellent swelling capacity and its biological characteristics, like biodegradability, impart bioavailability, drug targeting in a specific tissue and very weak antigenecity. IPN offers novel way to address delivery of hydrophobic and low bioavailable drug. Interpenetrating polymer networks are the polymeric blends showed superior performances over the conventional individual polymers and consequently, the range of applications have grown rapidly for such class of material.

IPN is defined as a combination of at least two polymers chains each in network form, of which at least one is synthesized and/or cross-linked in the immediate presence of the other without any covalent bonds between them. If only one component of the assembly is cross linked leaving the other in a linear form, the system is termed as semi- interpenetrating polymer networks[3,4]

### 1.1.1 Classification of IPN

**Based on Chemical Bonding**

Covalent Semi IPN:

A covalent semi IPN contains two separate polymer systems that are crosslinked to form a single polymer network.

NonCovalent Semi IPN:

A non-covalent semi IPN is one in which only one of the polymer systems is crosslinked

NonCovalent Full IPN**:**

A non-covalent full IPN is one in which the two separate polymers are independently crosslinked.

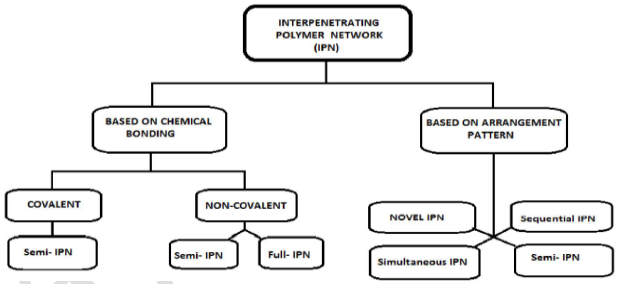


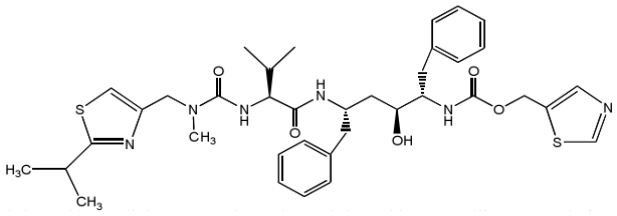

**Figure No. 1 Different types of IPN**

## 1.2 DRUG PROFILE:

**Ritonavir[5,6,7]**

Ritonavir is a member of the group of drugs as Anti-Retroviral. It is a protease inhibitor drug used against infection.

**Structure**



**Chemical name** : 10 hydroxy-2-methyl -5(1-methyl ethyl )-1-[2-(1-methyl ethyl)-4-Thiazolyl]-3-6-dioxo-8,11bis (phenymethyl)-2,4,7,12-tetraazatrid -ecan-13-oic acid, 5-thiazolymethyl ester.

**Molecular formula**: $C_{37}H_{48}N_6O_5S_2$

**Molecular weight:** 721.0

**Melting point** : 119-123$^0$ C.

**Description** : Ritonavir is an almost white to light –tan powder, odour slight and Characteristic.

**Category** : Antiretroviral.

**Solubility** : Practically insoluble in water, freely soluble in methanol and ethanol.

**Pka** : 3.48

**Log P** : 5.28

**Half- life** : 3-5 hr

**Storage** : Store protected from light and moisture.

**Mechanism of Action** : Ritonavir is a peptidomimetic inhibitor of both the HIV-1 and HIV-2 proteases. Inhibition of HIV protease renders the enzyme incapable of processing the gag-pol polyprotein precursor which leads to production of non-infectious immature HIV particles.

## 2.0 MATERIALS AND INSTRUMENTS

### 2.1 MATERIALS:

Ritonavir , Chitosan ,Hydroxy Propyl Cellulose (HPC) ,Hydroxy Propyl Methyl, Cellulose K100M (HPMC K100M),Polyvinyl Pyrrolidone (PVP),

Glutaraldehy ,Hydrochloric acid (HCl),Potassium Dihydrogen Phosphate ($KH_2PO_4$) ,Sodium Hydroxide (NaOH) and Light Liquid Paraffin

### 2.2 INSTRUMENTS:

Double Beam UV Spectrophotometer, FTIR Spectrophotometer, Electronic Weighing Balance ,Dissolution Test Apparatus,Peristaltic Pump,

Differential Scanning Calorimetry,Imaging System, Magnetic Stirrer, Heating Humidity Chamber and Scanning Electron Microscope

## 3. EXPERIMENTAL AND RESULTS

### 3.1 PREFORMULATION STUDY

#### 3.1.1 Characterization of Ritonavir: [8,9]

**i) Description:** Visual inspection of drug evealed that drug is a white colored crystalline solid.

**ii) Melting Point:** The melting point of the drug sample was determined by capillary method and found to be 119-123$^o$C, which complies with melting point reported in Merck Index (119-122$^0$C)

**Solubility:** Practically insoluble in water, Freely soluble in methanol and ethanol, soluble in isopropanol.

#### 3.1.2 Identification Tests for Propranolol HCl [8]:

#### 3.1.2.1 UV Absorption Spectrum of Ritonavir in Acid Buffer pH 1.2:

#### I) Preparation of 0.1 N HCL:

Acid buffer pH 1.2 was prepared by placing 8.5 ml of conc. HCl into 1000 ml volumetric

flask and making the volume upto the mark using distilled water. The pH was adjusted to 1.2 on the digital pH meter.

#### II) Scanning of Ritonavir in 0.1 N HCL:

Ritonavir (20mg) was accurately weighed and dissolved in10 ml 0.1 N HCL (pH 1.2) and clear solution was obtained. To this sufficient amount of the medium was added to make the volume to 100 ml. The resultant solution was diluted with the same medium (pH1.2) to obtained a concentration of 20 ug/ml and scanned between 200-400nm.

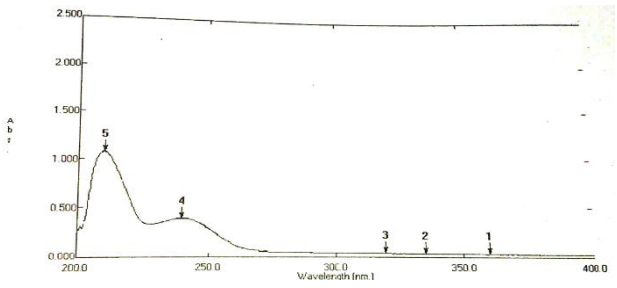



**Figure No 2**: Scanning of Ritonavir in 0.1N HCL (pH 1.2)

**Observation:** The λmax was found to be at 246 nm as shown in **Figure No. 8**

### 3.1.2.2 Preparation of Standard Calibration Curve of Ritonavir in Acid Buffer pH 1.2:

**Procedure:**

50 mg of Ritonavir was weighed accurately and dissolved in 50 ml of acid buffer pH 1.2, from this 1ml solution was withdrawn and diluted to 50 ml to get standard stock solution of 20 ug/ml. The stock was suitably diluted to get concentrations from 2 – 20 µg/ml and was analyzed at 246 nm to plot the standard calibration curve.

**Table no. 1: Standard Calibration Curve of Ritonavir in Acid Buffer pH1.2 at 246nm.**

| Sr. No. | Concentration (µg/ml) | Absorbance* |
|---|---|---|
| 1 | 2 | 0.244±0.007 |
| 2 | 4 | 0.352±0.006 |
| 3 | 6 | 0.487±0.008 |
| 4 | 8 | 0.609±0.007 |
| 5 | 10 | 0705±0.005 |
| 6 | 12 | 0.812±0.003 |
| 7 | 14 | 0.879±0.006 |
| 8 | 16 | 0.982±0.008 |
| 9 | 18 | 1.121±0.004 |
| 10 | 20 | 1.221±0.005 |

***Each value represent the mean ± standard deviation (n=3)**

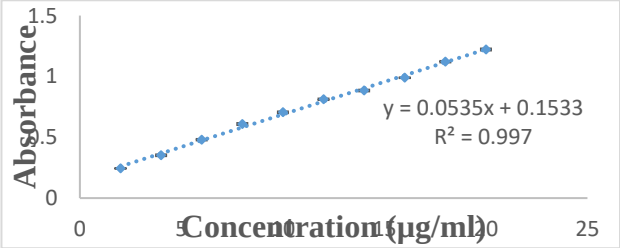



**Figure No.3: Standard Calibration Curve of Ritonavir in Acid Buffer pH 1.2**

**Observation:**

Equation of Regressed Line**:** y = 0.0535x+0.1533

Correlation Coefficient**:** ($R^2$) = 0.997

### 3.1.2.3 UV Absorption Spectrum of Propranolol HCl in Phosphate Buffer pH 6.8

**I) Scanning of Ritonavir in pH 6.8 :**

20 mg of Ritonavir was taken in 100ml volumetric flask. To that 5 ml of methanol was added and shaken well to dissolve the drug. The solution was made up to the mark with 6.8 pH phosphate buffer solutions. From the above solution 10 ml is diluted to 100ml with, 6.8 pH phosphate buffer solution to give 20 ug/ml concentration. From the above solution 1 ml is diluted to 10ml with, 6.8pH phosphate buffer solutions to give 2 ug/ml concentration. The prepared solution i.e., 2 ug/ml concentration was scanned for 200-400nm UV/ Visible spectrophotometer.

### 3.1.2.4 Standard Calibration Curve of Ritonavir in Phosphate Buffer pH 6.8 at 243 nm.

Accurately measured 50.0 ml of 0.2 M $KH_2PO_4$ solution and 22.4 ml of 0.2 M NaOH solution was added into 200.0 ml volumetric flask. The volume was made up to the mark with distilled water. The pH was adjusted to 6.8 on the digital pH meter.

**Procedure:**

50 mg of Ritonavir was weighed accurately and dissolved in 50 ml of acid buffer pH 6.8, from this 1ml solution was withdrawn and diluted to 50 ml to get standard stock solution of 20 ug/ml. The stock was suitably diluted to get concentrations from 2 – 20 µg/ml and was analyzed at 243 nm to plot the standard calibration curve.

**Table No 02: Standard Calibration Curve of Ritonavir in Phosphate Buffer pH6.8 at 43nm.**

| Sr. No. | Concentration (µg/ml) | Absorbance* |
|---|---|---|
| 1 | 2 | 0.065±0.008 |
| 2 | 4 | 0.140±0.007 |
| 3 | 6 | 0.243±0.006 |
| 4 | 8 | 0.334±0.008 |
| 5 | 10 | 0.448±0.004 |
| 6 | 12 | 0.534±0.006 |
| 7 | 14 | 0.672±0.003 |
| 8 | 16 | 0.769±0.004 |
| 9 | 18 | 0.847±0.003 |
| 10 | 20 | 0.921±0.005 |

***Each value represents the mean ± standard deviation (n=3)**

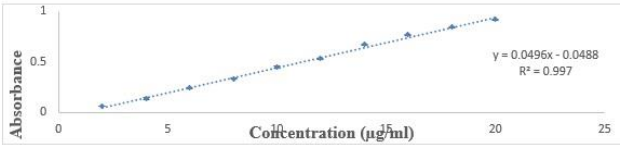



**Figure No. 04: Standard Calibration Curve of Ritonavir in Phosphate Buffer pH 6.8**

**Observation:**

Equation of Regressed Line**:** y = 0.0496-0.048

Correlation Coefficient**:** ($R^2$)

### 3.3 PREPARATION OF MICROSPHERES[10]

IPN microspheres were prepared using different ratios of Chitosan: HPC, Chitosan: HPMC K100M and Chitosan: PVP by using emulsion crosslinking method. Briefly, 2% (w/v) of Chitosan solution was prepared by dissolving in 2% (w/v) acetic acid in double-distilled deionized water and stirring it continuously until the attainment of a homogeneous solution. Different ratio HPC was then dispersed in different ratio of Chitosan solution and stirred. The drug Ritonavir was dissolved in the above polymer blend solution, which was added slowly to light liquid paraffin (100 g, w/w) containing 2% (w/w) span-80 under constant stirring at 1200 rpm speed for about 60 min. To this w/o emulsion, 5 ml of GA as a crosslinking agent containing 0.5mL of 1N HCl were added slowly and stirred for 3 h. The hardened microspheres were separated by filtration, wash repeatedly with n-hexane and distilled water to remove the unreacted GA. Similar Procedure for chitosan: HPMC K100M and chitosan: PVP was repeated. Solid microspheres obtained were vacuum dried at 40˚C for 24 h and stored in a desiccator until further use. Totally, nine formulations were prepared as per the formulation codes assigned in **Table No. 12**.

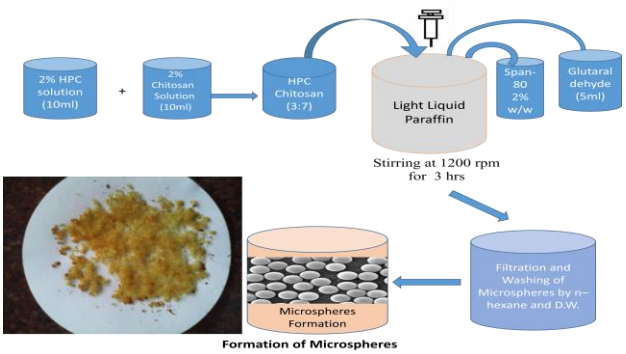



**Figure No. 5 Schematic representation of IPN Microspheres.**

**Table No. 03: Composition of IPN Microspheres.**

| Batch | Drug (mg) | Chitosan 2% w/v (ml) | HPC 2% w/v (ml) | HPMC K100M 2% w/v (ml) | PVP 2% w/v (ml) | Glutaraldehyde (ml) |
|---|---|---|---|---|---|---|
| P1 | 200 | 9 | 1 | - | - | 5 |
| P2 | 200 | 8 | 2 | - | - | 5 |
| P3 | 200 | 7 | 3 | - | - | 5 |
| P4 | 200 | 9 | - | 1 | - | 5 |
| P5 | 200 | 8 | - | 2 | - | 5 |
| P6 | 200 | 7 | - | 3 | - | 5 |
| P7 | 200 | 9 | - | - | 1 | 5 |
| P8 | 200 | 8 | - | - | 2 | 5 |
| P9 | 200 | 7 | - | - | 3 | 5 |

## 4.4: EVALUATION OF MICROSPHERES

### 4.4.1: Drug Content and Entrapment Efficiency[11, 12, 13]

Ritonavir microspheres 50 mg from each batch were digested in 50 ml of pH 6.8 phosphate buffer solution for overnight, and then sonicated for 15 min. After complete dissolution of microspheres drug content was determined by UV-visible spectrophotometer at 243 nm taking 6.8 phosphate buffer solution as a blank. The percent drug loading of microspheres was calculated using following equation

$$\% \text{ Actual Drug Loading} = \frac{\text{Encapsulated Drug (mg)}}{\text{Microspheres (mg)}} \times 100$$

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual Drug Loading}}{\text{Theoretical Drug loading}} \times 100$$

**Table No. 04:** Theoretical Drug Content (%), Actual Drug Loading (%) and Entrapment Efficiency (%) of Propranolol HCl Microspheres

| Batches | Theoretical Drug Content (%) | Actual Drug Loading* (%) | Entrapment Efficiency* (%) |
|---|---|---|---|
| P1 | 50 | 9.60±0.414 | 57.56±0.580 |
| P2 | 50 | 12.90±0.365 | 77.79±0.401 |
| P3 | 50 | 13.76±0.505 | 82.85±0.331 |
| P4 | 50 | 7.80±0.535 | 44.51±0.618 |
| P5 | 50 | 9.64±0.755 | 57.60±0.558 |
| P6 | 50 | 11.81±0.385 | 71.85±0.457 |
| P7 | 50 | 7.79±0.357 | 46.57±0.555 |
| P8 | 50 | 9.89±0.398 | 58.76±0.554 |
| P9 | 50 | 13.75±0.622 | 81.84±0.536 |

*** Each value represent the mean ± standard deviation (n=3)**

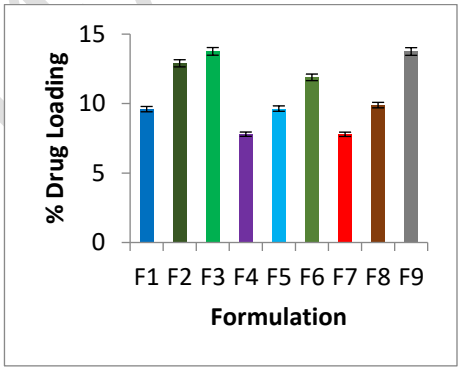



**Figure No. 06: % Actual Drug Loading of Formulation F1-F9.**

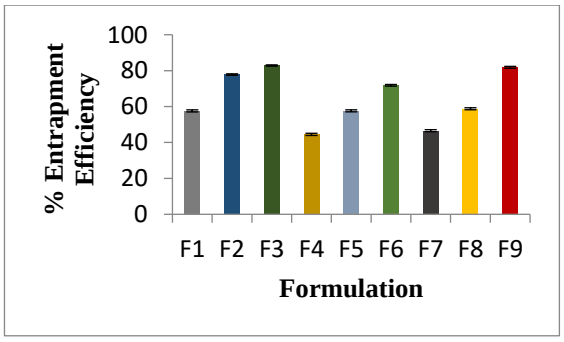



**Figure No. 07: % Entrapment Efficiency of formulation F1-F9**

### 4.4.2 Particle size analysis and morphological studies: [14,15,16]

The particle size and shape analysis of propranolol microspheres was done by Metzer optical microscope enabled with camera. About 200 particles were measured for particle size analysis and it was expressed as volume mean diameter in microns (SD), results are shown in **Table No. 9**

**Figure No. 08: Photomicrograph of Microspheres.**

**Table No 05: Mean Particle Size of Propranolol HCl microspheres.**

| Batches | Mean Particle Size (µm)* |
|---|---|
| P1 | 82.82±0.603 |
| P2 | 95.86±0.670 |
| P3 | 98.64±0.817 |
| P4 | 119.6±0.615 |
| P5 | 128.7±0.578 |
| P6 | 151.7±0.570 |
| P7 | 80.67±0.517 |
| P8 | 90.93±0.561 |
| P9 | 105.8±0.958 |

***Each value represent the mean ±± standard deviation (n=3)**

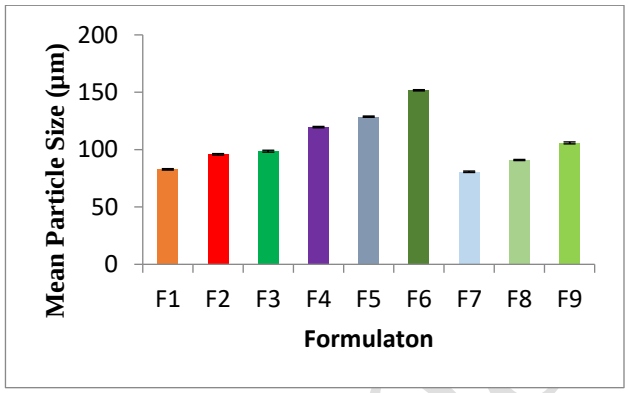



**Figure No. 09 Average Particle Size Analysis.**

### 4.4.3 Swelling Study[17,18]

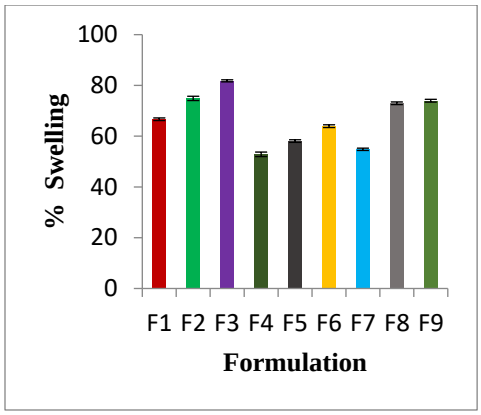
Water uptake of the cross-linked microspheres loaded with the drug was determined by measuring the extent of swelling of the matrix in pH 1.2 and phosphate buffer 6.8 solutions. The samples were allowed to swell in pH 1.2 buffer solution for 2 hr and then at pH 6.8 phosphate buffer for 10 hr. The excess surface adhered liquid drops were removed by blotting with soft tissue papers and the swollen microspheres were weighed to an accuracy of 0.01 mg using an electronic microbalance. The hydrogel microspheres were then dried in an oven at 50 $^0$C for 5 hr until there was no change in the dried mass of the samples.

$$\% \text{ water uptake} = \frac{\text{Mass of swollen microspheres} - \text{Mass of dry microspheres}}{\text{Mass of dry microspheres}} \times 100$$

**Table No. 06: Percent Water Uptake of Microspheres.**

| Sr. No. | Batch | % Swelling Study (% w/w)* | |
|---|---|---|---|
| | | pH 1.2 | pH 6.8 |
| 1 | P1 | 66.66±0.508 | 168.61±0.571 |
| 2 | P2 | 74.89±0.847 | 185.20±0.466 |
| 3 | P3 | 81.80±0.401 | 195.80±0.408 |
| 4 | P4 | 52.86±0.891 | 155.91±0.488 |
| 5 | P5 | 58.07±0.516 | 167.90±0.640 |
| 6 | P6 | 63.94±0.537 | 179.93±0.560 |
| 7 | P7 | 54.83±0.459 | 134.92±0.493 |
| 8 | P8 | 72.96±0.516 | 148.72±0.775 |
| 9 | P9 | 73.99±0.539 | 151.97±0.577 |

***Each value represent mean (n=3) observation ± S.D.**



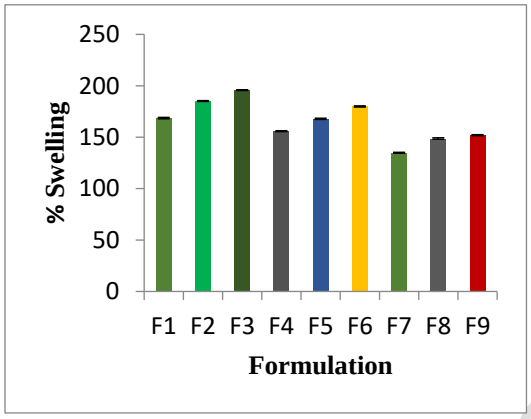




**Figure No. 10: % Swelling of Microspheres in pH 1.**
**Figure No. 11: % Swelling of Microspheres in pH 6.8**

### 4.4.4 *In* Vitro Drug Release Studies: [10, 19, 20]

Drug release from the IPN microspheres with different % drug loading and different polymer composition was investigated in 0.1 N HCl for the initial 2 h, followed by phosphate buffer pH 6.8 until the completion of dissolution. These experiments were performed using a fully automated dissolution tester coupled with a UV system (Double Beam UV Spectrophotometer Model -UV 2401 PC Shimadzu Corporation, Koyto, Japan.) equipped with six baskets at the stirring speed of 100 rpm. A weighed quantity of each sample was placed in 500 ml of dissolution medium maintained at 37 ˚C. Dissolution study is conducted initially 2hrs in acid buffer pH 1.2 and remaining 10 hr in phosphate buffer pH 6.8 During dissolution study 1 ml aliquot was withdrawn at different time intervals of 1 hr upto 12 hrs and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper no.42 and Propranolol HCl concentration was determined by UV spectrophotometer at λ max of 245.4 nm.

**Table No. 07: Percent Cumulative Drug Release of Batch F-1 to F-5.**

| Time (hr) | % Cumulative Drug Release | | | | |
|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 5.90±0.153 | 16.75±0.461 | 12.59±0.612 | 5.84±0.606 | 5.99±0.213 |
| 2 | 7.81±0.556 | 22.76±0.384 | 18.88±0.646 | 7.90±0.424 | 8.67±0.826 |
| 3 | 16.75±0.543 | 30.79±0.670 | 30.54±0.609 | 16.60±0.736 | 19.94±0.291 |
| 4 | 22.01±0.533 | 41.98±0.753 | 40.89±0.831 | 21.84±0.590 | 23.88±0.335 |
| 5 | 29.23±0.827 | 49.01±0.219 | 43.01±0.470 | 25.73±0.716 | 31.66±0.776 |
| 6 | 37.41±0.900 | 55.02±0.729 | 51.72±0.897 | 29.77±0.593 | 37.85±0.685 |
| 7 | 45.70±0.498 | 56.91±0.619 | 58.98±0.665 | 36.90±0.491 | 42.88±0.843 |
| 8 | 50.72±0.428 | 67.71±0.710 | 67.79±0.586 | 43.87±0.863 | 49.85±0.871 |
| 9 | 54.70±0.725 | 73.05±0.731 | 75.96±0.424 | 51.59±0.502 | 53.75±0.343 |
| 10 | 57.58±0.660 | 75.91±0.822 | 85.78±0.560 | 57.50±0.552 | 57.74±0.589 |
| 11 | 65.15±0.488 | 79.94±0.523 | 90.88±0.516 | 66.48±0.557 | 65.58±0.485 |
| 12 | 74.04±0.708 | 82.80±0.910 | 94.34±0.541 | 68.51±0.552 | 72.56±0.473 |

**Each value represent the mean ± standard deviation (n=3)**

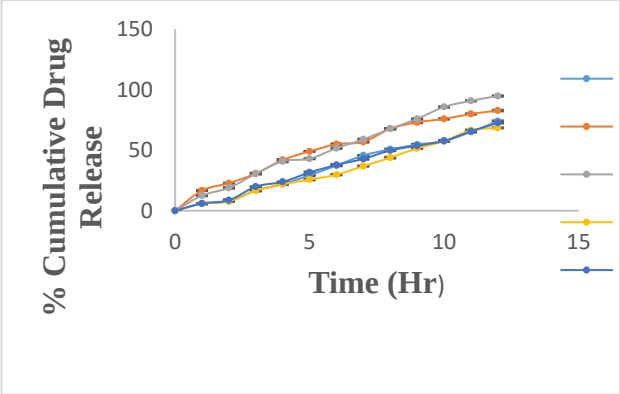



**Figure No. 12: *In vitro* Drug Release Profile of Formulation (F1-F5).**

**Table No. 08: Percent Cumulative Drug Release of Batch P-6 to P-7.**

| Time (hr) | % Cumulative Drug Release* | | | |
|---|---|---|---|---|
| | P6 | P7 | P8 | P9 |
| 0 | 0 | 0 | 0 | 0 |
| 1 | 9.52±0.618 | 9.77±0.231 | 8.67±0.494 | 9.57±0.543 |
| 2 | 11.66±0.544 | 13.71±0.459 | 12.76±0.674 | 11.81±0.384 |
| 3 | 21.65±0.576 | 25.81±0.438 | 26.80±0.500 | 26.63±0.517 |
| 4 | 31.86±0.386 | 32.87±0.345 | 35.86±0.568 | 36.83±0.646 |
| 5 | 38.88±0.544 | 40.69±0.471 | 42.74±0.963 | 39.91±0.282 |
| 6 | 41.69±0.495 | 47.59±0.811 | 48.70±0.805 | 44.61±0.560 |
| 7 | 49.84±0.343 | 49.61±0.562 | 54.95±0.235 | 45.87±0.379 |
| 8 | 55.59±0.540 | 50.55±0.602 | 56.47±0.644 | 53.88±0.325 |
| 9 | 67.48±0.546 | 54.79±0.488 | 61.60±0.796 | 55.83±0.591 |
| 10 | 72.65±0.424 | 67.76±0.719 | 68.63±0.537 | 66.53±0.589 |
| 11 | 74.97±0.125 | 68.60±0.557 | 75.42±0.687 | 73.87±0.759 |
| 12 | 84.88±0.697 | 73.91±0.355 | 78.55±0.568 | 80.63±0.563 |

*** Each value represent the mean ± standard deviation (n=3)**

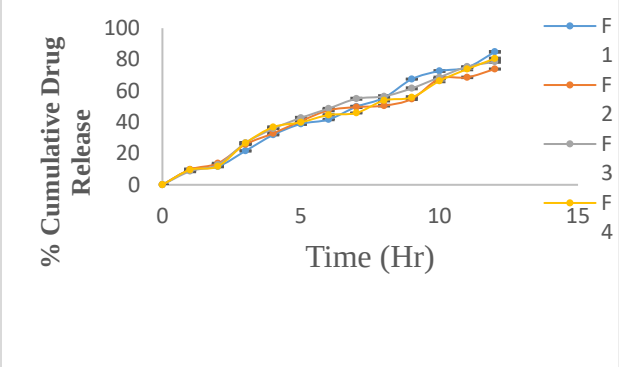



**Figure No. 13: *In vitro* Drug Release Profile of Formulation (F6-F9).**

### 4.4.5 Duration of Mucoadhesion:[21,22,23]

This is an important factor in the formulation of bioadhesive dosage forms capable of being retained on mucosal surfaces for extended period of time and must be given careful consideration.

**Method:** A freshly cut 5 cm long piece of pig nasal mucosa obtained from local abattoir within 1 hr of sacrificing the animal was cleaned by washing with isotonic saline solution. An accurate weight of the microspheres was mixed with sudan red and applied on mucosal surface which was attached over a polyethylene plate fixed at an angle at 40° relative to the horizontal plane. Phosphate buffer saline pH 7.4 warmed at 37°C was pumped peristatically, over the tissue at the rate of 5ml/min. The duration of complete washing of the microspheres was recorded.

**Table No. 09: Duration of Mucoadhesion for formulations P1-P9.**

| Sr. No | Formulation | Time (Hour)* |
|---|---|---|
| 1 | **F1** | 4.30±0.056 |
| 2 | **F2** | 6.40±0.045 |
| 3 | **F3** | 7.10±0.034 |
| 4 | **F4** | 5.02±0.082 |
| 5 | **F5** | 6.09±0.056 |
| 6 | **F6** | 7.06±0.031 |
| 7 | **F7** | 4.32±0.074 |
| 8 | **F8** | 4.36±0.049 |
| 9 | **F9** | 5.41±0.057 |

***Each value represent the mean ± standard deviation (n=3)**

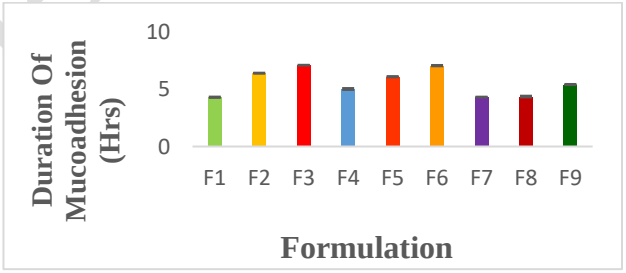



**Figure No. 14 Duration of Mucoadhesion.**

## 4.6: CHARACTERIZATION OF MICROSPHERE

### 4.6.1: Differential Scanning Calorimetry (DSC): [24,25,26]

A differential scanning calorimeter was used for thermal analysis of drug and physical mixture. Drug and its physical mixture were weighted directly in was weighed directly in the pierced DSC aluminum pan (Aluminum Standard 40 µl) and scanned at the temperature range of 25-400 °C and at heating rate of 10 °C/min. in nitrogen atmospheres at flow rate of 20 ml/min, thermogram obtained were observed for any interaction **Observation:**

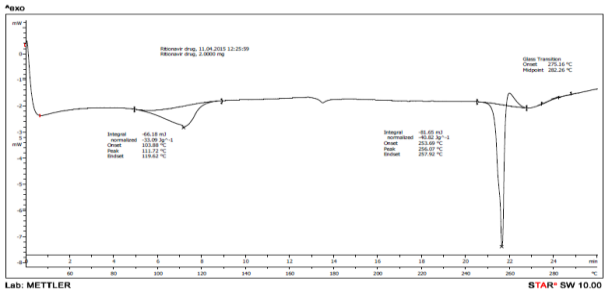



**Figure No. 15: Differential Scanning Calorimetry of Ritonvir.**

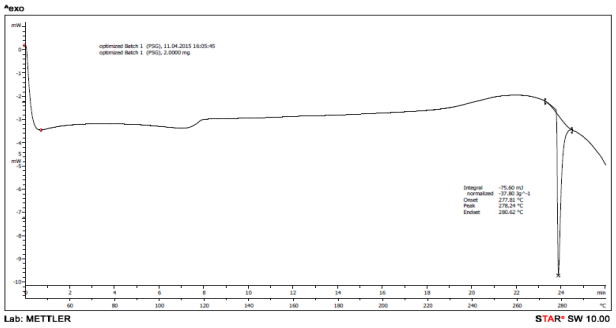



**Figure No. 16: Differential Scanning Calorimetry of IPN Microspheres containing Ritonavir.**

### 4.6.2 Scanning Electron Microscopy:[27,28,29]

The surface photography of the microparticles was examined using scanning electron microscopy. Microspheres were spread on a double sided adhesive plate, one side of which was stuck to glass slide. Excess microspheres were removed and the slide was kept on the sample holder and scanning electron micrograph was taken using an electron microscope. (JEOL, JSM-5200, Japan 15kv).

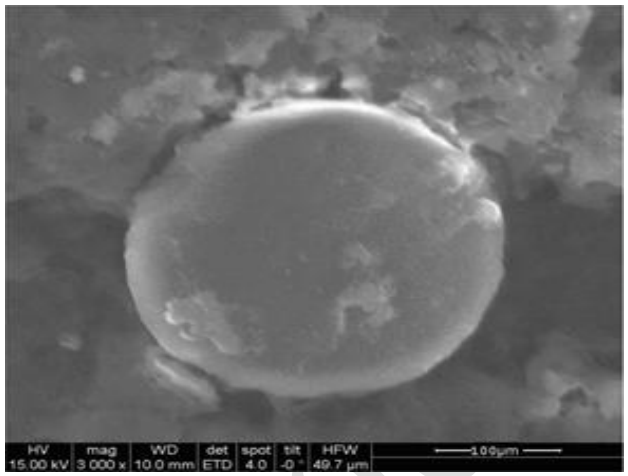



**Figure No. 17:** Scanning electron micrograph of IPN microsphere. (3000x)

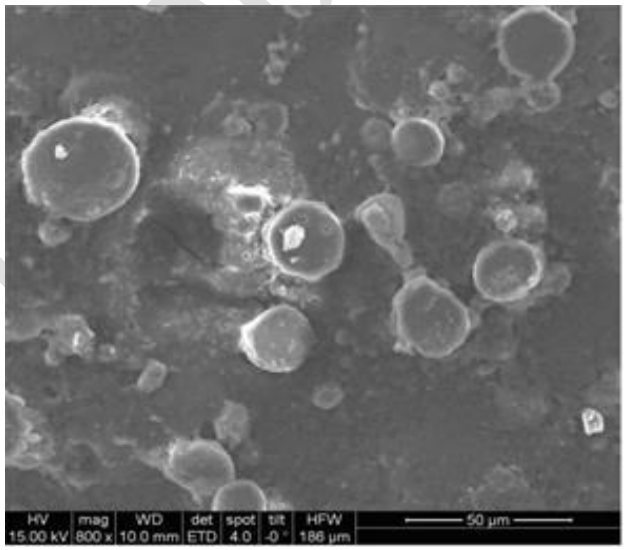



**Figure No. 18:** Scanning electron micrograph of IPN microsphere. (800x)

### 4.6.3 Treatment of *In vitro* Drug Release Data with Different Kinetic Equations

Drug release kinetics was assumed to reflect different release mechanisms of controlled release drug delivery systems. Therefore, five kinetics model were applied to analyze the *in vitro* data to find the best fitting equation.[26]

Zero order release equation;

$F_t = K_0 t$

Where Ft represents the fraction of drug released in time t and $K_0$ is the apparent rate constant of zero-order release constant.

First-order equation;

$In (l-F) = -K_1t$

Where F represents the fraction of drug released in time t and $K_1$ is the first-order release constant.

Higuchi equation;

$F = K_2t^{1/2}$

Where F represents the fraction of drug released in time t and $K_2$ is the Higuchi constant.

Hixson – Crowell equation;

$Q_0^{1/3} - Qt^{1/3} = kHC\ t$

Where $Q_0$ = Amount of drug released or dissolved at time t=0

& Qt = Amount of drug released or dissolved at time t.

Pappas equation;

$Mt/M^{\infty} = K_3t^n$

In Korsmeyer – Peppas equation Mt and $M^{\infty}$ are the amount of drug released at time t and$^{\infty}$, respectively and n is the diffusion coefficient. In spherical matrices, if n<0.5, a Fickian (case -l), 0.5 <n<1.0, a non-Fickian, and n> 1.0 a case-II (zero order) drug release mechanism dominates.

**Table No. 10: Kinetic Treatment of Dissolution Data of Formulations P1-P9.**

| Batch code | Parameters | Mathematical Models | | | | |
|---|---|---|---|---|---|---|
| | | Zero Order | First Order | Hixon – Crowell | Korsemeyer – Peppas | Higuchi Plot |
| F1 | $R^2$ | 0.996357 | 0.890292 | 0.963663 | 0.0601608 | 0.925209 |
| | Slope | 6.194010 | 0.122877 | 0.632631 | 0.9850403 | 0.082137 |
| | Intercept | 1.238681 | 0.633199 | 1.671547 | 0.9813264 | 0.367306 |
| F2 | $R^2$ | 0.983140 | 0.770975 | 0.899750 | 0.26242400 | 0.981101 |
| | Slope | 6.657197 | 0.102203 | 0.591477 | 0.64356406 | 0.048813 |
| | Intercept | 10.26373 | 0.946349 | 0.096197 | -0.4978806 | 0.133089 |
| F3 | $R^2$ | 0.996601 | 0.899386 | 0.939286 | 0.30978593 | 0.959281 |
| | Slope | 7.879780 | 0.112896 | 0.672469 | 0.85362485 | 0.047383 |
| | Intercept | 4.402857 | 0.874242 | 0.636435 | -0.62538719 | 0.254090 |
| F4 | $R^2$ | 0.996128 | 0.897854 | 0.970980 | 0.05887184 | 0.927343 |
| | Slope | 5.894835 | 0.120790 | 0.613532 | 0.98470434 | 0.083090 |
| | Intercept | 2.097472 | 0.617377 | 1.580162 | -0.98289484 | 0.362121 |
| F5 | $R^2$ | 0.997691 | 0.875929 | 0.956443 | 0.12587592 | 0.922426 |
| | Slope | 6.011648 | 0.119325 | 0.614519 | 1.05758418 | 0.072574 |
| | Intercept | 0.109340 | 0.666421 | 1.826488 | -0.96559104 | 0.380417 |
| F6 | $R^2$ | 0.996744 | 0.855884 | 0.952972 | 0.140835125 | 0.928868 |
| | Slope | 7.013186 | 0.117059 | 0.652258 | 0.870813402 | 0.059557 |
| | Intercept | 1.049340 | 0.761528 | 2.133757 | -0.77843682 | 0.339584 |
| F7 | $R^2$ | 0.985728 | 0.818420 | 0.920882 | 0.2057465 | 0.946937 |
| | Slope | 5.961703 | 0.108026 | 0.581676 | 0.90300633 | 0.056779 |
| | Intercept | 5.434395 | 0.814651 | 2.478090 | -0.7466749 | 0.296250 |
| F8 | $R^2$ | 0.988227 | 0.828121 | 0.926625 | 0.270985515 | 0.922136 |
| | Slope | 6.518461 | 0.113070 | 0.619059 | 1.055329423 | 0.050108 |
| | Intercept | 4.823846 | 0.801232 | 2.424536 | -0.80646067 | 0.386536 |
| F9 | $R^2$ | 0.988615 | 0.828946 | 0.930190 | 0.247197138 | 0.912432 |
| | Slope | 6.328681 | 0.111182 | 0.605470 | 1.00297097 | 0.04873 |
| | Intercept | 4.025604 | 0.796986 | 2.372682 | -0.78386427 | 0402402 |

### 4.6.4. Stability study

Formulation P3 selected as optimized formulation as it gave desirable drug release hence it was kept for stability study. Stability study of an optimized formulation was carried out by storing the microspheres (wrapping in aluminum foil) at 40± 2 $^0$C and 75 ± 5% relative humidity for 3 months. At an interval of 1 month, the microspheres were examined for % Drug Loading, % Entrapment Efficiency and *in vitro* release data.

**Table No. 11 Percent Drug Loading and Percent Entrapment Efficiency study of Batch P3 kept for stability at 40 ± 2˚C/75 ± 5 % RH**

| Temperature and %RH | Parameters evaluated | Duration | | | |
|---|---|---|---|---|---|
| | | 0 month | 1 month | 2 month | 3 month |
| 40°C ± 2 °C 75 % ± 5 % RH | % Drug Loading | 13.790±0.505 | 13.83±0.452 | 13.81±0.456 | |
| | % EE | 82.77±0.331 | 83.01±0.395 | 82.89±0.324 | |

## 5. DISCUSSION AND CONCLUSION

## 5.1 DISCUSSION

### 5.1.1 PREFORMULATION STUDIES:

**a. Identification Test:**

The drug sample was characterized on the basis of physicochemical and spectral analysis to examine its authenticity. The results confirmed it to be the pure samples of propranolol HCl. The procured polymer samples were also characterized and confirmed to the reported values**.**

**b. UV Scanning and Standard Calibration Curve of Propranolol HCl:**

Scanning of Propranolol HCl was done in acid buffer pH 1.2 and phosphate buffer pH 6.8 λmax was found to at 288.2 nm as shown in Figure No. 6, 8. Standard calibration curve of Propranolol HCl in both media, obeyed Beer-Lambert's law in the concentration range of 2-20 µg/ml. Results are shown in **Table No. 1, 2** and **Figure No.2, 3.**

The obtained linear regression equation is as follows

| | |
|---|---|
| pH 1.2 Acid Buffer | Linear Regression Equation y = 0.018x + 0.043 |
| | Slope ($R^2$) = 0.998 |
| pH 6.8 Phosphate Buffer | Linear Regression Equation y = 0.019x + 0.033 |
| | Slope ($R^2$) = 0.996 |

**c. Drug- Polymer interaction**

FTIR of the drug confirmed the presences of all prominent peaks were at wave numbers 3278.76 $cm^{-1}$ ( Secondary NH Stretching), 3053.11 $cm^{-1}$ (Aromatic CH stretching), 2964.39 $cm^{-1}$ and 2837 $cm^{-1}$ (Aliphatic asymmetric and symmetric C-H stretching),1577.66 $cm^{-1}$ (N-H deformation), 1456.16 $cm^{-1}$ (C-H deformation), 1107.06 $cm^{-1}$ (C-O-C structure) indentifying its authenticity.

FTIR Spectra of drug, polymer and physical mixture of drug with polymer. From the results it can be concluded that, all principle peaks of drug were retained in physical mixture hence there was no interaction between drug and polymer.

### 5.1.2 FORMULATION OF IPN MICROSPHERES

In the present study Propranolol HCl loaded IPN microspheres were prepared by crosslinking with glutaraldehyde using chitosan as crosslinking polymer and HPC, HPMC K100M, Na CMC as neutral polymer. The prepared microspheres were evaluated for % drug loading, % EE, mean particle size, % swelling behavior and % drug release.

### .5.2.3 EVALUATION OF MICROSPHERES

**a) Drug Loading and % Entrapment Efficiency:**

The % drug loading was found to be in the range (30.63%-38.3%) for HPC, (25.10%-30.92%) for HPMC K100M, and (29.68%-36.73%) for Na CMC.

The maximum % drug loading and % Entrapment efficiency was found for batch P1 (HPC) i. e. 38.3%, and 76.6% resp. This may be due to accumulation of more amount of drug in rigid polymeric network during formation of microspheres.

The % Entrapment efficiency was found to be in the range between (61.26%-76.6%) for HPC, (50.2%-61.84%) for HPMC K100M, and (59.63 %-73.41%) for Na CMC. The % EE showed depends on nature and content of neutral polymer. By increasing amount of neutral polymer a slight decrease in % Entrapment efficiency was observed which may be due to the formation of loose network that allow for leaching out of more of drug particles during microspheres preparation. These findings are supported by **Rokhade AP *et al.*[17]**

Results are shown in **Table No. 4** and **Figure No.06, 07.**

**b) Particle Size Analysis:**

The mean particle size of all microspheres was in range of 68-164 µm. Results are shown in **Table No.05** and **Figure No. 08,09.**

From the results it can be depicted that particle size of obtained microspheres shows dependence on nature and concentration of polymeric blend.

The particle size was found to be higher for batch P3 (96.43µm) than batch P1 (68.98µm), batch P6 (163.25µm) than batch P4 (128.33µm), batch P9 (103.03µm) than batch P7 (82.01µm). This could be due to higher amount of HPC, HPMC and Na CMC present leading to higher viscosity in polymer solution, thereby producing bigger droplets during emulsification that were later hardened in presence of glutaraldehyde. This result was supported by **Patil SA *et al.*[27]**

According to Arshadi[65] various manufacturing parameters such as apparatus design, type of stirrer, stirring speed, viscosity of emulsion phase and the emulsifier concentration affects the particle size. As concentration of HPMC increases in the formulation of batch P4 to P6, the particle size also increases from 128.33µm to 163.25µm due to more viscous solution which is difficult to pass through syringe and difficult to break droplets during stirring. All microspheres were distributed in range of 68-164 µm MPS

The particle size was observed in following order, HPMC > Na CMC > HPC

As the amount of neutral polymer increases, viscosity of polymeric solution also increases hence particle size was found to be increase. This results was supported by **Mallikarjuna B *et al.*[42]**

### c) Swelling Study:

The formulations containing higher amount of HPC, HPMC, Na CMC exhibit higher swelling[8]. For instance, the % swelling of batch P3 (210%) exhibits higher swelling than batch P1 (159%) for HPC, batch P6 (181%) exhibits higher swelling than batch P4 (149%) for HPMC, batch P9 (191%) exhibits higher swelling than batch P7 (139%) for Na CMC, due to higher amount of more hydrophilic nature of HPC, HPMC, Na CMC than Chitosan, which allows the IPN matrix to absorb higher amount of water.

As the concentration of chitosan in the polymeric blend increase leads to significant decrease in swelling was observed. Such a reduction in swelling may be due to formation of rigid network at higher concentration of chitosan which may affect water uptake.

From the results it can be stated that the microspheres prepared from HPC as a neutral material showed higher swelling this may be due to hydrophilic nature of HPC.

The batch P3 showed higher % swelling in pH 1.2 Acid Buffer and pH 6.8 Phosphate Buffer and it was found 78% and 210% respectively. Results are shown in **Table No. 06** and **Figure No. 10, 11.**

### d) *In Vitro* Drug Release:

The drug release was found to be increase from batch P1-P3(75.68%, 78.89%, 93.12%) and batch P4-P6(62.88 %, 72.21%, 76.47%) and batch P7-P9 (69.56%, 71.98, 81.99%) Polymer-drug interactions are considered to be responsible for controlling *in vitro* release of propranolol HCl from the IPN microspheres, but the extent of such interactions depends upon the properties and nature of the polymers in a blend IPN system as well as the blend composition. The effect of IPN blend ratio for formulations P1 to P9 is showed in figure No.27, 28. HPC, HPMC, Na CMC are neutral polymers, whereas Chitosan is crosslinking polymer. The formation of IPN from Chitosan and neutral polymers in the presence of GA is believed to involve electrostatic interaction, hydrophobic association and hydrogen bonding. Because Chitosan is highly protonated in acidic solution (pH 1.2), the cationic Chitosan and in the presence of neutral HPC and GA, the IPN formed remains stable in the dissolution media and

triggers the release of Propranolol HCl drug showing the burst release at acidic pH, which slows down in alkaline pH media. Typically, the complete release of propranolol HCl was achieved upto 12 hrs for batch P3 (containing 7:3). Here, the mechanism would be that hydrophilic Chitosan and HPC chains allow water molecules to penetrate into the IPN network. The hydration force between these chains in aqueous buffer media seems to be responsible for the observed swelling and thus, controlling the release of propranolol HCl.

Release of drug was depended upon the amount of neutral polymers (i.e. HPC, HPMC, Na CMC), and ratio of polymers (e.g. Chitosan: HPC)

The % cumulative release is quite fast and larger at higher amount of HPC, where as the release is quite slower at lower amount of HPC. This result was supported by **Mallikarjuna B *et al.*[42]**

The HPMC was found to be retards the drug release. This may in turn reduces the frequency of dosing, thereby improving the patient compliance. This result was supported by **Sandhu NR *et al.*[42]**

Batch P3 shows higher drug release was found 93.12% upto 12hrs. Results are shown in **Table No. 07, 08** and **Figure No. 12, 13.**

### e) Duration of Mucoadhesion:

As the concentration of HPC and HPMC increases, duration of mucoadhesion was found to be increase. Higher duration of mucoadhesion was found for batch P3. No significant effect was found for batches containing Na CMC, it may be due to electrostatic interaction between the polymers. Results are shown in **Table No. 09** and **Figure No. 14**

### f) Differential Scanning Calorimetric Study:

The DSC was used to study thermal transition occurring during heating under inert microspheres.

The thermograph of Propranolol HCl and formulation shows that there was no change in melting point which confirms that there was neither change in the crystallinity of Propranolol HCl nor any interaction. From the thermograph of formulation it confirmed that the drug is successfully entrapped in the microspheres as the peak of drug was not observed. From the results it can also be concluded that there was no major interaction between Propranolol HCl, HPC and chitosan used in the preparation of microspheres.

Results are shown in **Figure No.15, 16.**

### g) Scanning Electron Microscopy Study:

From the SEM photography it was observed the formulated optimized microsphere (P3) was found to be spherical shaped without forming agglomeration and their surfaces are slightly rough. Results are shown in **Figure No. 17, 18.**

### h) Kinetic Study:

The P3 formulation is showing correlation coefficient 0.997 for zero order .While value of slope from Korsemeyer - Peppas is 0.622 which indicates Non Fickian drug release and follow zero order release. Thus it can be concluded that the formulation is showing release zero order**.** Results are shown in **Table No.10**

### i) Stability Study:

Formulations P3 showed good stability with no significant change in % drug loading, % EE and in *in vitro* drug release after stability study at 40$^{o}$C ± 2$^{o}$C and 75 % ± 5 % RH, for period of 3 months. Results are shown in **Table No. 11**

## 5.2 CONCLUSION:

Sustained release IPN microspheres containing Propranolol HCl were successfully prepared by using Emulsion Crosslinking Method. Nature and polymeric ratio were found to be important parameters affecting the drug release particle size and on swelling behavior. The concept of controlled and targeted delivery is well establish

for oral and parenteral use. In this study, effect of polymer on release of propranolol HCl for different polymeric blends and their ratio was observed so from this study it can be concluded that chitosan and HPC in 7:3 ratio able to sustained the release drug for 12 hrs.

## 6.0 SUMMARY

Literature survey reveals that Propranolol HCl, an antihypertensive drug has a short half-life and therefore it is need to prepare sustained release formulation. This multiparticulate drug delivery system has various advantages over the unit dosage forms.

The IPN microspheres were prepared by using emulsion crosslinking method. In that the chitosan is used as crosslinking polymer while Hydroxy propyl cellulose (HPC), Hydroxy propyl methyl cellulose (HPMC) and sodium carboxy methyl cellulose (Na CMC) were used as neutral polymer by using glutaraldehyde as crosslinking agent and span 80 as emulsifying agent.

Drug was scanned under UV spectroscopy and maximum absorbance was found at 288.2 nm in both pH 1.2 and pH 6.8 medium. Both drug and polymer were investigated for interaction by FTIR spectroscopy.

In the present work nine batches were prepared by varying the different ratio of polymer concentration in each batch. First three batches (P1-P3) contain chitosan: HPC and (P3-P6) contain chitosan: HPMC while (P7-P9) contain chitosan: Na CMC having polymer ratio (9:1, 8:2, 7:3). The formulated microspheres were subjected for various evaluation parameters such as % Drug loading, % EE, Mean particle size, % Swelling study, and *In vitro* release study. On the basis of obtained results it can be stated that by varying polymer blend and their ratio it significantly affect the drug entrapment, particle size and *in vitro* release. From the result batch P3 gave desired release profile hence it was selected as a optimized batch and continued for further study. The batch P3 showed 93.12% drug release, 96.43µm particle size and 61.26% entrapment efficiency. Scanning electron microscopy of optimized batch P3 showed spherical shape with slightly rough surface.

The stability studies were carried out on optimized formulation P3 at $40^0$ C± $2^0$ C and 75% ± 5% RH for three months. The microspheres were evaluated for percent drug loading, percent drug entrapment efficiency and for percent cumulative drug release for 0, 30, 60 and 90 days. No significant changes in percent drug loading, percent drug entrapment efficiency and drug release, and were obtained and hence it was concluded that the optimized batch (P3) was stable.

In this study, effect of polymer on release of propranolol HCl for different polymeric blends and their ratio was observed so from this study it can be concluded that chitosan and HPC in 7:3 ratio able to sustained the release drug for 12 hrs

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# HOW TO CITE THIS ARTICLE

Ms. J. D. Walde. Mr. H. S. Kanhere. (2020). Formulation and Evaluation of Interpenetrating polymer network Microspheres containing ritonavir. *International Journal for Pharmaceutical Research Scholars, 9(1); 01-17.*



 

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