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PREPARATION & EVALUATION OF IPN HYDROGEL BY SYNTHESIZED CARBOXY METHYL DERIVATIVE OF GUM KARAYA & LOCUST BEAN GUM; EXAMINATION OF SUSTAINED ORAL DELIVERY POTENTIAL OF CARVEDILOL IN VIVO

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ABSTRACT

Most popular and convenient route of drug delivery is oral dosage form. But modified release formulations are being developed for better patient compliance and also improving clinical efficacy of the drug. Recent years have seen the use of interpenetrating polymer network (IPN) gain importance in controlled drug delivery. So for our work we have synthesized carboxy methyl derivatives of gum karaya and locust bean gum and also prepared and evaluated IPN hydrogel using the same. For enhancement of this same work we have examined sustained oral delivery potential of carvedilol in vivo. The percentage yield, drug entrapment efficiency, particle size measurement, FE-SEM analysis, drug polymer interaction, DSC, XRD, in vitro release study, swelling study and mucoadhesion study were done to determine the characteristics of the IPN hydrogel beads were done. In vivo hypotensive activity of pure drug suspension and drug loaded IPN hydrogel beads in mice were established. The study revealed the exact combination in which the IPN hydrogel beads

were able to entrap almost 100% drug and provide sustained release of carvedilol over a period of 8 hours in simulated intestinal fluid (pH 6.8). The in vivo study in animal model suggested that the IPN formulation could reduce the blood pressure for a much longer duration than with the pure drug suspension.

Keywords: Interpenetrating polymer network (IPN), drug, hydrogel, controlled drug delivery, in-vivo, FE-SEM analysis, DSC, XRD

INTRODUCTION

Sustained drug delivery systems are designed to achieve prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose **[1]**. Sustained release systems include any drug-delivery system that achieves slow Release of drug over an extended period of time. If the systems can provide some control, whether this is of a temporal or spatial nature, or both, of drug release in the body, or in other words, the system is successful at maintaining constant drug levels in the target tissue or cells, it is considered a controlled-release system.

MERITS OF SUSTAINED DRUG DELIVERY SYSTEM

1. The frequency of drug administration is reduced.
2. Patient compliance can be improved.
3. Drug administration can be made more convenient as well.
4. The blood level oscillation characteristic of multiple dosing of conventional dosage forms is reduced.
5. Better control of drug absorption can be attained, since the high blood level peaks that may be observed after administration of a dose of a high availability drug can be reduced.
6. The characteristic blood level variations due to multiple dosing of conventional dosage forms can be reduced.
7. Reduction in fluctuation in plasma drug concentration and hence more uniform pharmacological response.
8. Less frequent dosing causes improved patient compliance **[2]**.

Demerits of Drug Delivery System

1. Probability of dose dumping.
2. Reduced potential for dose adjustment.
3. Cost of single unit higher than conventional dosage forms.
4. Increase potential for first pass metabolism.
5. Requirement for additional patient education for proper medication.

6. Decreased systemic availability in comparison to immediate release conventional dosage forms.
7. Poor in vitro and in vivo correlations **[3]**

Advantages of Biopolymer-Based Drug Delivery System

1. Recently, bio-polymers are being extensively studied for the design of control drug delivery system.
2. Non-toxic
3. Biodegradable
4. Biocompatible
5. Presence of large variety of functional groups amenable for structural modifications

Hydrogel

Hydrogel is 3-D polymer network systems can imbibe large amount of water, resembles tissue-like consistency and swell without dissolving. Hydrogels can be developed using biopolymers in an eco-friendly environment for drug delivery applications **[4]**.

Benefits and limitations of hydrogels

General benefits

- Biocompatible
- Can be injected in vivo (in a whole, living organism) as a liquid that then gels at body temperature
- Protect cells
- Good transport properties (such as nutrients to cells or cell products from cells)
- Timed release of medicines or nutrients
- Easy to modify
- Can be biodegradable or bio absorbable

- **General limitations**
  - High cost
  - Low mechanical strength
  - Can be hard to handle
  - Difficult to load with drugs/nutrients
  - May be difficult to sterilize
  - Non-adherent

Interpenetrating polymer network

An IPN is a composite of at least two polymers, exhibiting varied characteristics, which is obtained when at least one polymer network is synthesized or cross-linked independently in the immediate presence of the other **[5]**.

Merits of IPN

1. IPN is also attractive in producing synergistic properties from the component polymers.
2. IPN hydrogel is expected to have an improved capability of immobilizing a drug.
3. IPN enhance the mechanical properties of the final product

Classification of IPN (Figure 1)

1. Covalent Semi IPN- A covalent semi IPN contains two separate polymer systems that are cross-linked to form a single polymer network.
2. Non Covalent Semi IPN- A non covalent semi IPN is one in which only one of the polymer system is cross linked.
3. Non Covalent Full IPN- A non covalent full IPN is a one in which the two separate polymers are independently cross linked.
4. Sequential IPN- In sequential IPN the second polymeric component network is polymerized following the completion of polymerization of the first component network.
5. Novel IPN- Polymer comprising two or more polymer networks which are at least partially interlocked on a molecular scale but not covalently bonded to each other and cannot be separated unless chemical bonds are broken.
6. Semi IPN- If only one component of the assembly is cross linked leaving the other in a linear form, the system is referred as semi IPN.
7. Simultaneously IPN- Simultaneously IPN is prepared by a process in which both component networks are polymerized concurrently, the IPN may be referred to as a simultaneously IPN **(Figure 2)**.

Gum Karya

Gum karaya is a vegetable gum produced as an exudate by trees SterculiaurensRoxb belonging to family Sterculiaceae. Chemically, gum karaya is an acid polysaccharide composed of the sugars galactose, rhamnose and galacturonic acid. It is a high molecular-weight polysaccharide **[6] (Figure 3)**.

Locust Bean Gum

Locust bean gum is a galactomannan vegetable gum extracted from the seeds of the carob tree (Ceratoniasiliqua), Family Leguminosae, mostly found in the Mediterranean region. The carob seed consists of three different parts: the husk surrounding the seed, the germ (protein) and the endosperm (gum). It consists chiefly of high-molecular weight hydrocolloidal polysaccharides, composed of galactose and mannose units combined through glycosidic linkages, which may be described chemically as galactomannan **(Figure 4)**.

Morphology- Locust bean gum occurs as a white to yellow-white powder. The bean, when made into powder, is sweet—with a flavour similar to chocolate.

Solubility- It is dispersible in either hot or cold water, forming a sol having a pH between 5.4 and 7.0, which may be

converted to a gel by the addition of small amounts of sodium borate **(Figure 5)**.

Drug Profile (CARVEDILOL)

Carvedilol, is a non-selective beta blocker used for treating mild to severe congestive heart failure (CHF), left ventricular dysfunction (LVD) following heart attack in people who are otherwise stable, and for treating high blood pressure **[8] (Figure 6)**.

Brand Names: Coreg, Coreg CR

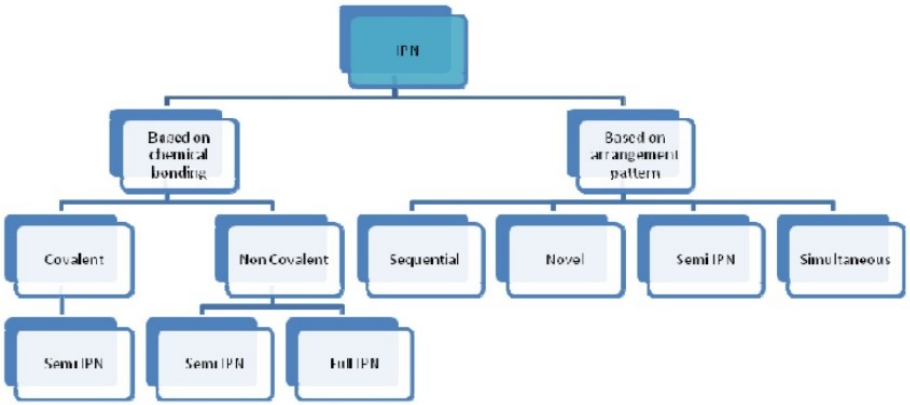

Figure 1: Different form of IPN

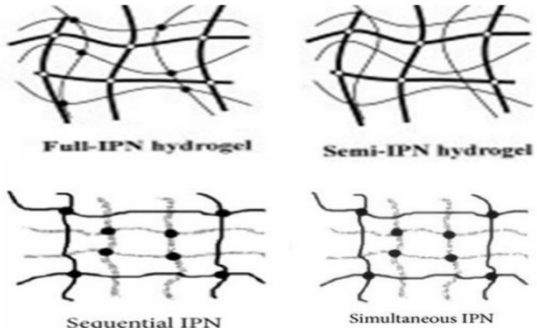

Figure 2: Structure of Different type of IPN

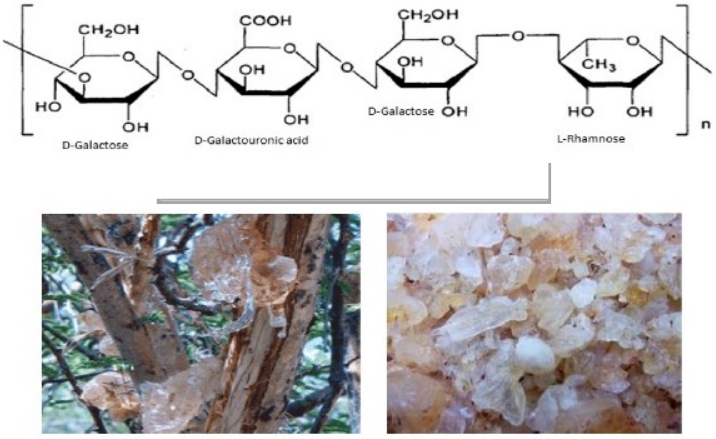

Figure 3: Structure/Images of Karaya Gum

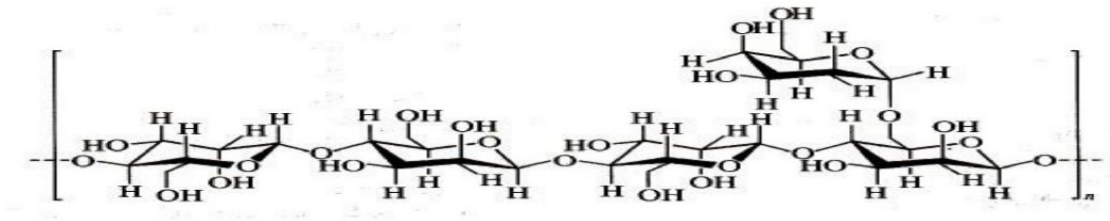

Figure 4: Structure of Locust bean Gum

Figure 5: Images of Locust bean Gum

IUPAC Name: 1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy) ethylamino] propan-2-ol

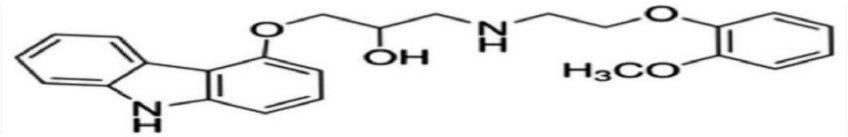

Figure 6: Chemical structure of carvedilol

Table 1: Some properties of carvedilol

Molecular Weight	406.482 g/mol	**Melting point**	114-115°C
Bioavailability	25-35%	**Protein binding**	98%
Metabolism	liver	**Excretion**	Urine (16%), Feces (60%)
Biological half-life	7-10 hr.	**Volume of distribution**	115 L
Plasma clearance ranges	500 to 700 ml/min.	**logP**	4.19
pK_a (Strongest Acidic)	: 14.03	**pK_a (Strongest Basic)**	8.74

- Solubility: Freely soluble in dimethylsulfoxide; soluble in methylene chloride, methanol; sparingly soluble in ethanol, isopropanol; slightly soluble in ethyl ether. The water solubility of carvedilol is 1.668g/L. High solubility of carvedilol was noticed at low pH (545.1-2591.4 µg/ml within the pH range 1.2-5.0) and low solubility was found at high pH (5.8-51.9 µg/ml within the pH range 6.5-7.8).
- Absorption: Carvedilol is rapidly and extensively absorbed following oral administration, with an

absolute bioavailability of approximately 25% to 35% due to a significant degree of first pass metabolism.

- Side effects Common carvedilol side effects may include weakness, dizziness, and diarrhea, dry eyes, tired feeling or weight gain. This is not a complete list of side effects and others may occur.
- Dosage- Carvedilol comes in tablets ranging from of 3.125 milligrams (mg) to 25 mg. It's also available as an extended-release capsule.
- Medical use - Carvedilol is indicated in the management of congestive heart failure (CHF), commonly as an adjunct to angiotensin-converting-enzyme inhibitor (ACE inhibitors) and diuretics. It has been clinically shown to reduce mortality and hospitalizations in people with CHF. The mechanism behind its positive effect when used long-term in clinically stable CHF patients is not fully understood, but is thought to contribute to remodeling of the heart, improving upon its structure and function. In addition, carvedilol is indicated in the treatment of hypertension and to reduce risk of mortality and hospitalizations in a subset of people following a heart attack. It can be used alone or with other anti-hypertensive agents. In the 2013 guideline, it is recommended as the drug of choice in people with histories of CHF and/or myocardial infarction.
- Pharmacodynamics- Carvedilol is a nonselective beta-adrenergic blocking agent with alpha1-blocking activity and is indicated for the treatment of hypertension and mild or moderate (NYHA class II or III) heart failure of ischemic or cardiomyopathic origin. Carvedilol is a racemic mixture in which nonselective beta-adrenoreceptor blocking activity is present in the S(-) enantiomer and a-adrenergic blocking activity is present in both R(+) and S(-) enantiomers at equal potency. Carvedilol has no intrinsic sympathomimetic activity. The effect of carvedilol's beta-adrenoreceptor blocking activity has been demonstrated in animal and human studies showing that carvedilol (1) reduces cardiac output in normal subjects; (2) reduces exercise and/or isoproterenol-induced tachycardia and (3) reduces reflex orthostatic tachycardia **[9]**.
- Pharmacokinetics- Carvedilol is about 25% to 35% bioavailable

following oral administration due to extensive first-pass metabolism. The compound is metabolized by liver enzymes, CYP2D6 and CYP2C9 via aromatic ring oxidation and glucuronidation, then further conjugation by glucuronidation and sulfation. Compared to the parent compound, the three active metabolites exhibit only one-tenth of the vasodilatation of the parent compound. However, the 4-hydroxyphenyl metabolite is about 13-fold more potent in beta-blockade than the parent compound **[10]**.

Objective of the Work

Oral dosage forms has long been the most popular and convenient route of drug delivery. There are many obvious reasons for this, not the least of which would include acceptance by the patient and ease of administration. Various types of modified release formulations have been developed to improve the patient compliance and also clinical efficacy of the drug. The sustained release oral dosage forms have been demonstrated to improve therapeutic efficacy by maintaining steady state drug plasma concentration. Sustained release preparations are helpful to reduce the dosing frequency and side effects of drugs and improve patient's convenience. The use of hydrophilic polymers from natural origin, especially the polysaccharides have been the focus of current research activity in the design of particulate matrix devices due to their non-toxic, biocompatible, biodegradable nature and broad regulatory acceptance. On the other hand oral controlled release multiple unit dosage forms are becoming more popular than single unit dosage forms as they spread uniformly throughout the gastrointestinal tract, avoiding the vagaries of gastric emptying and different transit rates, resulting in a more uniform drug absorption and reduced local irritation when compared to single unit dosage forms. In recent years, interpenetrating polymer network (IPN) hydrogels have also gained considerable attention for controlled drug delivery. Interpenetration of the two networks may result in a higher mechanical strength than in the homopolymer network. Furthermore, IPNs provide free volume space for the easy encapsulation of drugs in their network structure. A large number of polysaccharides such as xanthan gum, guar gum, locust bean gum, pectin have been used as hydrophilic matrices to investigate release behaviour of drugs. In order to enrich the resources, there is a quest for new polysaccharides at one

hand, and modification of existing ones on the other hand. Native polysaccharides owing to their diverse chemical composition and functional groups are amenable to chemical modifications through chemical derivatization, chemical cross-linking or ionic cross-linking and thus tailor-made materials are obtained which can be used to modulate drug release. Cross-linking is a suitable procedure to control swelling of hydrophilic matrices and to regulate drug release. In contrast to covalent cross-linking, ionic cross-linking is a simple and mild procedure, and more acceptable to regulatory agency from the aspect of safety.

Recently IPN systems of a native or modified LBG (Acrylamide grafted, carboxymethylated) and other polysaccharides such as alginate and synthetic polymer polyvinyl alcohol have been investigated for controlled delivery of a wide variety of drugs. Till date, there are no reports on IPN combination of LBG and karaya gum.

Therefore, the objective of this present work is:

- To synthesize carboxymethyl derivatives of gum karaya and Locust bean gum
- To prepare IPN hydrogels using modified gum karaya and Locust bean gum.
- To evaluate the properties of hydrogel particles.
- To examine sustained oral delivery potential of carvedilol in vivo

METHODS

Preparation of pH 6.8 phosphate buffer solution

In a 1000 ml beaker, 6.808 gm of potassium dihydrogen phosphate and 1.8 gm of sodium hydroxide were mixed well and water was added to make up the volume **[11]**.

Preparation of pH 1.2 HCl buffer solution

In a 1000 ml beaker, 8.33 ml of Conc. HCl are mixed well and water was added up to make up the volume. The solution was adjusted to pH 1.2 **[12]**.

Determination of λ_{max} of Carvedilol in phosphate buffer solution (pH 6.8)

Stock solution was prepared by dissolving accurately weighed (Mettler balance, Mettler Toledo, AB 204-S, Switzerland) 10 mg of carvedilol in 100 ml methanol to make a final concentration of 100 µg/ml. From the stock solution, 0.2 ml of sample was withdrawn and diluted to 10 ml with pH6.8 phosphate buffer solution. The resulting solution (2µg/ml) was scanned from 200-400 nm using a UV spectrophotometer (Thermo Scientific, USA) to determine the λ_{max} of carvedilol in

phosphate buffer solution. The wavelength of maximum absorption was found at 284 nm **[13]**.

Determination of λ_{max} of Carvedilol in HCl solution (pH 1.2)

Stock solution was prepared by dissolving accurately weighed (Mettler balance, Mettler Toledo, AB 204-S, Switzerland) 10 mg of carvedilol in 100 ml methanol to make a final concentration of 100µg/ml. From the stock solution, 0.2 ml of sample was withdrawn and diluted to 10 ml with HCl solution (pH 1.2). The resulting solution (2µg/ml) was scanned from 200-400 nm using a UV spectrophotometer (Thermo Scientific, USA) to determine the λ_{max} of carvedilol in HCl buffer solution. The wavelength of maximum absorption was found at 284 nm **[14]**.

Standard curve of Carvedilol in phosphate buffer solution (pH 6.8)

Accurately weighed, 10mg of carvedilol was dissolved in 100 ml of methanol. A series of standard solution with a concentration range of 2-10 µg/ml were prepared from the initial stock solution by diluting with phosphate buffer medium (pH 6.8). UV absorbance of standard solution was measured by UV spectrophotometer at 284 nm. Standard curve of absorbance versus concentration of carvedilol (µg/ml) has been presented. Each experiment was carried out in triplicate **[15]**.

Standard curve of Carvedilol in HCl solution (pH 1.2)

Accurately weighed, 10mg of carvedilol was dissolved in 100 ml of methanol. A series of standard solution with a concentration range of 2-10 µg/ml were prepared from the initial stock solution by diluting with pH 1.2 HCl buffer medium. UV absorbance of standard solution was measured by UV spectrophotometer at 284 nm. Standard curve of absorbance versus concentration of carvedilol (µg/ml) has been presented. Each experiment was carried out in triplicate **[16]**.

Carboxymethylation of polysaccharides

- Karaya gum (KG)- KG (2.2gm) was washed with 25ml of methanol for 2h to remove organic impurities. Accurately weighed 2gm of washed KG was dispersed in 6.7ml of ice-cold deionized water containing 3.024gm sodium hydroxide. Then aqueous solution of monochloroacetic acid (1.5gm dissolved in 3.3ml of deionized water) was slowly added into alkaline slurry of KG over a period of 1h, maintained at 15–18˚C. The temperature of the mixture was raised slowly up to 60–65˚ C and stirred for another hour. The wetted mass was washed with 50ml of methanol and filtered. The residue

was washed further with three successive amounts of 20 ml 80% methanol for 15 min and filtered. Finally the filtered mass was washed with methanol and air-dried.

- Locust beam gum (LBG)- LBG (2.2gm) was washed with 25ml of methanol for 2h to remove organic impurities. The LBG (2gm) was dispersed in 4ml of water and heated to 80°C for 15 min for hydration of polymer. Then 6.7ml cold NaOH (45% w/v) solution was added into the hydrated polymer. Then aqueous solution of monochloroacetic acid (3.3ml 45.04% w/v) was slowly added into alkaline polymer reaction mixture over a period of 1h, maintained at 15–18˚ C. The temperature of the mixture was raised slowly to 60–65˚ C and stirred for at least another 1 h. The final product was purified following the method describe earlier (Section 3.7.1) **[17]**.

Preparation of interpenetrating hydrogel beads

Weighed quantity of Carvedilol was dispersed in 10 ml of water and stirred magnetically for 10 min for its uniform dispersion. Required amount of carboxymethyl LBG was added into the drug dispersion under continuous magnetic agitation. Once the modified LBG was dissolved, pre- weighed carboxymethyl KG was dispersed. The stirring was continued for 1h. The drug-polymer mixture was filled in 10 ml glass syringe and was added into aluminium chloride solution drop by drop through 21G needle. The droplets were incubated for 10min and the hydrogel beads were formed instantaneously. The beads were isolated by filtration, washed with distilled water and dried. Different drug polymer ratio was used to prepare hydrogel beads. The composition of the hydrogel beads are represented in **Table 2 [18]**.

Table 2: Composition of the hydrogel beads

Formulation code	CM-LBG:CM-KG (mass ratio)	Concentration of AlCl3 (% w/v)	Drug loading (20%w/w)
F1	4.5:0	3	112.5
F2	3.5:1.0	1	112.5
F3	3.5:1.0	2	112.5
F4	3.5:1.0	3	112.5
F5	0:4.5	3	112.5

Evaluation

Percentage yield

The percentage yield of hydrogel beads of various compositions were computed using the mass of final product (hydrogel beads) after drying with respect to the initial total weight of the drug and polymers used for preparation of hydrogel beads. The

percentage yield was calculated according to the following formula,

Percentage yield = (weight of final product hydrogel beads / total weight of drug and polymers used) × 100

Drug Entrapment efficiency

Accurately weighed 10 mg hydrogel beads of each formulation were allowed to disintegrate into 250 ml phosphate buffer (PH6.8) solution for overnight in Orbital Shaker bath (Lunar, Amalgamated suppliers, Kolkata, India). The solution was warmed and then filtered through WhatmanTM filter paper (No. 41). The filtrate was analyzed for drug content using UV spectrophotometer at 284 nm (Thermo Fisher Scientific, USA). Each determination was made in triplicate and entrapment efficiency was calculated as follow:

Drug Entrapment Efficiency (%) = Actual drug content theoretical drug content / theoretical drug content) ×100

Particle size measurement

Fifty hydrogel beads from each formulation were observed under digital microscope (Olympus CH20i) at 10X magnification. Before that, the eye piece micrometer was calibrated using standard stage micrometer. 40 divisions of ocular micrometer were equal to 100 divisions of stage micrometer. The particle diameter was calculated by using the following relationship.

Particle Diameter (µm) = (100 /40) ×number of eye piece division of each particle×10

FE-SEM analysis

The hydrogel beads were examined under FE-SEM Supra 55 (Carl Zeiss, Germany) instruments attached with Energy Dispersive Microanalyzer (Oxford Liquid Nitrogen free SDD X MAX 50 EDS) at 100-140X magnification. The instrument was operated at a resolution of 0.8 nm at 15 KV with acceleration voltage of 30 KV. Schottky Field Emission Electron Gun was used for emission of electrons. The samples were kept in saturated vapor of palladium to make the particles' surface conductive. Then, the samples were mounted on FE-SEM instrument and imaged with a secondary electron detector.

Drug-polymer interaction

Drug-polymer compatibility was examined by FT-IR analysis. KBr disks of pure drug, drugloaded and drug–free hydrogel beads were scanned at a resolution of 4cm-1 in a Perkin Elmer FTIR spectrometer (Spectrum 100, USA) from 4000 to 450 cm-1.

Differential scanning calorimetry (DSC) analysis

The samples (3.5-5mg) of pure carvedilol, powdered carvedilol-loaded hydrogel beads and drug-free hydrogel beads were scanned in PerkinElmer instrument (Pyris 1, USA) from 25°C to 125°C at a speed of 10°C/min. Nitrogen was purged at flow rate of 20ml/min. Indium was used as a reference for calibration of heat flow.

X-ray diffraction analysis

X-rays of pure drug, drug-loaded and drug-free hydrogel beads were traced in a wide angle X-ray diffractometer (Ultima III, D/Max 2200, Rigaku Corporation, Japan) using Cu-Kα-radiation as a source of x-ray. The crystallograms were recorded at a speed of 5° per min over a diffraction angle (2θ) range of 10–30°. The instrument was set up with a tube voltage of 40 kV and current of 30mA.

In vitro release study

The release of carvedilol from the hydrogel beads was carried out using 6 station paddle type dissolution rate test apparatus (VEEGO, VDA-6D, Mumbai, India). Accurately weighed100mg of particles of each formulation was placed in 900ml of HCl solution (pH1.2). The temperature of the dissolution vessel was set at 37±0.5°C. The paddle was rotated at 100rpm. At definite time interval 10ml sample was withdrawn and immediately replaced the same volume of fresh HCl solution (pH1.2). The experiment was continued up to 2h in HCl solution. The same procedure was adopted to carry out in-vitro drug release test in phosphate buffer solution (pH6.8) up to 8h. The percentage drug release was calculated and plotted against time. The in vitro drug release data up to 60% was fitted into Korsmeyer-peppas model equation in order to evaluated drug release mechanism from the hydrogel beads.

$$M\propto=kt$$

Where Mt/M∞ is the fractional solute release at time t, k is a constant which incorporates the structural and geometric characteristics of the device, and n is diffusion exponent. The mechanism is Fickian type when the value of n ≤0.43 and non-Fickian or anomalous when the value of n lies within 0.43-0.85 and case II transport when n = 0.85.A value of n greater than 0.85 is indicative of super case II transport mechanism.

Swelling study of hydrogel beads

The drug-free hydrogel beads of known weight (10 mg) were placed in a beaker containing 50ml HCl solution (pH 1.2) for a period of 2h. At pre-determined time interval (10, 20, 30, 45, 60, 90,120 min), the swollen hydrogel beads were separated from the swelling medium, blotted with tissue paper to remove surface water and weighed (Mettler Toledo AB204-S, Switzerland). The hydrogel beads were then put into the medium for further swelling. This process was continued up to 2h. The same procedure was followed up to 4h to evaluate swelling kinetics of the hydrogel beads in phosphate buffer solution (pH 6.8). The degree of swelling of the particles was calculated using the following equation:

$$Swelling\ Degree\ (\%) = \frac{w_t - w_0}{w_0} \times 100$$

Where, Wo and Wt symbolize the weight of the hydrogel beads at time zero and t, respectively.

Muco adhesion study of hydrogel beads

The mucoadhesion test was performed in both HCl solution (pH1.2) and phosphate buffer (pH 6.8). The freshly excised pieces of goat intestinal mucosa (2×3 cm) were mounted onto glass slides with cyanoacrylate glue. Fifty hydrogel beads were placed onto the mucosal tissues and the glass slides were tied onto the arm of a USP tablet disintegrating test apparatus. The arm was set to provide a slow and regular up-down movement (32-40 cycles/min) in a vessel containing 900 ml of fluid at 37°C. At predetermined time interval, the hydrogel beads adhered to the mucosal tissue was counted and the percentage mucoadhesion was calculated by the following formula

Mucoadhesion(%) =(Number of hydrogel beads adhered /Number of hydrogel beads applied)×100

Pharmacodynamics of the hydrogel formulation

Male Swiss albino mice weighing around 20-22 gm were housed in a temperature and light controlled room (23±2°C; 12h light/dark cycle), with free access to feed and water. After 4th week the blood pressure was measured for all the three groups to confirm hypertension.

Table 3: Composition of high fat diet

Component	gm / kg of diet
Maize starch	367
Lard	316
Cassien	255
Vitamin and mineral mixture	61
Sodium chloride	1

All the procedures were in agreement to Institutional Animal Ethics Committee (955/RO/A/2006/CPCSEA-2006 approved via GCTS/IAEC/2017/March/05) Fifteen mice were randomly divided into three groups of 5 animals each. Group 1 was treated as control (fed with normal water), Group 2 (high fat diet) received pure carvedilol suspension at a dose of 7.38 mg/kg BW and Group 3 (high fat diet) was treated with the optimized formulation (38.20 mg hydrogel beads equivalent to 7.38 mg carvedilol) via oral gavages. 20% fructose solution was given to Group 2 and Group 3 animals instead of water.

The dose was calculated using the following equation

Animal dose (mg/kg)=Human equivalent dose[HED](mg/kg)× (Human Km (37) /Animal km (3))

Following induction of hypertension, the treatment was commenced with pure drug suspension and optimized drug-loaded formulation. On the day of experiment, the blood pressure of the mice was measured at 1h interval up to 8h by non-invasive tail

cuff method using B.P IITC Life Science, Washington instrument, USA fitted with iWORKS software. One way ANOVA analysis was done to determine the statistical significant difference between control and tested groups. The difference was considered significant when $p<0.05$.

Analytical Monitoring

The standard curves of carvedilol in phosphate buffer (pH6.8) and HCl solution (pH1.2) were constructed to estimate the drug concentration in test samples **(Table 4, 5; Figure 9, 10)**. Before that the standard drug solution obtained in different media were scanned in UV-Vis spectrophotometer to determine the λ_{max}. The wavelength for maximum absorption was noted at 284nm at both media. The scanning reports are shown in **Figure 7 and Figure 8**.

2. Formation & Properties of IPN Hydrogel Beads

Preliminary experiments revealed that the native LBG and KG were unable to form isolatable hydrogel beads in their own capacity in contact with trivalent aluminium ions. Hence the carboxymehtyl groups were introduced to render them ability to form hydrogels in presence of trivalent metal salts. It was found that carboxymethyl KG failed to form hydrogel beads itself but formed insoluble complexes with aluminium ions. On contrary, carboxymethyl LBG formed hydrogel beads when an aqueous solution of modified LBG was dropped into different concentration of aluminium chloride. However, only carboxymethyl LBG hydrogel beads retained only about 65% of the fed drug. Hence, it was thought to use a combination of carboxymethyl LBG and carboxymethyl KG for the preparation of IPN hydrogel beads using AlCl3 as cross-linking solution in order to modify the drug retention capacity and release characteristics. The morphological characteristics were examined by field emission scanning electron microscopy (FE SEM) and the elemental composition was assessed by EDX spectra **(Figure 11 and Figure 12)**. The elemental composition is given in **Table 6**.

Depending upon the concentration of AlCl3, the drug entrapment efficiency of the hydrogel particles reached to almost 100%. Highest drug entrapment efficiency was found at 3% $AlCl_3$. The effect of $AlCl_3$ on particle diameter was also noticed. The particle diameter gradually decreased with increase in concentration of $AlCl_3$ in the gelation medium, perhaps due to greater cross-linking of the dual polymers. The mucoadhesion of the hydrogel particles were less in acidic medium than that observed at weakly alkaline medium. The mucoadhesive property of the particles in acidic medium reversed in weakly alkaline pH. The data are shown in **Table 7**.

Drug Release and Swelling Kinetics

In-vitro drug release profiles of the hydrogel beads in HCl solutions (pH 1.2) are displayed in **Figure 13**. The hydrogel beads formulations (F1) released about 60% drug in 2h. The IPN beads treated with 1% AlCl3 (F2) released almost its entire content within 45min. However, the IPN beads treated with 2% and 3% AlCl3 (F3 and F4, respectively) released about 85% and 65% of the entrapped drug at the end of 2h. So the data suggested that the use of CMKG in the IPN structure did not prohibit the rapid release of carvedilol in acidic medium. Only the IPN beads treated with 3% AlCl3 were found to resist the rapid diffusion of carvedilol in acidic medium compared to IPN formulation F2 and F3 treated at low concentration of AlCl3. The in-vitro release profiles of the hydrogel bead formulation in phosphate buffer (pH 6.8) are represented in **Figure 13**. In compassion to the percentage drug release obtained in acidic media, the amount of drug release was found less in phosphate buffer solution (pH 6.8) within a same time frame of 2h. About 21%, 56%, 32% and 14% drug was released in phosphate buffer solution (pH 6.8) for the F1, F2, F3 and F4 formulation, respectively. Overall, the drug release rate weakly medium was slower relative to that observed in acidic medium. Only CM-LBG beads provided 75% drug release in 8h. Irrespective of AlCl3 concentration, the IPN beads were able to maintain a sustained drug release over a period of 8h. However, with the increase in AlCl3 concentration up to 3% (w/v), the most sustained drug release profile was attained in case of F4 formulation, reinforced with 3% AlCl3, perhaps due to higher cross-linking density of the hydrogel matrix.

The swelling kinetics of hydrogel beads in HCl solution (pH 1.2) and phosphate buffer solution (pH 6.8) is illustrated in **Figure 14**. The drug release data in acidic and alkaline medium corroborated well with the swelling tendency of the particles. Among the formulation only CM-LBG (F1) formulation swelled less. Among the AlCl3 treated formulation (F2, F3, F4). F2 formulation exhibited the highest swelling degree probably due low cross-linking density in the hydrogel matrix at the end of 1.5h. The maximum degree of 126% was observed and therefore, the swelling degree decline due to dissolution of poly and consequent weight loss. However, F3 and F4 formulation the swelling degree was gradually increases up to 2h, reaching swelling degree of 118% and 104%.In a time frame of 1.5h., the swelling degree was less increase of 3% $AlCl_3$ treated formulation (F4) in solution of pH 1.2. In phosphate buffer solution (pH 6.8) swelling percentage for all the formulation was

gradually increases up to 4h and the lowest swelling degree was noted for the 3% $AlCl_3$ treated formulation (F4) with respect to 2h. The swelling degree was 126%, 335%, 234% and 90% respectively for the F1, F2, F3 and F4 formulations. It was noteworthy that despite higher swelling degree of the hydrogel beads in phosphate buffer solution (pH 6.8) the drug release was faster than acidic medium. It has been reported that the solubility of carvedilol in acid solution was 545.1-2591.4 µg/ml and alkaline solution 5.8-51.9 µg/ml. Therefore, the higher solubility of HCl medium was responsible for higher drug release rate in acidic media despite lower swelling property of the beads. Swelling is a continuous process of transition from unsolvated glassy or partially rubbery state to a relaxed rubbery region. When a hydrogel in its initial state is in contact with solvent molecules, the latter attacks the hydrogel surface and penetrates into the polymeric network. In this case, the un solvated glassy phase is separated from rubbery hydrogel region with a moving boundary. Regularly the meshes of the network in the rubbery phase will start expanding, allowing other solvent molecules to penetrate within the hydrogel network.

The In-vitro drug release data up to 60% were fitted into Korsmeyer-Peppas model to determine the physical mechanism involve in the process of drug release from the hydrogel particles. The values of n and k were determined by using least square regression analysis. In case of phosphate buffer solution (pH 6.8) the formulation F1-F3 exhibited the values of diffusion co-efficient within 0.43-0.85. Hence the drug release from the particles was said to follow anomalous transport from their hydrogel structure **(Table 8)**. That is the drug release occurred due to a combination of simple diffusion and polymer chain relaxation. The gel characteristic constant gradually increased and the lowest k value was obtained for the F4 formulation. Regardless of the variation in hydrogel composition the drug release mechanism was super case II transport from the particles in HCl solution (pH 1.2) Hence, the drug releasing in acidic media was controlled by erosion and swelling mechanism and strong indication of zero order kinetics **(Table 8)**. Korsmeyer-Peppas modeling of drug release data up to 60% in HCl solution (pH 1.2) and phosphate buffer solution (pH 6.8).

In case of Fickian or Case I transport, the polymer chains have a high mobility and the water penetrates easily in the rubbery network. Therefore, the solvent diffusion rate, R_{diff}, is clearly slower than the polymer chain relaxation rate, R_{relax}, ($R_{diff} << R_{relax}$). In case of non-Fickian diffusion, which appears when the glass

transition temperature (Tg) of polymer is well above the experimental temperature. In this situation, the polymer chains are not adequately mobile to permit urgent penetration of water into the polymer core . Depending on the relative rates of chain relaxation and diffusion, they commonly classified the non- Fickian diffusion to two subsections: "Case II transport" and "anomalous transport". Case II transport is dominated when the diffusion is very rapid compared to relaxation (R_{diff}>>R_{relax}), with relaxation occurring at an observable rate. The anomalous transport is observed when the diffusion and relaxation rates are comparable (R_{diff}≈R_{relax}). By determining the diffusional exponent, n, one can gain information about the physical mechanism controlling drug release from a particular device. For the case of anomalous transport, developed the following model to describe the release behaviour of dynamically swelling hydrogels.

$$Mt/M\infty = k_1 t + k_2 t^{0.5}$$

This expression describes the release rates in terms of relaxation-controlled transport process, k1t, and the diffusion-controlled process, $k_2 t^{1/2}$.

To define the relative contribution of each of the phenomena were further resolved by fitting in vitro drug release data into Peppas-Sahlin model. The values of k1 and k2 were determined by nonlinear regression analysis using Prism software (Trial version), the values presented in **Table 9**. In all the formulation from F1 to F3 the values of k2>> k1 hence, the dominating physical phenomena is simple diffusion mechanism. But in case of F4 formulation k1 >>k2therefore, polymer chain relaxation was the dominant mechanism for drug release. The negative value of k2 simplify negligible contribution for simple diffusion phenomena.

Examination of Physical Form of Drug

The differential scanning calorimetry (DSC) and x-ray diffraction patterns of pure carvedilol, carvedilol-loaded and carvedilol–free hydrogel beads are displayed in **Figure 15 and Figure 16**, respectively. In DSC thermogram of pure drug, one exothermic peak at 90.93°C and two endothermic peaks at 98.90°C and 117.54°C are found. The endothermic peak at 98.90°C could be due to loss of water in the sample and the peak at 117.54°C corresponded to melting point of the drug. However, no exothermic or endothermic transitions were noted in the thermograms of carvedilol-loaded and carvedilol–free hydrogel beads. Intense peaks were observed for the pure carveilol in the x-ray of pure carvedilol, but no intense peaks were noted for the x-ray scans of carvedilol-loaded and carvedilol–free hydrogel beads. This suggested that the drug in entrapped form lost its crystalline

nature and transformed into amorphous form.

Drug -Polymer Interaction

In the infrared spectra of carvedilol, the characteristics absorption peaks of the functional groups were observed at different frequencies. The peak corresponding to N-H bending appeared at 1505 cm-1. The wave numbers at 3331 cm-1 and 3491 cm-1 was ascribed to the N-H stretching. C-N stretching was observed at 1332 cm-1. The peaks at 1054 cm-1 and 1016 cm-1 could be due to alkyl aryl ethereal stretching. C-O stretching of alcohol group and CO stretching of epoxides were found at 1181 cm-1 and 1220 cm-1, respectively. The hyroxyl stretching was noted at 3599cm-1 (Figure 11). Similar peaks were noted at 1505 cm-1, 3362 cm-1, 3534 cm-1, 1333 cm-1, 1053 cm-1, 1022 cm-1, 1128cm-1, 1219cm-1, 3534cm-1, respectively in the IR spectrum of drug-loaded hydrogel beads. No significant differences were observed in the respective characteristics peaks of the drug when it was entrapped in IPN hydrogel beads. Only C-O stretching of alcoholic group and alcoholic hydroxyl stretching was shifted towards lower wave numbers i.e. from 1181 cm-1 to 1128cm-1 and 3599 cm-1 to 3534 cm-1 **(Figure 17)**. This could be due to the formation of weak H-bonds between –OH functional groups of drug and the polymers. Overall, no strong drug-polymer interaction was evident.

***In Vivo* Hypotensive Activity**

In case of pure drug suspension, the systolic pressure reduced to maximum 31.85% at the end of 6h. Thereafter, the blood pressure gradually rose up. The diastolic pressure also reduced to a maximum of 35.90% and thereafter, the BP reduction tendency was similar tothat observed in case of systolic pressure reduction. On contrary, the drug-loaded formulation exhibited its blood pressure lowering effect slowly and gradually up to 7h. Thereafter, the blood pressure lowering effect of the drug started to diminish and had tended to recover to normal blood pressure level. The difference in percent reduction of systolic and diastolic blood pressure with respect to control was statistically significant ($p < 0.5$). **Figure 18**. Data for in vivo hypotensive activity of pure drug suspension and drug-loaded IPN hydrogel beads in mice.

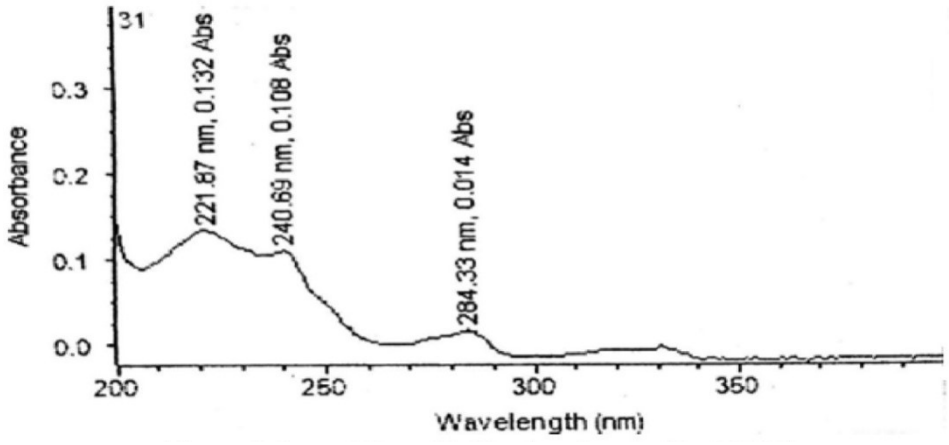

Figure 7: λ_{max} Carvidilol in phosphate buffer (pH 1.2)

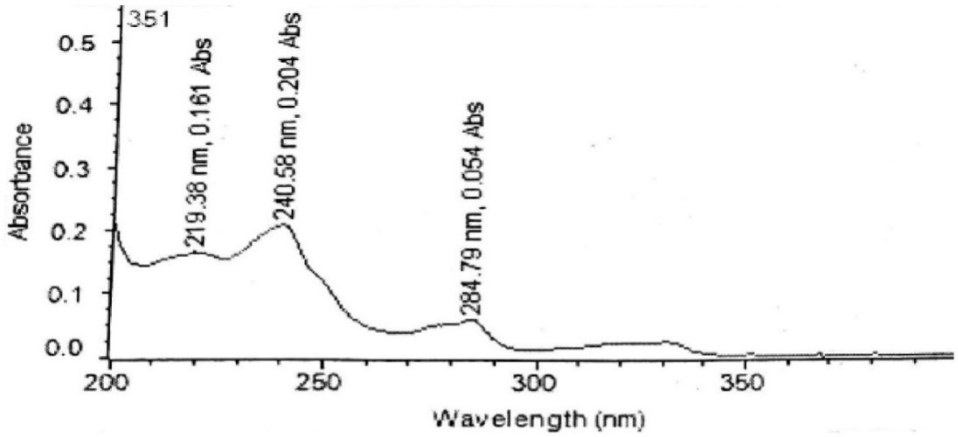

Figure 8: λ_{max} Carvidilol in HCl Solution (pH 6.8)

Table 4: The data for standard curve of carvedilol in pH 6.8 phosphate buffer solution

Conc. (µg/ml)	Absorbance (1)	Absorbance (1)	Absorbance (3)	Absorbance (4)	Mean	Stddev
0	0	0	0	0	0	0
2	0.047	0.066	0.045	0.069	0.056	0.0125
4	0.091	0.102	0.110	0.085	0.097	0.0112
6	0.143	0.136	0.149	0.125	0.138	0.0103
8	0.186	0.180	0.171	0.181	0.179	0.0062
10	0.263	0.227	0.231	0.236	0.239	0.0162

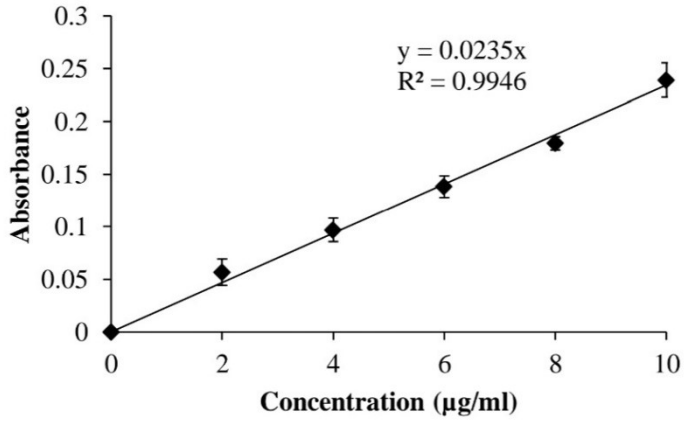

Figure 9: Standard curve of Carvedilol in pH6.8 phosphate buffer solution

Table 5: The data for standard curve of carvedilol in pH 1.2 HCl solution

Conc. (µg/ml)	Absorbance (1)	Absorbance (1)	Absorbance (3)	Absorbance (4)	Mean	Stddev
0	0	0	0	0	0	0
2	0.055	0.044	0.048	0.049	0.049	0.0045
4	0.090	0.095	0.095	0.098	0.094	0.0033
6	0.157	0.157	0.148	0.141	0.150	0.0077
8	0.209	0.194	0.214	0.215	0.208	0.0096
10	0.236	0.257	0.240	0.235	0.242	0.0102

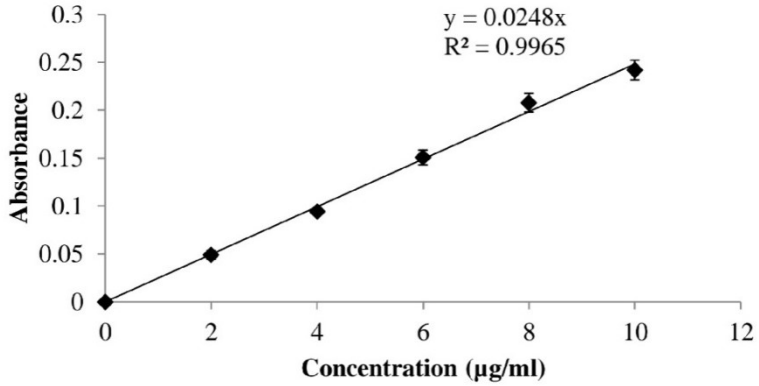

Figure 10: Standard curve of Carvedilol in pH6.8 phosphate buffer solution

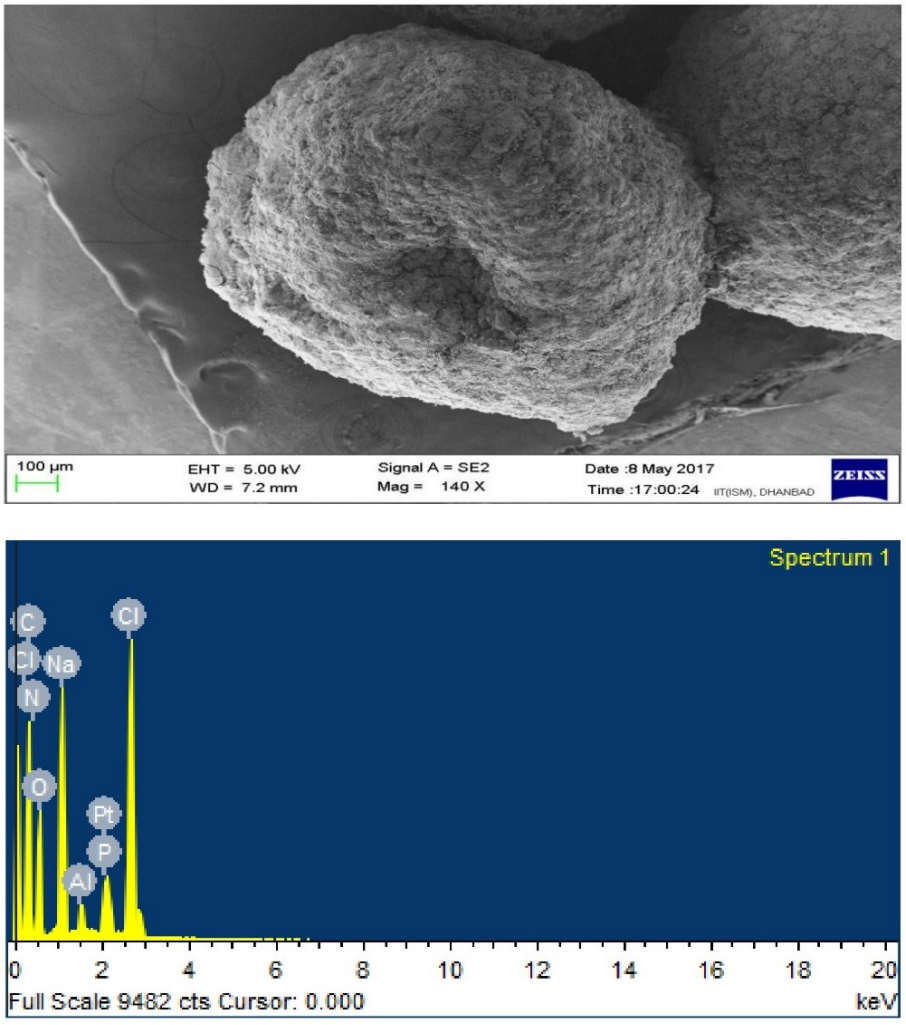

Figure 11: FE-SEM image and EDX spectrum of drug-free hydrogel bead

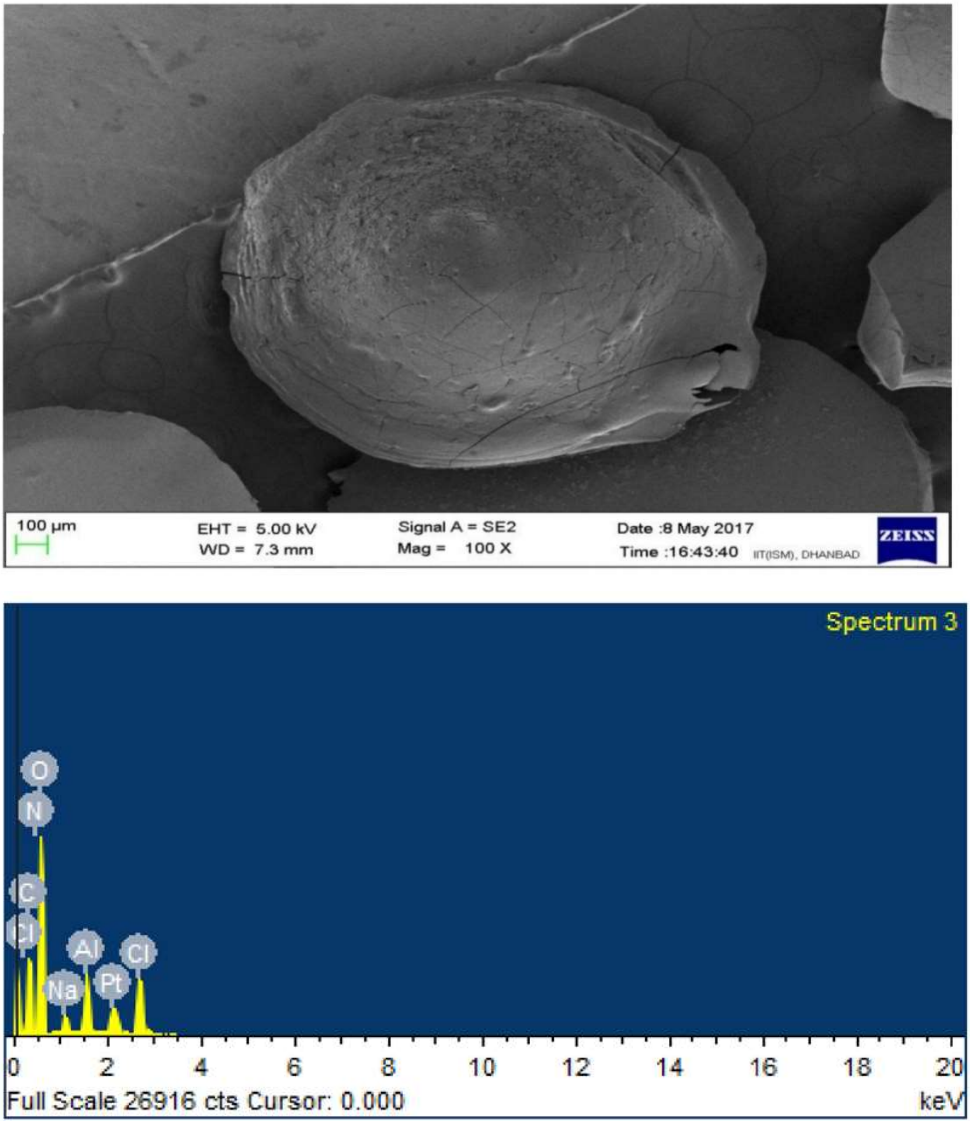

Figure 12: FE-SEM image and EDX spectrum of drug-loaded hydrogel bead

Table 6: Elemental composition of drug-free and drug-loaded hydrogel beads

Elements in drug-free hydrogel beads	Weight %	Atomic %	Elements in drug-loaded hydrogel beads	Weight %	Atomic %
C	44.2	60.48	C	27.14	37.89
N	3.42	4.03	N	1.24	1.49
O	19.78	20.39	O	49.80	52.20
Na	8.91	6.40	Na	1.66	1.21
Al	0.81	0.50	Al	4.59	2.85
Cl	16.28	7.58	Cl	7.80	3.69

Table 7: Particle diameter, drug entrapment efficiency (%) and mucoadhesion (%) properties of IPN hydrogel beads

Formulation Code	Particle diameter (µm)	Drug entrapment efficiency (%)	Mucoadhesion (%)	
			(pH1.2)	(pH6.8)
F1	1413±136	65.12±2.95	-	-
F2	2060±106	86.70±1.75	18%	20%
F3	1457±129	96.65±1.41	12%	26%
F4	1235±76	99.74±1.87	10%	50%

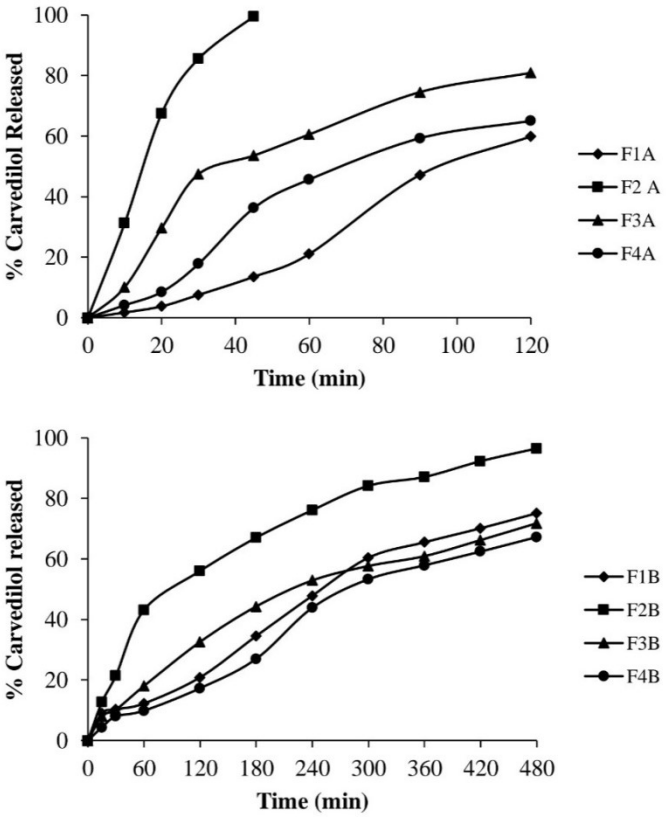

Figure 13: In vitro carvedilol release profiles in HCl solution (pH 1.2) (top) and phosphate buffer solution (pH 6.8) (bottom)

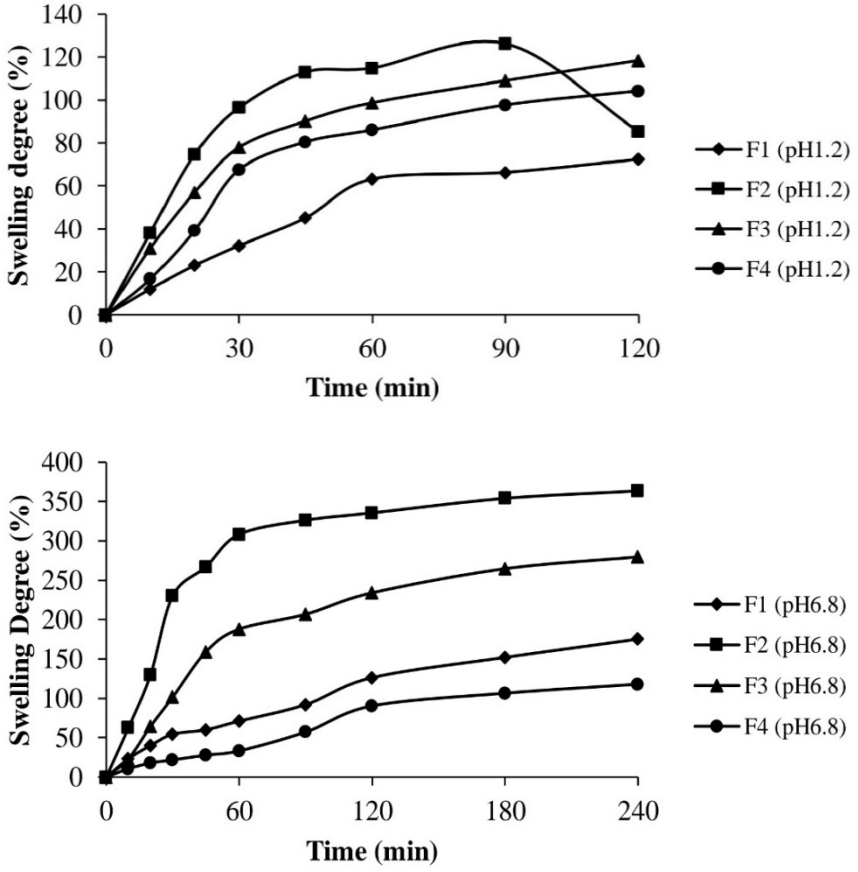

Figure 14: Swelling kinetics of IPN hydrogel beads in HCl solution (pH1.2) (top) and phosphate buffer solution (pH6.8) (bottom)

Table 8: In-vitro drug release data

Formulation code	HCl solution (pH1.2)			Phosphate buffer solution (pH6.8)		
	Gel characteristic constant (k)	Diffusion exponent (*n*)	Correlation coefficient (r^2)	Gel characteristic constant (k)	Diffusion exponent (*n*)	Correlation coefficient (r^2)
F1	0.0004	1.489	0.993	0.0116	0.654	0.912
F2	0.0314	1.012	0.997	0.0199	0.704	0.974
F3	0.0125	0.994	0.914	0.0112	0.693	0.987
F4	0.0020	1.301	0.977	0.0041	0.827	0.975

Table 9: Peppas-Sahlin Model Modeling of Drug Release Data in Phosphate Solution (pH 6.8)

Formulation code	Phosphate buffer solution (pH6.8)		
	Relaxation-controlled transport (k_1)	Diffusion-controlled (k_2)	Correlation coefficient (r^2)
F1	0.1717	0.3989	0.9815
F2	0.1256	3.740	0.7771
F3	0.0676	2.136	0.9857
F4	0.1699	-0.1317	0.9825

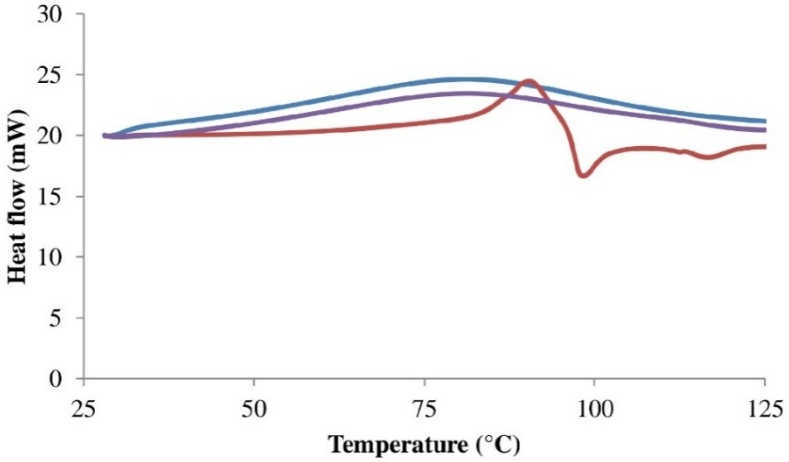

Figure 15: DSC patterns of pure drug, drug-loaded formulation (F4) and drug-free formulation

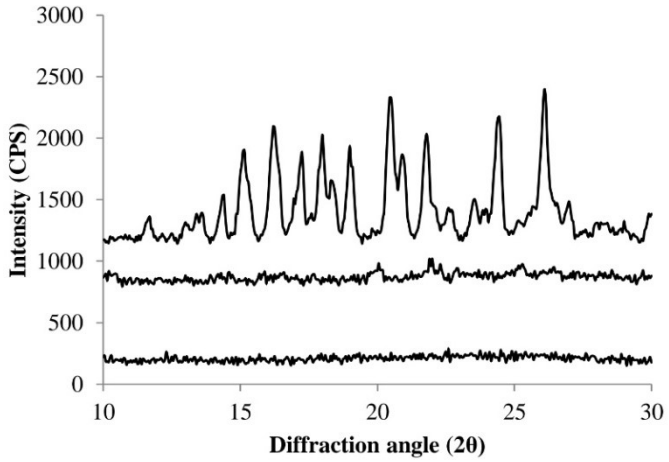

Figure 16: x-ray diffraction patterns of pure drug, drug-loaded formulation (F4) and drugfree formulation

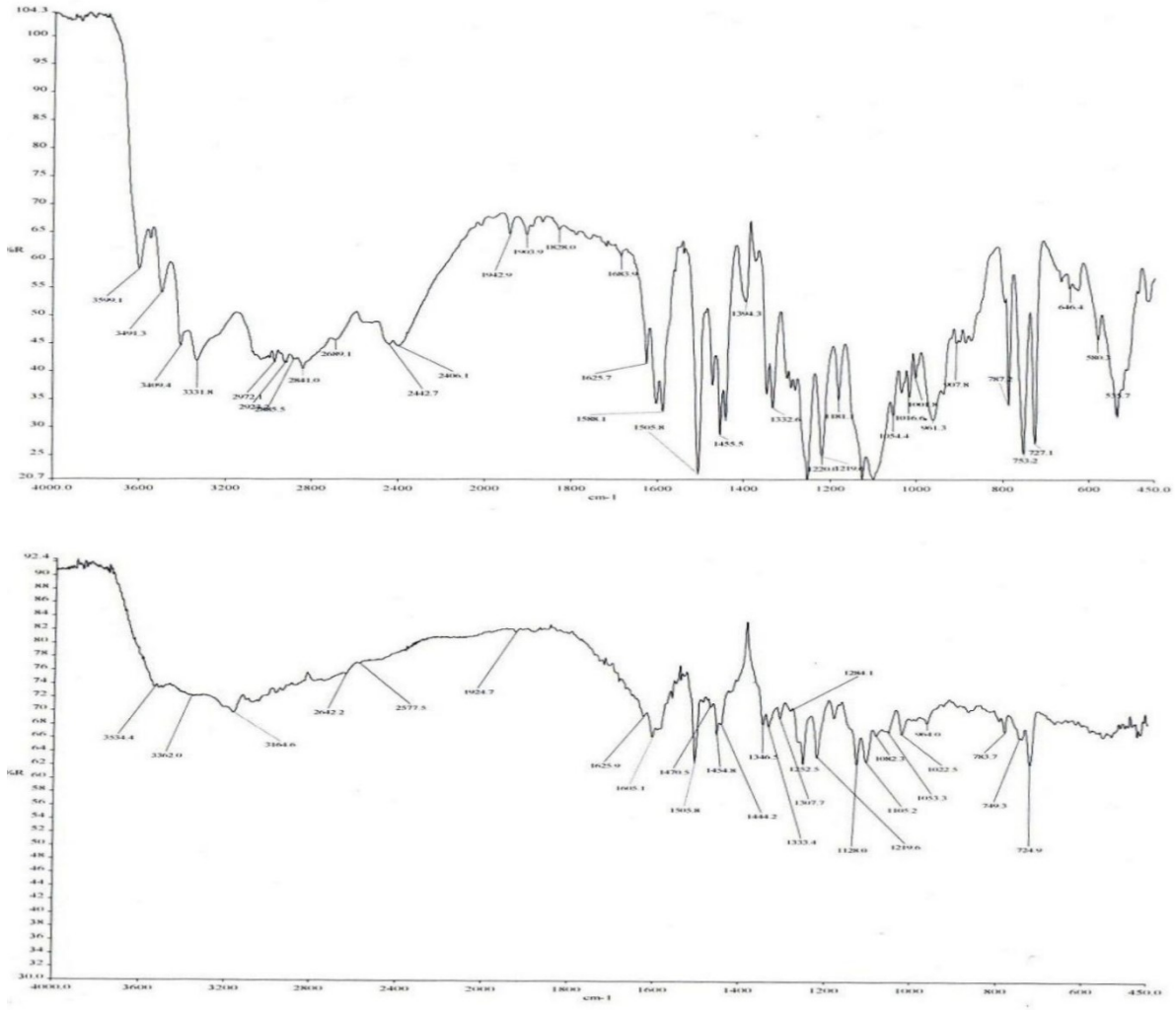

Figure 17: FTIR Spectra of Pure Drug (top) and Drug-loaded Hydrogel Beads (bottom)

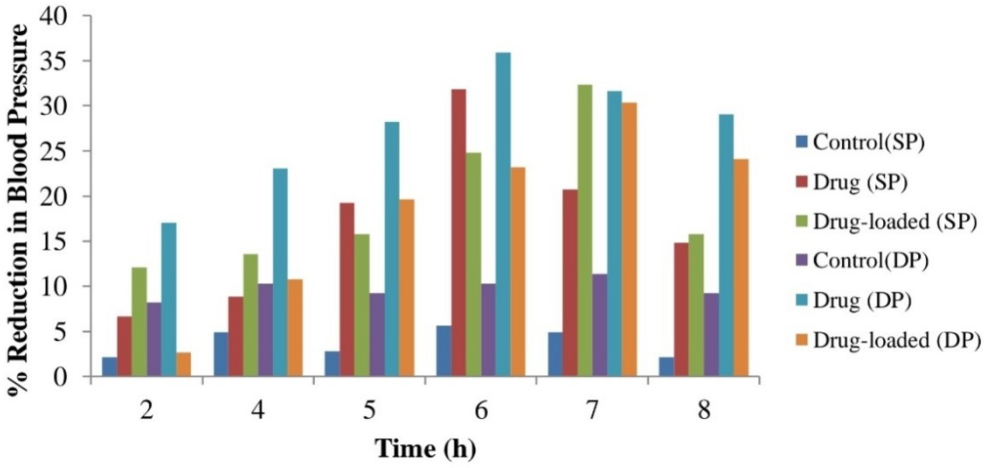

Figure 18: *In Vivo* Hypotensive Activity

CONCLUSION

The study revealed that CM-KG could not form spherical hydrogel beads alone in presence of $AlCl_3$ solution, but its aqueous solution from insoluble fibers. On contrary, CM-LBG solution did form spherical

hydrogel beads in presence of trivalent aluminum ions. However, CM-LBG hydrogel beads is failed to retain 100% drug, fed during preparation of the particles. The use of CM-KG in combination with CM-LBG form spherical hydrogel beads in presence of aluminum ions. Depending upon the aluminum ion concentration the IPN hydrogel beads were able to entrap almost 100% drug at 3% $AlCl_3$ concentration All the IPN beads provided sustained release of carvedilol over a period of 8 hrs in simulated intestinal fluid (pH 6.8). The slowest drug release profile was achieved with the formulation cross-linked with, 3% $AlCl_3$ solution. The formulation cross-linked with 1% and 2% $AlCl_3$ caused rapid diffusion of drugs in acidic media, however the formulation of cross-linked with 3% $AlCl_3$ suppressed the rapid diffusion of drug in acidic media. Following entrapment of carvedilol lost its crystallinity. No significant drug polymer interaction was evident. In vivo hypotensive activity study in animal model suggested that IPN formulation could reduce the blood pressure for a longer duration than that achieved with the pure drug suspension. Overall, the IPN exhibited controlled drug release property and potential in vivo hypotensive activity. A focus should be given to suppress the drug release in acidic media to make this novel IPN system an effective oral controlled released system. The future work further encompasses the pharmacokinetic study and toxicity study before clinical application of this IPN hydrogel bead system.

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Conflict of Interest: The authors declear that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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