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PREPARATION AND EVALUATION OF SUSTAINED RELEASE FORMULATION OF BOSENTAN

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ABSTRACT
The main objective of the present study was to prepare sustained release capsules of Bosentan which constitute an innovative dosage form that overcome the problems of large frequency of dosing. In the view of enhancing bioavailability, an attempt has been made to prepare sustained release capsules and to know the effect of different types of polymers. Polyelectrolyte complex was prepared by using cationic polymer like PVP and anionic polymer like Carbopol. Different PVP grades such as PVP k_{30} and k_{90} were used. The capsules were subjected to pre compression and evaluation parameters. The selected formulation was subjected to stability studies as per ICH guidelines. The effect of polymers loading in In- Vitro drug release and the mechanism of release was studied by different mathematical models. This can be adjusted by maintaining the concentration of the suitable polymers. Capsules showed no appreciable changes with respect to appearance, drug content and dissolution profiles on storage for 45 days at accelerated stability conditions. It was found that the Bosentan drug release depends on combination of two polymers (Carbopol and PVP k90 or k30) which modifies the release profile by controlling water penetration and leads to drug release. The objective was to achieve drug release at 24 hours. It was concluded that sustained release capsules of Bosentan can be successfully formulated by polyelectrolyte complex method with improved patient compliance. And also it can be concluded that among all the formulations Carbopol and PVP k90 or k30 combinations was considered as the optimised formulations in the present research work.

KEYWORDS: Bosentan, Hypertension, Sustained release, PVP, Carbopol, Polyelectrolyte Complex.

INTRODUCTION
Hypertension (HTN or HT), also known as high blood pressure (HBP), is a long term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure usually does not cause symptoms. Long term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss and chronic kidney disease.[1]

Capsules are solid dosage forms in which the medicaments enclosed within gelatine shells. The medicament may be a powder, a liquid, or a semisolid mass. Hard shell capsule sizes range from No.5, the smallest, to No.000, which is the largest.[2],[3]

Polyelectrolyte complexes are prepared by oppositely charged polymers which have electrostatics interaction in aqueous solution. Polyelectrolyte complexes (PECs) are an ideal tool for achieving the drug more soluble, stable, controlled and sustained release. Polymer compounds that contain net positive and negative charge at neutral pH called as polyelectrolyte.[4], [5]

Bosentan is an Endothelin receptor antagonist that belongs to a class of highly substituted pyrimidine derivatives, with no chiral centres.

It is poorly soluble in water (1.0 mg/100 mL) and in aqueous solutions at low pH (0.1 mg/100 mL at pH 1.1 and 4.0; 0.2 mg/100 mL at pH 5.0). Solubility increases at higher pH values (43 mg/100 mL at pH 7.5).

It is available as 62.5 mg and 125 mg film-coated tablets for oral administration, two times per day as immediate release formulation. Bosentan is a specific and competitive antagonist at Endothelin receptor types ETA and ETB. Bosentan has a slightly higher affinity for ETA receptors than for ETB receptors.

As immediate release preparations are taken two times a day, sustained release capsules for once daily will increase patient compliance.[6], [7]

Polyelectrolyte complexes were used for sustained delivery of Isosorbide Mononitrate[8], Bosentan Monohydrate[9] and Verapamil.[10]

Literature reveals sustained release formulations for Bosentan using microspheres.

➢ MATERIALS

- Drug Bosentan was purchased from Pure Chem Pvt. Ltd, Gujarat. $PVPk_{30}$, $PVPk_{90}$ and Carbopol were purchased from Essel Fine Chem, Mumbai. Magnesium Stearate was purchased from Sd Fine Chem Limited, Mumbai. Ethanol and HCl were purchased from Finar Limited, Ahmedabad.

Lactose was purchased from Finar Limited, Ahmedabad.

METHODOLOGY

- **Determination of λ_{max}**

20 mg of Bosentan was accurately weighed and transferred to 10 ml volumetric flask and dissolved in 5 ml of Ethanol; And volume is made upto 10ml with 0.1N HCL (=2000 µg/ml) (Stock I solution). 1 ml of this solution was diluted with 10 ml of 0.1N HCL (=200 µg/ml) using 10ml volumetric flask (Stock II solution) and scanned on a UV visible spectrophotometer between 190 to 390 nm. Same procedure is followed to check the λ_{max} in 7.4 pH buffers also. The maxima obtained in the graph were considered as λ_{max} for the pure drug solution in respective pH solutions.

- **Standard graph of Bosentan in 0.1N HCl**

As described above stock II solution of Bosentan was prepared (200 µg/ml). From this solution subsequent dilutions were made in 0.1N HCl in order to get 2, 4, 6, 8, 10, 12, 14, 16, 18 µg/ml. Absorbance of these solution were measured at λ_{max} 263nm using UV-Visible Spectrophotometer and standard cure was plotted.

- **Standard graph of Bosentan in Phosphate buffer PH 7.4**

Similarly stock II solution and further serial dilutions were prepared using 7.4 Buffer and absorbance's of the samples were measured at 269 nm.

- **Drug-Excipients Compatibility study**

Compatibility of the Drug with the excipients is determined by subjecting the physical mixture of the drug and the excipients of the formulation to infrared absorption spectral analysis (FTIR). Any changes in chemical composition of the drug after combining it with the excipients were investigated with I.R. spectral analysis.

Procedure

Weighed amount of drug or physical mixture of drug and polymer or polyelectrolyte complex of drug were mixed with 100 mg of potassium bromide (dried at 40-500^{0}C) the mixture was taken and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR Spectrophotometer.

- **Formulation Development**[11], [12]

As per the literature controlled release formulation of Bosentan 125mg can be administered once daily. Immediate release formulations were administered two times daily at dose of 62.5mg.[13]

Preparation of Polyelectrolyte Complex of Bosentan

Controlled release capsules of Bosentan were prepared by polyelectrolyte complex technique using variable concentrations of charged polymers like Carbopol, PVP k30, PVP k90 (Poly vinyl pyrrolidone).

a) Preparation of gels of both polymers and Mixing

Specified quantities of all materials were weighed and then active ingredient and polymers were dissolved in Ethanol separately. Carbopol gel was taken onto the magnetic stirrer. Drug solution was mixed with PVP gel solution and added to Carbopol gel drop by drop using syringe and stirred for one hour. Oppositely charged polymers react with each other. Complex will form which precipitated out from the solution.

In this step, Carboxylic group of Carbopol react with Amine group of PVP and form Amide group ($CONH_2$).

b) Filtering and Drying

After one hour stirring, filter the product using whatman filter paper, then dry in hot air oven at 50^{0}C. After drying, complex was weighed and % yield was calculated.

C) Screening the complex into pellets or granules

The complex was passed through a # 10 sieve to prepare the granules.

d) Sizing the granulation

The above granules were passed through sieve number # 20 to get smaller granules.

e) Adding lubricant and diluent blending

The granules were mixed with Lactose which was passed through 40 sieves which acts as diluent and mixed for 10 min. Magnesium Stearate was passed through #80 sieves and added to the above blend which acts as a lubricant and mixed for one min.

f) Filling into capsule

Granules were manually filled into body of capsule and fixed with cap.

Composition of Formulations

ABLE 1: Composition of Formulations F1_F6.

Composition (mg)	F1	F2	F3	F4	F5	F6
Bosentan	120	120	120	120	120	120
Carbopol	200	266.6	133.4	133.3	177.7	88.93
PVPk30	200	133.4	266.6	133.3	88.93	177.7
Lactose	24	24	24	22.4	22.4	22.4
Magnesium Stearate	6	6	6	6	6	6
Total	550	550	550	415	415	415

TABLE 2: Composition of Formulations F7_F12.

Composition (mg)	F7	F8	F9	F10	F11	F12
Bosentan	120	120	120	120	120	120
Carbopol	400	533.33	266.66	266.66	355.5	177.7
PVPk30	400	266.66	533.33	266.66	177.7	355.5
Lactose	24	24	24	20	20	20
Magnesium Stearate	6	6	6	6	6	6
Total	950	950	950	680	680	680

TABLE 3: Composition of Formulations F13_F21.

Composition (mg)	F13	F14	F15	F16	F17	F18	F19	F20	F21
Bosentan	120	120	120	120	120	120	120	120	120
Carbopol	133.33	177.7	88.8	200	266.6	133.3	266.6	355.5	177.7
PVPk90	133.33	88.8	177.7	200	133.3	266.6	266.6	177.7	355.5
Lactose	22.4	22.4	22.4	24	24	24	20	20	20
Magnesium Stearate	6	6	6	6	6	6	6	6	6
Total	415	415	415	550	550	550	680	680	680

ASSAY

The prepared powder blend equivalent to 10mg of Bosentan was weighed from each formula and transferred into 50ml volumetric flask and 50ml of ethanol was added to it and sonicated for 10 minutes. The undissolved matter was removed by filtration through what man no.1 filter paper. 1 ml of above solution is taken and diluted with 10 ml Phosphate buffer 7.4 pH.

The absorbance of the solution was checked by UV-spectrophotometer at 269 nm and amount of drug present was calculated using standard graph.

IN-VITRO DISSOLUTION PROFILE[14]

In-vitro dissolution testing is important in the development of solid dosage forms. It provides decisive information on formulation selection, the critical processing variables. For in-vitro evaluation of sustained release drug delivery systems, the ideal dissolution testing should closely mimic the in-vivo conditions with regard to pH, bacteria and types of enzymes, enzymatic activity, fluid volume and mixing intensity. Apparently, such dissolution specifications will be very difficult, if possible at all, to be standardized and validated. Nonetheless, several dissolution methodologies were reported in the literature for the testing of sustained release drug delivery systems.

Dissolution studies were carried out by using USP II (Basket) type dissolution test apparatus (basket method). Capsules were placed in a basket so that the capsule can be immersed completely in dissolution media and not float. In order to simulate the pH changes along the GI tract, two dissolution media with 0.1N HCL and 7.4pH buffer were sequentially used as per sequential pH change method. When performing experiments, the 0.1 N HCl (1.2 pH) was used for initial 2 h (since the average gastric emptying time is 2 h) then removed and the fresh pH 7.4 phosphate buffer was added. 900ml of the dissolution medium was used at each time. Rotation speed was 100 rpm and temperature was maintained at 37 ± 0.5 0C. 5 ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analysed at 269 nm by UV absorption spectroscopy.

Evaluation and characterisation of PEC

- **X- Ray Diffraction (XRD)[15], [16]**

X-ray diffraction (XRD) relies on the dual wave/particle nature of X-rays to obtain information about the structure of crystalline materials. A primary use of the technique is the identification and characterization of compounds based on their diffraction pattern.

The para focusing (or Bragg-Brentano) diffractometer is the most common geometry for diffraction instruments.

1. Bosentan
2. Carbopol
3. PVP k90

4. Polyelectrolyte Complex of Carbopol and PVP k90 without Bosentan
5. Polyelectrolyte Complex of Carbopol and PVP k90 with Bosentan were taken.

- **Fourier transforms infrared spectroscopy (FT-IR)[17]**

Fourier transforms infrared spectroscopy was performed on each of the samples to determine the structure of the organic compounds and to identify the presence of specific functional groups within a sample. The IR spectra of the pure Bosentan, Blank PEC, Pure PVP, Pure Carbopol and PEC contains Bosentan were recorded using KBr pellet techniques in FT-IR spectroscopy. In order to characterize the formation of PEC, overlapping spectra of blank PEC, overlapping spectra of pure PVP and pure Carbopol over Blank PEC were performed.

Overlapping spectra of Bosentan over PEC containing Bosentan was studied to check whether Bosentan has not reacted in the PEC formulation.

- **Differential scanning calorimetry (DSC)[18], [19]**

Calorimetry is the science of measuring heat changes from chemical reactions or physical events.

DSC is a key thermal analysis technique that measures heat changes that occur in the biomolecule during a controlled increase or decrease in temperature, making it possible to study materials in their native state.

Glass Transitions Temperature[20]

Glass transition temperature (Tg) is an important tool used to modify physical properties of drug and polymer molecules. Tg is shown by certain crystalline as well as amorphous solids. During the process of heating, some solid gets melted and if quench cooled, instead of crystallizing, gets converted to amorphous solid form appearing as that of glass. This glass formation is seen because of the dynamic arrest of molecules forming a disordered state at Tg. The molecules/atoms in glassy state are subject to only vibration and not translational and rotational motion. Mainly, at Tg, conversion of glassy (vitrified, amorphous) solid to rubbery (viscous liquid) takes place.

DSC done with below samples:
1. Polyelectrolyte Complex of Carbopol and PVP k90 without Bosentan
2. Polyelectrolyte Complex of Carbopol and PVP k90 with Bosentan.

- **Overlapping IR Spectra of Drug and Polymer**

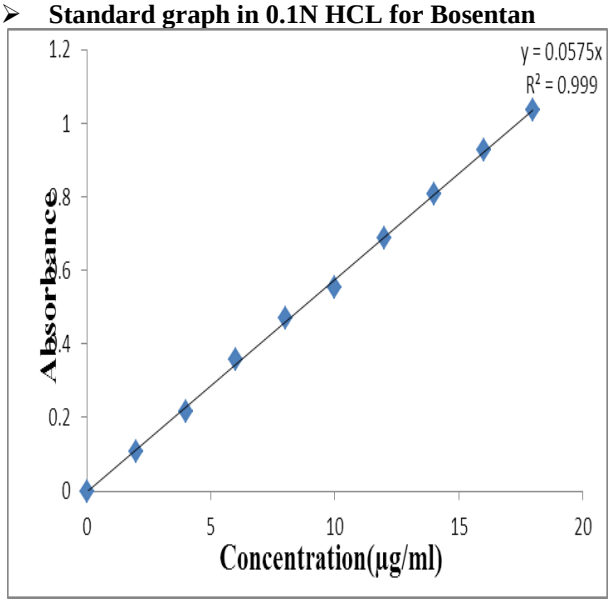
1. Overlapping of IR Spectrum of PVP k90 Over Complex without Bosentan**:**
2. Overlapping of IR Spectrum of PVP k90 and Carbopol over Complex without Bosentan:
3. Overlapping of IR Spectrum of Pure Bosentan over Complex containing Bosentan:

RESULT

- **Determination of λmax of Bosentan in 0.1N HCl and Phosphate Buffer pH 7.4**

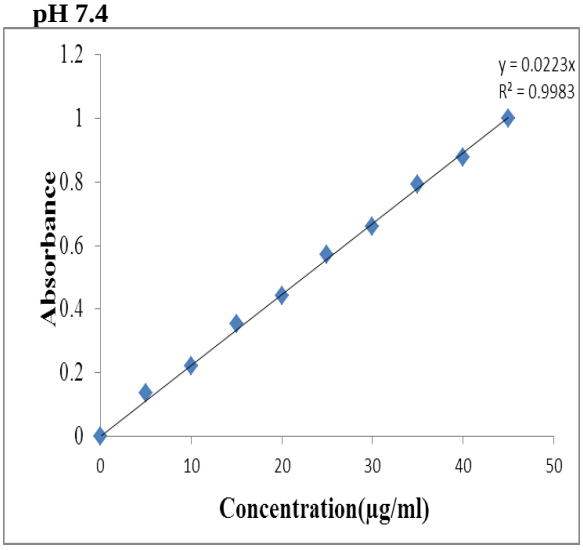
1. 263 nm in 0.1N HCl.
2. 269 nm in Phosphate buffer 7.4 pH.

- **Standard graph in 0.1N HCL for Bosentan**



"Fig. 1": Standard graph of Bosentan in 0.1N HCl.

- **Standard graph of Bosentan in phosphate buffer pH 7.4**



"Fig. 2": Standard graph of Bosentan in phosphate buffer PH 7.4.

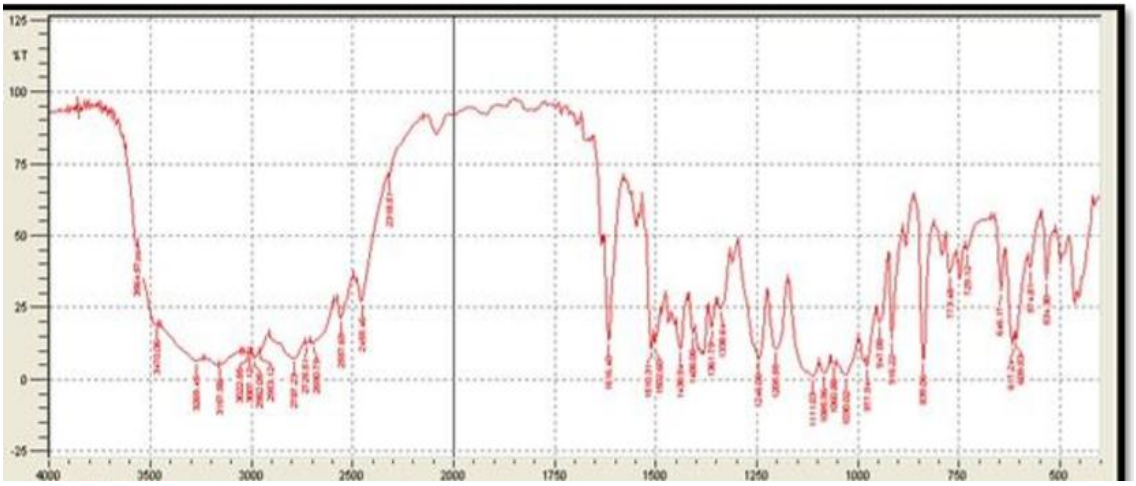
➢ **FTIR of pure drug Bosentan**

TABLE 4: FTIR of pure drug Bosentan.

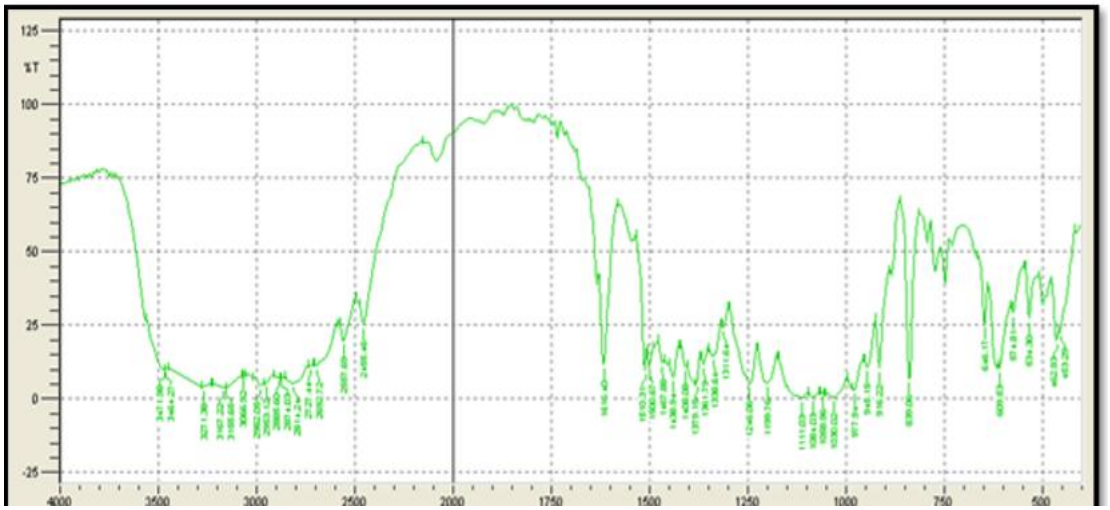
Functional Group	Actual Values(cm–1)	Values obtained(cm–1)
N-H group Stretching	3500-3310	3483.56
OH group Stretching	1350-1260	1342.5
C-H(CH3) Stretching	1465-1370	1450.52
C=C Stretching	1660	1666.25
C-H, Stretching, Aromatic	3300	3346.61
Presence of aromatic rings	860-640	798.56
C-O group Stretching	1150	1158.9
C-N Stretching	1380	1390.72
CH2 Stretching	1460	1464.88
C=C Bending	690-900	871.85

➢ **Melting Point Determination**

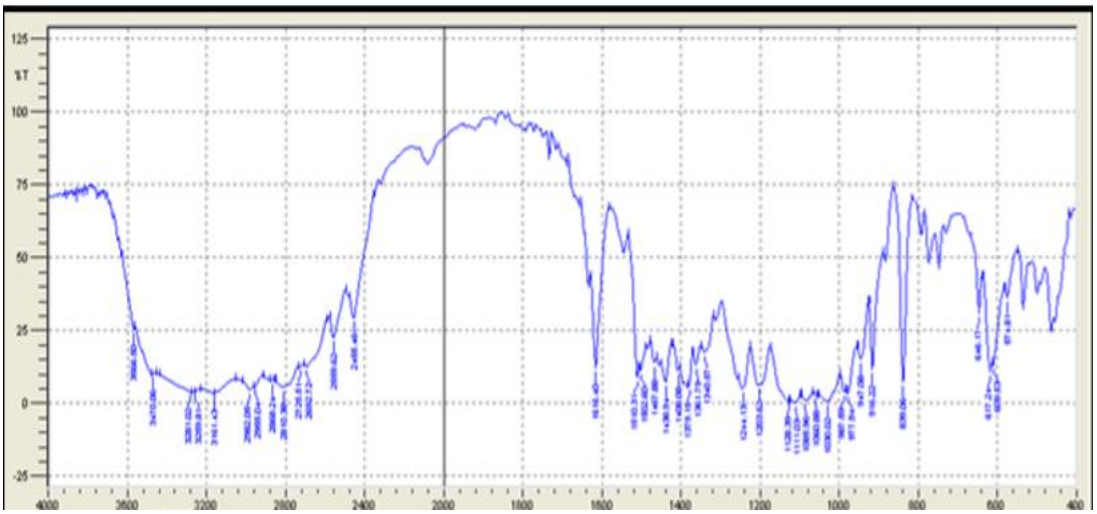
Melting point of **Bosentan** was determined by capillary method. The melting point of **Bosentan** was found to be **138^{0}C.**



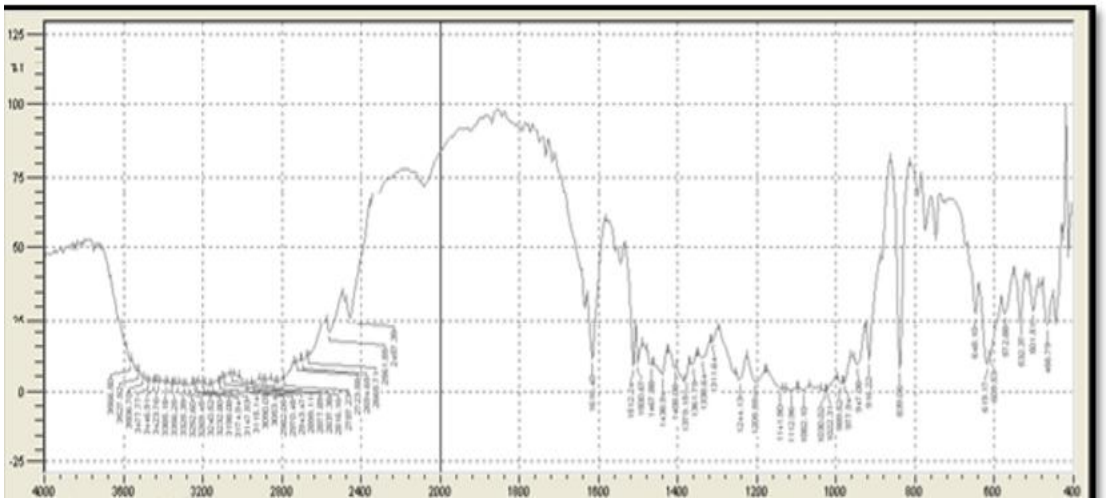
"Fig. 3": IR data of Pure Bosentan.



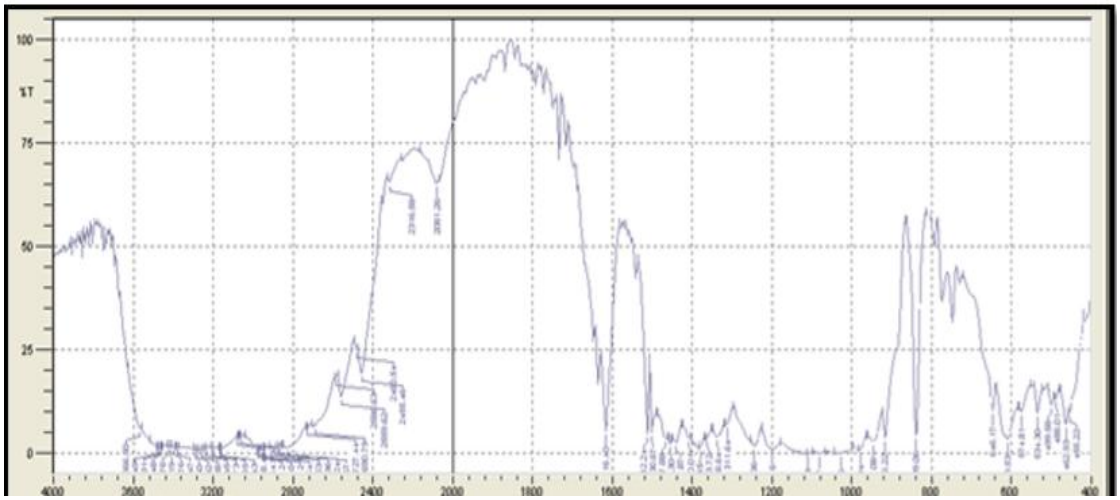
"Fig. 4": IR data of Bosentan and Carbopol.



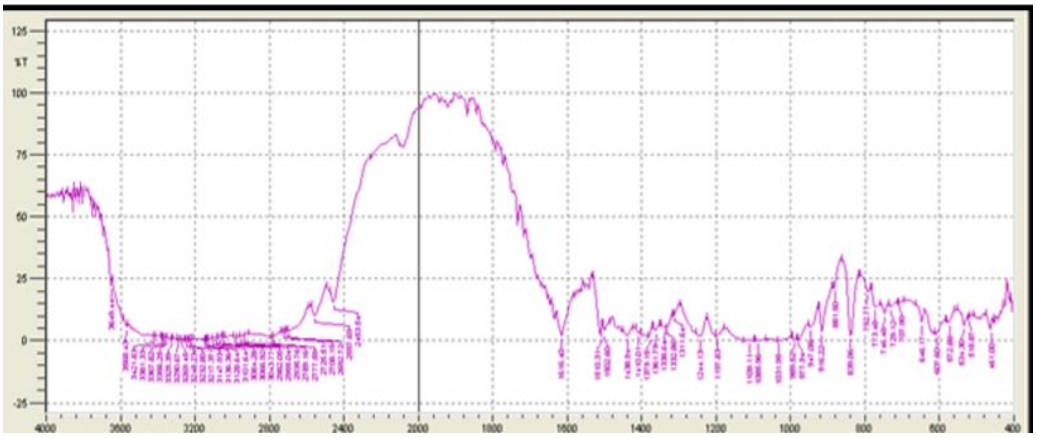
"Fig. 5": IR data for Bosentan and PVP k90.



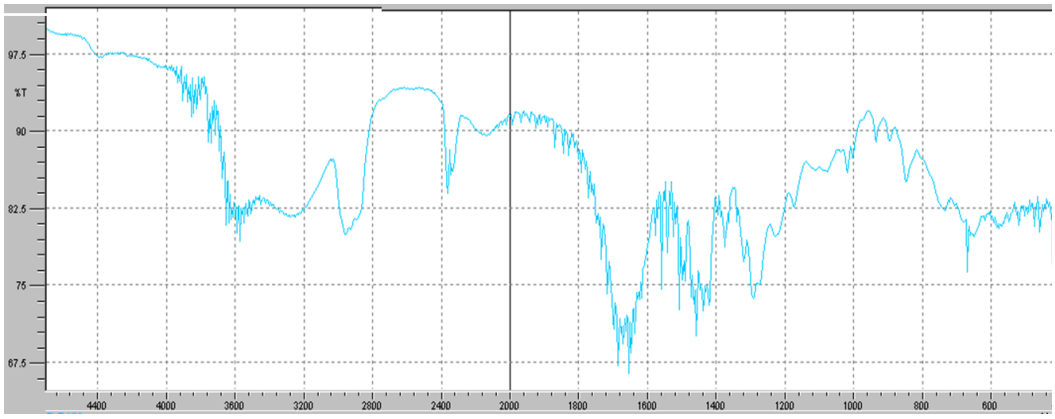
"Fig. 6": IR data for Bosentan and PVP k30.



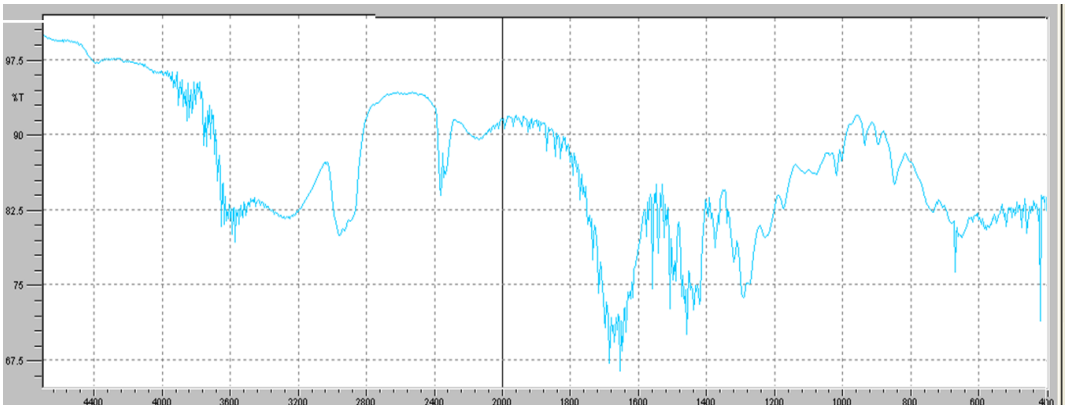
"Fig. 7": IR data for Polyelectrolyte Complex of Carbopol and PVP k90 with Bosentan.



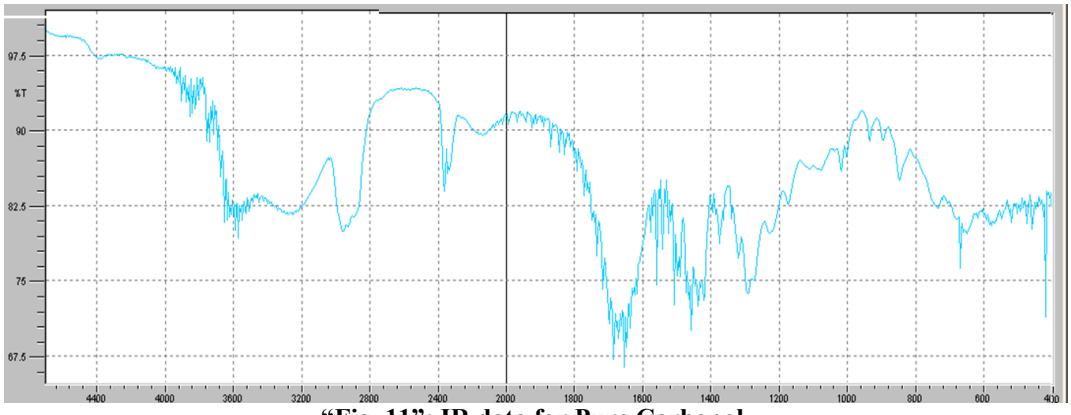
"Fig. 8": IR data for Polyelectrolyte complex of Carbopol and PVP k30 with Bosentan.



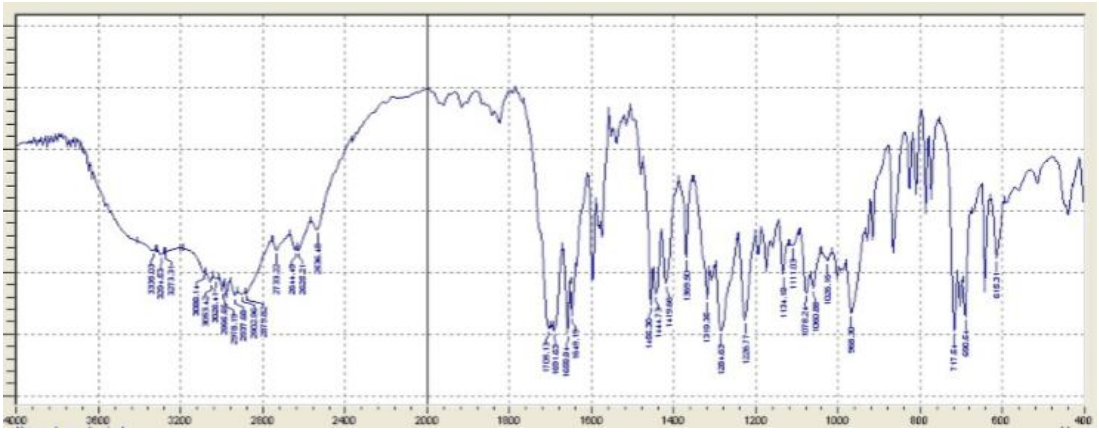
"Fig. 9": IR data for Pure PVP k90.



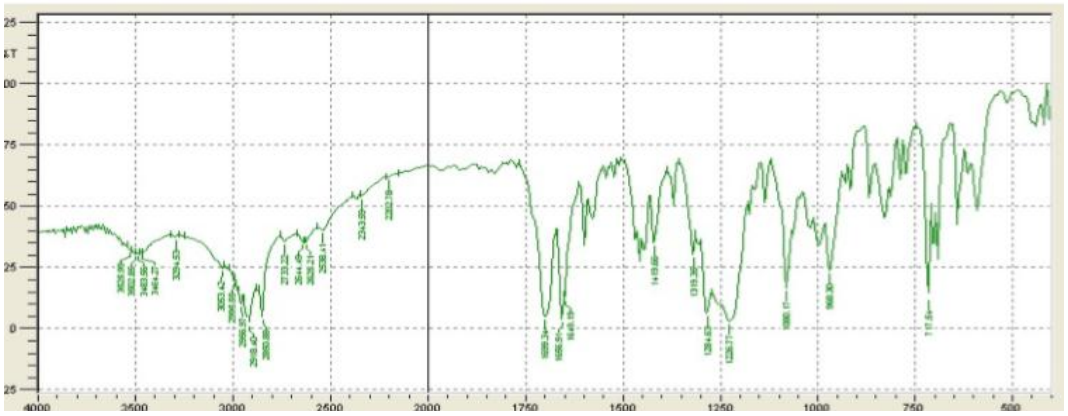
"Fig. 10": IR data for Pure PVP k30.



"Fig. 11": IR data for Pure Carbopol.



"Fig. 12": IR data for Polyelectrolyte Complex of Carbopol and PVP k90 Without Bosentan.



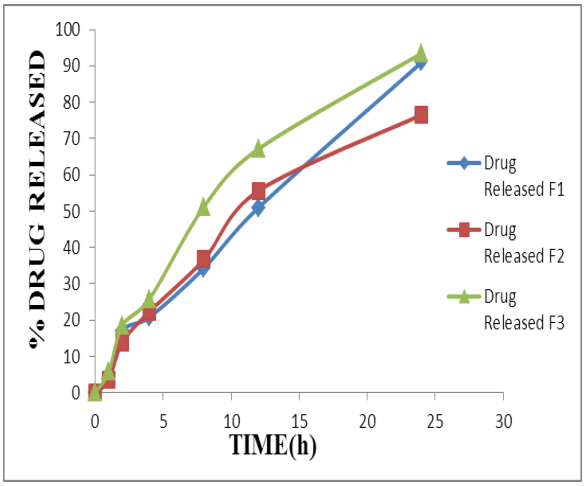
"Fig. 13": IR data for Polyelectrolyte Complex of Carbopol and PVP k30 Without Bosentan.

TABLE 5: Assay and Weight Variation test for Formulations F1_F10.

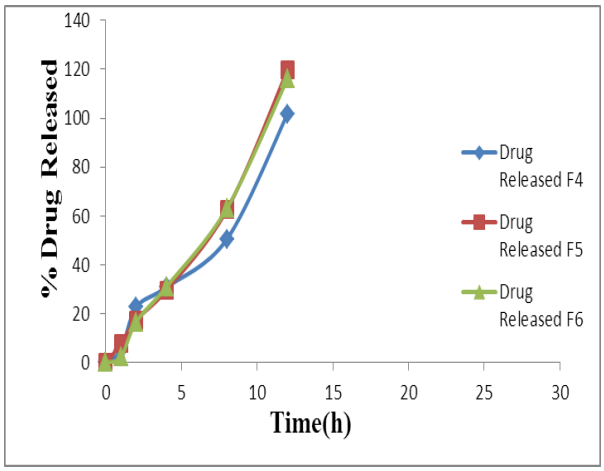
Formulation No.	Assay(Drug Content) mg (%)	Weight Variation(mg) n = 20
F1	117.861±0.349 (98.217%)	647±0.447
F2	117.234±0.406 (97.695%)	648±1.09
F3	118.8±0.175 (99%)	643±0.97
F4	117.528±0.084 (97.94%)	506±1.555
F5	119.92±0.124 (99.93%)	507±1.011
F6	117.094±0.111 (97.578%)	506±1.123
F7	118.188±0.234 (98.49%)	1054±0.978
F8	116.76±0.437 (97.3%)	1051±0.75
F9	118.6±0.613 (98.833%)	1053±0.711
F10	112.8±0.565 (94%)	778±1.37

TABLE 6: Assay and Weight Variation Test for Formulations F11_ F21.

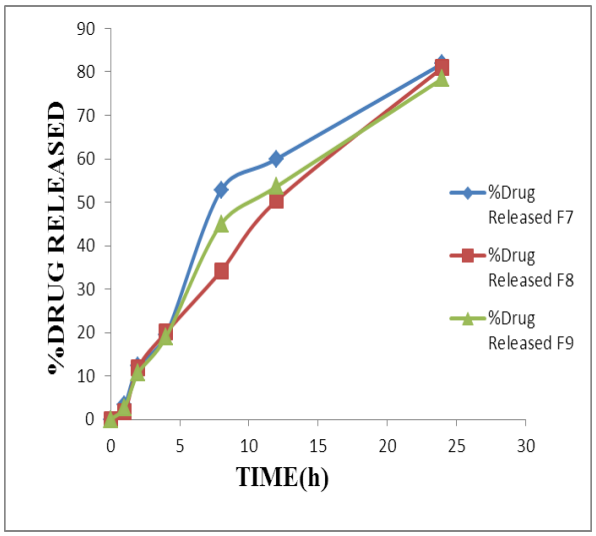
Formulation No.	Assay(Drug Content) mg (%)	Weight Variation(mg) n= 20
F11	117.098±0.250 (97.581%)	780±1.64
F12	114.999±0.481 (95.832%)	773±0.98
F13	118.717±0.702 (98.93%)	503±1.054
F14	118.838±0.270 (99.931%)	508±1.497
F15	115.708±0.812 (96.423%)	507±1.11
F16	113.342±0.255 (94.451%)	645±0.591
F17	119.558±0.139 (99.631%)	643±0.435
F18	116.323±0.705 (96.935%)	644±1.342
F19	118.111±0.361 (98.425%)	780±1.634
F20	115.312±0.893(96.093%)	777±1.76
F21	118.941±1.39 (99.117%)	774±1.505



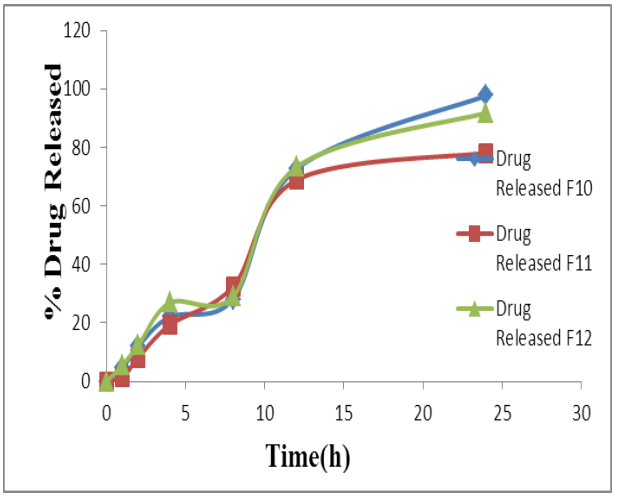
"Fig. 14": In-Vitro Dissolution Profile of Formulations F1, F2, F3.



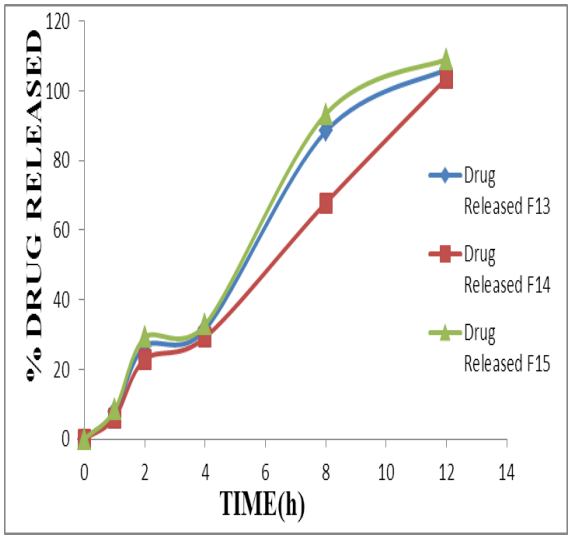
"Fig. 15": In-Vitro Dissolution Profile of Formulations F4, F5, F6.



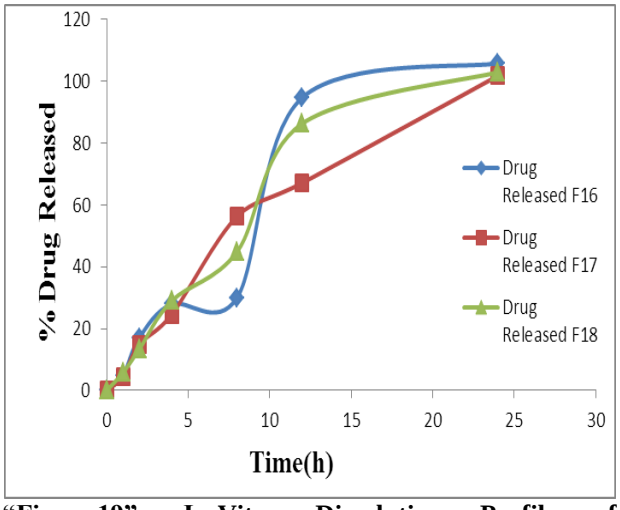
"Fig. 16": In-Vitro Dissolution Profile of Formulations F7, F8, F9.



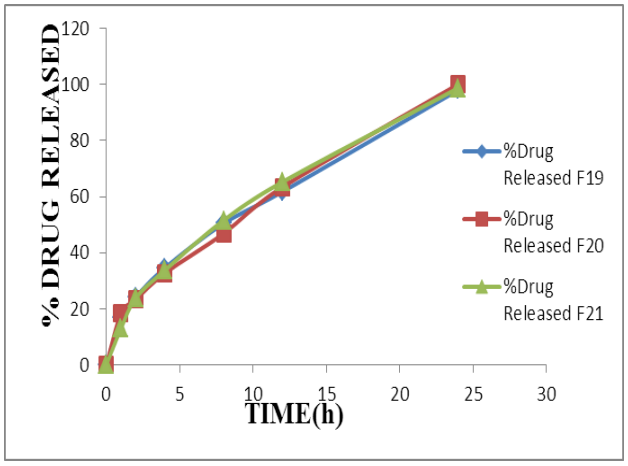
"Fig. 17": In-Vitro Dissolution Profile of Formulations F10, F11, F12.



"Fig. 18": In-Vitro Dissolution Profile of Formulations F13, F14, F15.

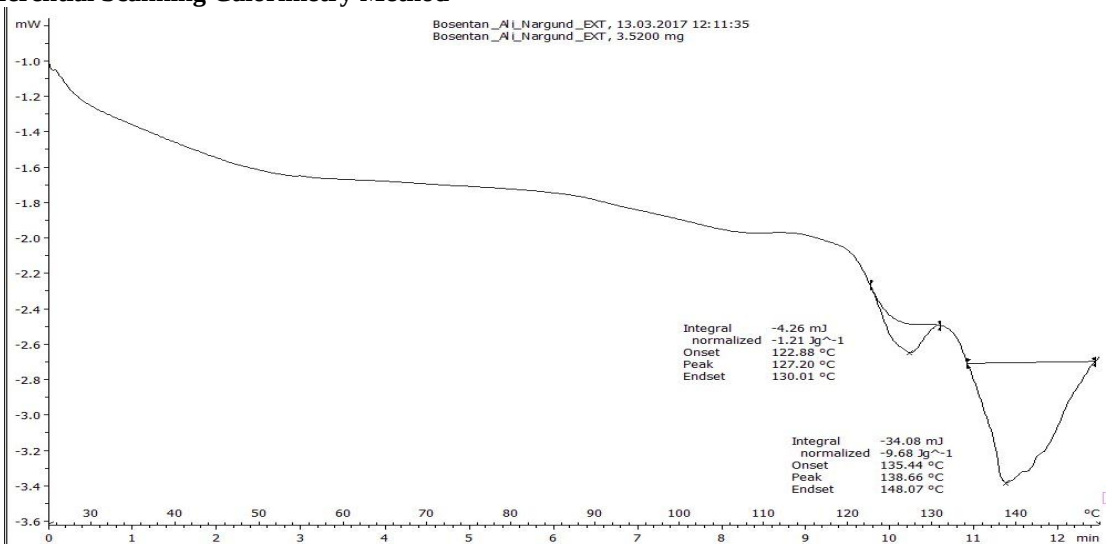


"Fig. 19": In-Vitro Dissolution Profile of Formulations F16, F17, F18.

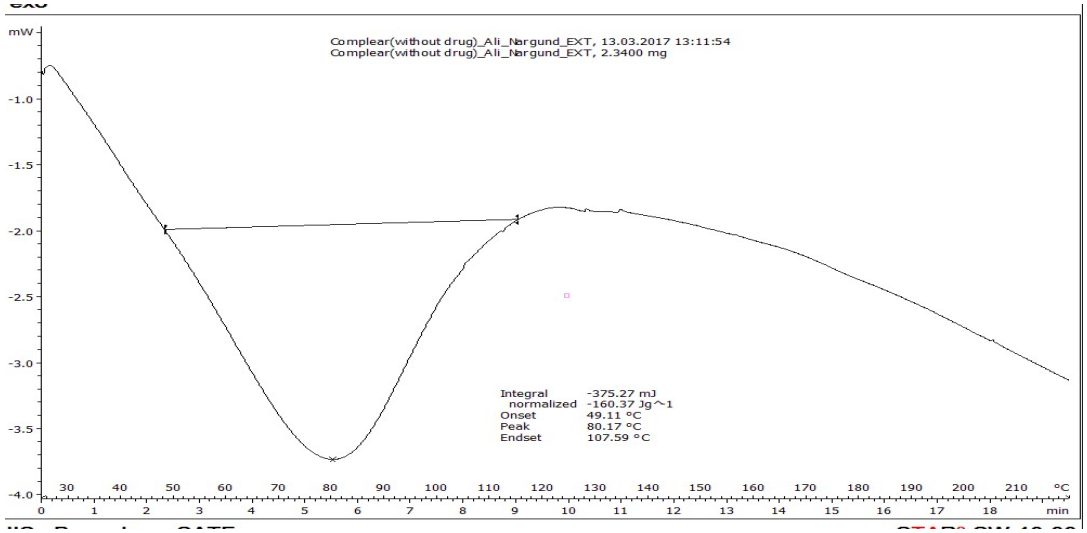


"Fig. 20": In-Vitro Dissolution Profile of Formulations F19, F20, F21.

- **Differential Scanning Calorimetry Method**

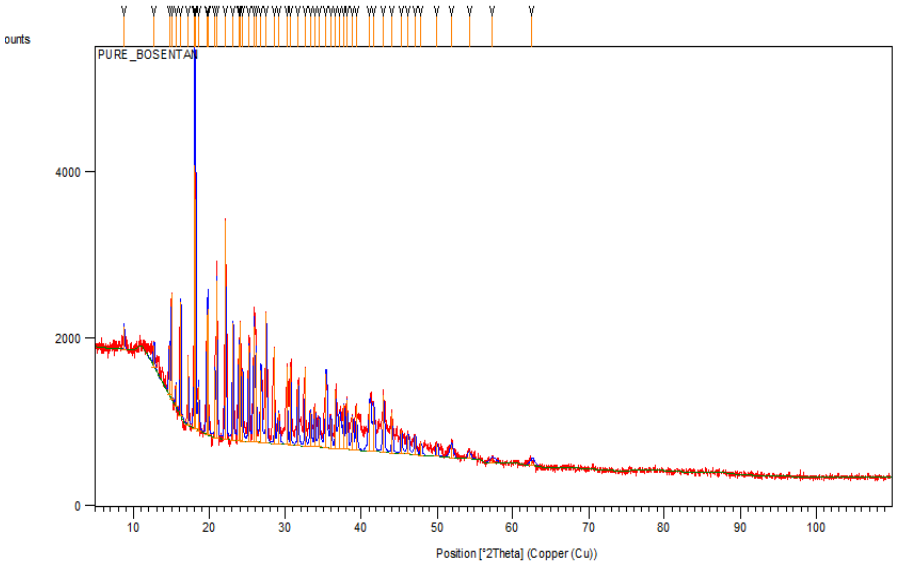


"Fig. 21": DSC for Complex of Carbopol and PVP k90 With Bosentan.

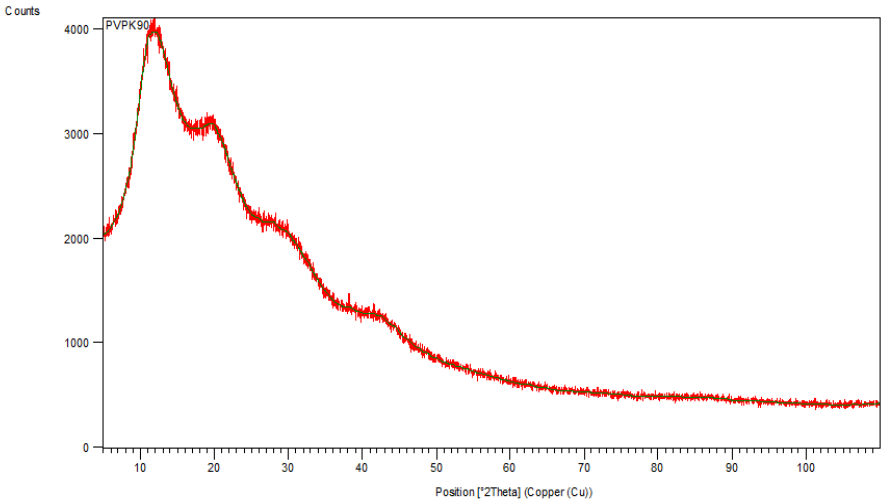


"Fig. 22": DSC for Complex of Carbopol and PVP k90 Without Bosentan.

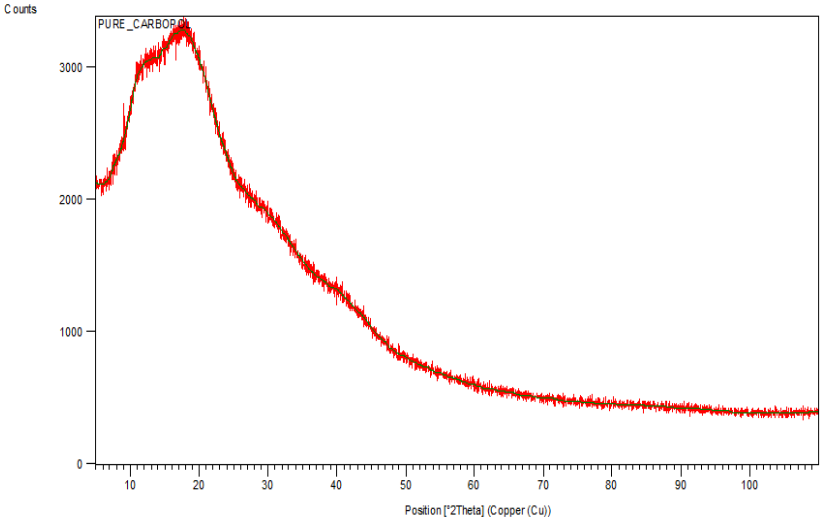
- **Powder X-Ray Diffraction Method**



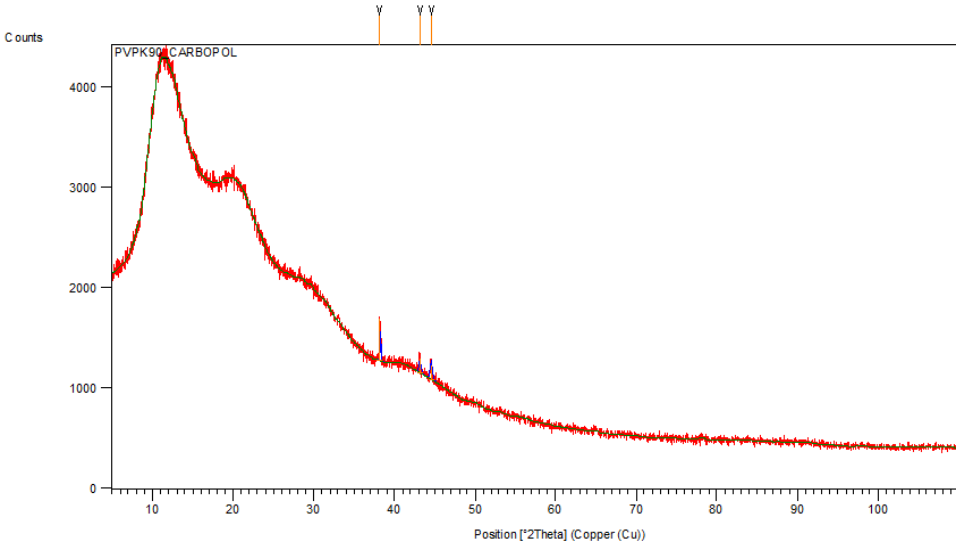
"Fig. 23": XRD for Pure Bosentan.



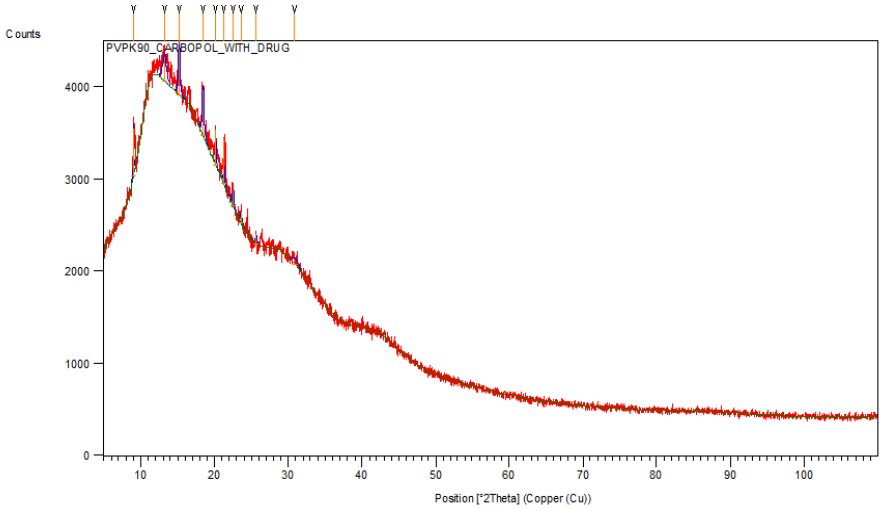
"Fig. 24": XRD for Pure PVP k90.



"Fig. 25": XRD for Pure Carbopol.



"Fig. 26": XRD for Complex of PVP k90 and Carbopol Without Bosentan.



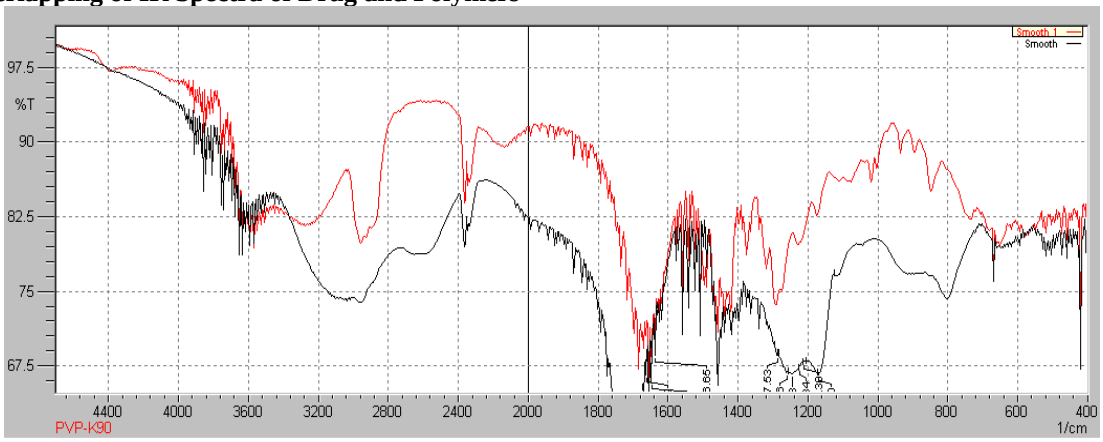
"Fig. 27": XRD for Complex of PVP k90 and Carbopol With Bosentan.

TABLE 7: Mathematical Modelling of Drug Release Profile of Formulations F21_F20_F19.

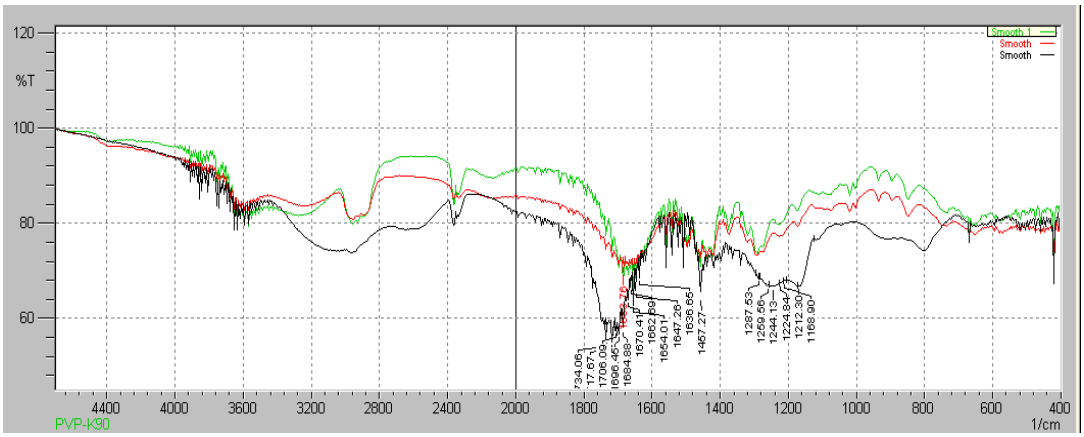
Formulation	Zero Order Plot	First Order Plot	Higuchi Plot	Korsmeyer's - Peppas Plot		Mechanism of Drug Release
	R^2	R^2	R^2	R^2	N	Non-Fickian
F21	*0.9664*	*0.9816*	*0.9852*	*0.9889*	*0.6573*	
Formulation	**Zero Order Plot**	**First Order Plot**	**Higuchi Plot**	**Korsmeyer's - Peppas Plot**		**Mechanism of Drug Release**
	R^2	R^2	R^2	R^2	N	Fickian Diffusion
F20	*0.8297*	*0.9544*	*0.9913*	*0.9779*	*0.4967*	

Formulation	Zero Order Plot	First Order Plot	Higuchi Plot	Korsmeyer's - Peppas Plot		Mechanism of Drug Release
	R^2	R^2	R^2	R^2	N	Non-Fickian
F19	*0.8403*	*0.995*	*0.9885*	*0.9974*	*0.5365*	

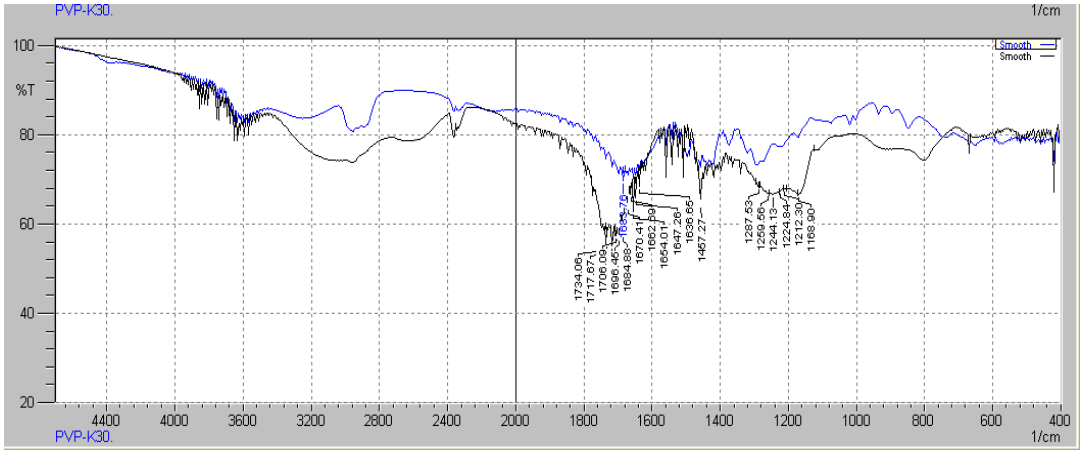
➢ **Overlapping of IR Spectra of Drug and Polymers**



"Fig. 28": Overlapping of IR Spectrum of PVP k90 Over Complex without Bosentan.



"Fig. 29": Overlapping of IR Spectrum of PVP k90 and Carbopol over Complex without Bosentan.



"Fig. 30": Overlapping of IR Spectrum of Pure Bosentan Over Complex containing Bosentan.

DISCUSSION

In the present work, an attempt has been made to formulate sustained release capsules of Bosentan by preparing polyelectrolyte complex method using different polymers like Carbopol, PVP k30, PVP k90.

In the present study 21 formulations with variable concentration of polymer were prepared and evaluated for physicochemical parameters, in vitro release studies.

Identification of Bosentan was carried out by Infra-Red Absorption Spectrophotometry. Spectra obtained were compared with those spectra given in USP. The obtained IR spectra match with Bosentan spectra in USP, which indicates the purity of Bosentan.

FTIR techniques have been used here to study the physical and chemical interaction between drug and excipients used. In the present study, it has been observed that there is no chemical interaction between drug and the polymer used. It was observed that there

were no changes in the main peaks of Bosentan in FTIR spectra of mixture of drug and polymers, which indicated, there were no physical interactions because of some bond formation between drug and polymers. NH_2 groups of PVP and COOH groups of Carbopol are missing in Blank PEC and additionally peak due to $CONH_2$ was observed.
Wavenumber of COOH: 1740 cm^{-1}
Wavenumber of NH_2: 3350 cm^{-1}
Wavenumber of $CONH_2$: 3300 cm^{-1}

Overlapping of IR Spectra of Drug and Polymers
To conform that PEC has formed by interaction between NH2 group of PVP and COOH group of Carbopol overlapping spectra of IR was carried out. Overlapping spectra of PVP or Carbopol over Blank PEC indicated the new peak formation due to CONH2 because of interaction of peaks due to NH2 group of PVP and COOH group of Carbopol. When as were missed these Amino group of PVP and COOH group of Carbopol, this indicates that PEC has formed due to interaction between oppositely charged polymers.

X-ray diffract gram of a) **Pure Bosentan**, b) **Drug loaded complex** and c) **Unloaded complex** are presented in **"Fig. 23, 27, 26"** respectively. Bosentan drug has showed the characteristic intense diffraction patterns due to its crystalline nature. These peaks are not present in the Pure PVP or Pure Carbopol patterns indicate both are amorphous. The diffraction patterns of PEC containing drug again showed similar to pure drug diffraction but at a lesser intensity indication. The drug is dispersed at a molecular level in a crystalline nature through amorphous PEC formed by PVP and Carbopol. XRD indicates that drug is retained in crystalline nature after incorporated into PEC.

DSC thermo grams of **Blank Polyelectrolyte Complex and Drug Loaded Polyelectrolyte Complex** is showed **in "Fig. 22, 21"** respectively.

When PEC containing Bosentan subjected for DSC shown peaks at **138^0C** which is observed as melting point of Pure Bosentan indicating that drug was uniformly dispersed in PEC at the molecular level and drug has not interacted with polymers. Purity of the drug is maintained.

Formulation **F21** followed first order model (**R^2= 0.9816**) for kinetics of release. Mechanism of diffusion was found to be Non-Fickian Diffusion type. **(n=0.6573).**

Formulation **F20** the model fits for Higuchi diffusion and the mechanism of diffusion was found to be Fickian Diffusion type as **n** value was **0.4967**. Formulation followed first order model (**R^2= 0.9544**) for kinetics of release.

Formulation **19** followed first order model (**R^2= 0.995**) for kinetics of release. Mechanism of diffusion was found to be Non- Fickian Diffusion type as n value was **0.5365**.

All the 21 formulations of prepared capsule of Bosentan were subjected to In- Vitro release studies. These studies were carried out using USP dissolution apparatus type-II, in 0.1N HCl for 2 h and followed by using pH 7.4 Phosphate Buffers for 24 h as dissolution media. The results obtained from In-Vitro release studies were plotted as % drug release vs. time.

In-vitro release profiles of capsules during studies were found to have very good sustaining efficacy.

F1, F2, F3 were planned using 400 mg of PEC with 120 mg of drug with varying proportions of Carbopol: PVP k30 in 1:1, 2:1, 1:2 respectively. The result obtained were 90.985%, 76.52%, 93.457% at the end of 24 h which indicated the release was controlled and all drug was not release at 24 h.

Next trial were planned to increase the drug release at 24h by reducing the polymer amount to 267 mg with varying proportions of Carbopol: PVP in the ratio of 1:1, 2:1, 1:2 respectively. The results obtained 101.78%, 116.125%, 119.68% respectively at the ends of 12 h which indicated that amount of PEC incorporated was not sufficient to extend the release upto 24h.

To extend the release upto 24 h, further trials were taken by doubling the polymer amount for F1-F6 as F7-F12 the obtained result indicated that amount of polymer was too high so that all the drug was not release upto 24 h. Only range of 78-97.6% of drug release was obtained.

As the required % of drug release was not achieved using PVP k30 in combination with Carbopol in PEC Complex, PVP k90, a higher viscosity grade was planned for complex formation.

F13, F14, F15 were formulated using PVP k90 polymer 266.66 mg of polymer complex with 120 mg of drug similar to F4, F5, F6 using PVPk30. Here also the all the drug release was observed upto 12h. The results indicated in a similar way with PVP k30.

F16, F17, F18 were planned by increase the polymer amount to 400 mg for 120 mg of the drug. All the drug released at 24h but the release at 12h was comparatively more.

So further F19, F20 and F21 were formulated using 533 mg of polymer complex with 120 mg of drug where % drug release was 98.11, 100.172, 98.793 at 24 h respectively.

As the observed release was gradual increased these formulations compared to F16, F17, F18, these set of formulations were considered to optimum formulations.

In all the trials using 1:1, 2:1, 1:2 ratio of Carbopol to PVP, it was observed that 2:1 ratio drug release was less compared 1:1 and 1:2, which could be due to higher viscosity of Carbopol compared to PVP.

Comparing the results of dissolutions of PVP k30 with PVP k90 (F4, F5, F6 versus F13, F14, F15) or (F1, F2, F3 versus F16, F17, F18) or (F10, F11, F12 versus F19, F20, F21), not much change in the drug release was observed. So with change in the viscosity grade of PVP, not much change in the drug release was observed. Instead more drug release was observed in case of F19, F20 and F21.

CONCLUSION

- Drug estimation by U.V spectroscopy was developed. Bosentan showed linearity between 2- 18 µg/ml in 0.1N HCl at 263nm and 5- 45 µg/ml in Phosphate buffer 7.4 pH at 269 nm.
- The drug content was uniform in all the formulations of capsules prepared.
- IR spectroscopic studies indicated that the drug is compatible with all the excipients.
- The total quantities of polymers influence the release of drug from the formulation. As the polymer quantity was increased, the drug release rates were found to be decreased.
- Formulations **F19, F20** and **F21** shown comparatively good release than the other formulations and the drug release upto 24h.
- Release of drug from formulation is decreased when Carbopol is used compared to PVP because of more viscosity.
- Pellets prepared by polyelectrolyte complex method containing drug had a slab shape with a smooth surface.
- All the formulations showed angle of repose within the range of **22.9^0** to **29.98^0**. Results indicating that they are having good flow properties.
- The % drug content was found to be **94%** to **99.93%.**
- Short-term stability studies indicated no appreciable changes in the drug content and in vitro drug release rates of formulation F19, F20 and F21.
- Controlled release of Bosentan can be prepared using Carbopol and PVP k90 for the controlled release of Bosentan capsules.
- The release of drug was influenced by the viscosity grades of polymer like PVP k30 and PVP k90. As the viscosity of polymer is increased, the release of drug decreased.
- All the formulations were remained in integrity but formulations **F4**, **F5**, **F6**, **F13**, **F14** and **F15** showed drug release within 12 hrs. Other formulations were showed drug release at the end of 24 hrs.
- Based on the encouraging results, the sustained release capsules of Bosentan can be considered suitable for clinical use in the treatment of Hypertension, where a quick onset of action for a dosage form is desirable along with the convenience of administration.
- All the formulations showed Disintegration Time within the range of **71.36** to **218.88** second.
- Hence formulations **F19**, **F20** and **F21** are considered as best formulations based on In-Vitro drug release.
- Sustained release of Bosentan capsules can be prepared using Carbopol and PVP for the sustained release of Bosentan capsules.

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