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Formulation and invitro evaluation of gastro retentive based micro beads of valsartan by ionic gelation technique

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ABSTRACT

The aim of the present work was to prepare and evaluate floating microbeads of Valsartan. The use of natural polymers in dosage form design has received considerable attention, especially from the viewpoint of safety. Among the polymers, Low Methoxy Pectin (LMP), and copolymer Locust bean gum (LBG), Tamarind kernel Powder (TKP) and Gum Ghatti (GG). Microbeads formulation is based upon the interaction between the polymer (LMP) and cross linking agent. LMP is an anionic polymer, which can be easily cross linked with $CaCl_2$. Microbeads were formulated in different ratio using LMP:LBG, LMP:TKP, LMP:GG as retarding agents and 10% of Calcium chloride ($CaCl_2$), as cross linking agents by employing Ionic Gelation Technique. The complexation between Ca^{+2} ions with LMP along with natural copolymer leads to retard the release of the drug. Floating time and floating lag time, drug Entrapment efficiency and *in-vitro* dissolution studies were also carried out. Formulation (PTa) containing (LMP 3%+TKP3%) was found to give a maximum entrapment efficiency of 96.68% and an optimum drug release was 95.1% in eight hours (in pH 1.2 media). The *invitro* data was explored with zero order, first order, Higuchi and Korsmeyer equations. The release kinetics was found to be governed by the type and content of the polymer (LMP) and copolymer. Formulation (PTa) shows zero order release kinetics with higher (r^2) values. By suitable modulation could be developed floating microbeads for this type of drug. Hence, Floating microbeads of Valsartan can be developed by using low methoxy polymer along with the natural polymers.

KEY WORDS: Microbeads, Gelation Technique, Entrapment Efficiency, Higuchi and Korsmeyer

INTRODUCTION

Floating drug delivery systems (FDDS) are good novel drug delivery systems which retain in the gastric pH for a prolonged period of time without affecting the gastric emptying rate. The approaches for prolongation of gastric residence time are floating drug delivery systems, low density systems, raft systems incorporating alginate gels, bio adhesive/muco adhesive system, super porous hydro gels, magnetic systems. Floating system was first developed by Davis (1968), these are the low density systems that have sufficient buoyancy to float and remain in stomach for prolonged period of time. The development of oral drug delivery systems for a specific drug involves the drug optimization of the dosage form and characteristic of gastro intestinal physiology. The floating microspheres not only prolongs the gastric retention time but also controls the space in the stomach by maintaining the delivery system positioned at a steady site and there by properly delivering the drug . The floating microspheres enhance bioavailability and improve pharmacokinetic and pharmacodynamics profiles of the drugs by retaining the drug reserve in stomach and to release the drug in controlled manner so as to achieve zero order release kinetics for a prolonged period of time. Gastro retentive drug delivery systems(GRDDS) extending the absorption phase of drugs which show a limited and narrow absorption window at the upper part of gastro intestine tract or

drugs in the gastro duodenum and reduces wastage of drug and improves solubility of drugs that are less soluble in a high pH environment. Microspheres of biodegradable and non biodegradable-polymers are designed for sustained release depending upon the final application.

MATERIALS AND METHOD

Valsartan was gift sample from Hetero drugs pvt. ltd, Hyderabad. Low methoxy pectin used as polymer was gift sample from Krishna Pectins Pvt. Ltd, India. Locust bean gum and Tamarind kernel powder used as copolymers were gift samples from R.A chem. Pvt Ltd, Hyderabad. Another copolymer Gumghatti was gift sample from Krystal Colloids Pvt Ltd, Mumbai. Sodium bicarbonate &Glacial Acetic Acid used as Gas generating mixture, Calcium chloride used as cross linking agent were gift samples from SD Fine Chemicals Pvt Ltd, Mumbai. All the chemicals and reagents used were of analytical or pharmacopoeial grade.

FORMULATION OF FLOATING MICROBEADS

PREPARATION OF VALSARTAN LOADED MICROBEADS

Valsartan loaded microsphere formulations were prepared by using Low methoxy pectin (LMP) as polymer and Locust Bean Gum (LBG), Tamarind kernel Powder (TKP) and Gum Ghatti (GG) as copolymer employing Orifice Ionic Gelation technique. This method involves the use of the syringe with a needle. The size of microbeads formed was dependent on the size of needle used and viscosity of the pectinate solution. The interaction between Pectin and Calcium chloride was used to prepare Calcium Pectinate microbeads. The active ingredient, Valsartan (40mg) was dispersed in the LMP along with of copolymer i.e, LBG, TKP, GG. To the solution 220mg of Sodium bi carbonate was added. The formulations are coded as shown in Table given below. This solution was mixed thoroughly with a stirrer to form viscous dispersion. The resulting foam solution was then added manually drop wise into calcium chloride (10% w/v) solution through a syringe with a needle of size no 23G. The added droplets were retained in calcium chloride solution for 15 minutes to complete the curing reaction and to produce rigid microbeads. The microbeads were collected by decantation and then washed thoroughly with distilled water and dried at 45^{o}C for 4 hours.

Drug + Polymer mixture

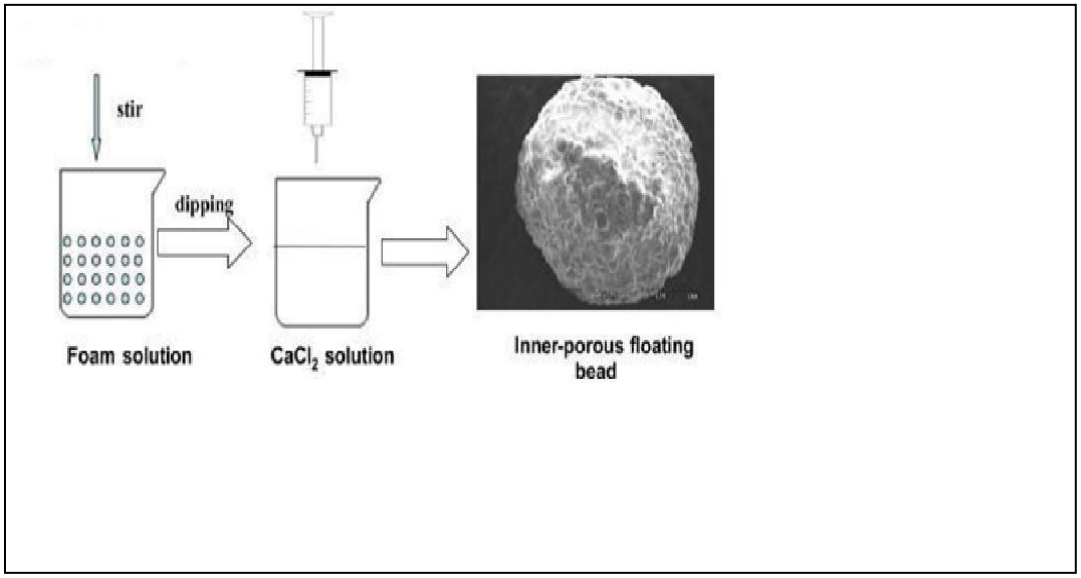


Fig 1: Preparation of gastroretentive microbeads of valsartan

Table 1: Formulation of gastroretentive microbeads of Valsartan

Ingredients (mg)	Pectin and LBG				Pectin and TKP			Pectin and Gum Ghatti			
	PLa	PLb	PLc	PLd	PTa	PTb	PTc	PGa	PGb	PGc	PGd
Valsartan	40	40	40	40	40	40	40	40	40	40	40
Low Methoxy Pectin	2.5%	3%	3.25%	3.5%	3%	1%	1%	3%	3%	3.25%	3.5%
Locust bean gum	1.5%	1%	0.75%	0.5%	_	_	_	_	_	_	_
Tamarind kernel powder	_	_	_	_	3%	2%	3%	_	_	_	_
Gum ghatti	_	_	_	_	_	_	_	3%	1%	.75%	.5%
$CaCl_2$ (10%) (ml)	100	100	100	100	100	100	100	100	100	100	100
Aceticacid (10%) (ml)	10	10	10	10	10	10	10	10	10	10	10
Sodium bicarbonate	220	220	220	220	220	220	220	220	220	220	220

EVALUATION OF GASTRORETENTIVE MICROBEADS

PRODUCTION YIELD (%)

The production yield of microbeads of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microbeads and % production yields were calculated as per the formula mentioned below.

$\% PY = W_O / W_T \times 100$

PY = Production Yield; W_O = Practical mass (microbeads); W_T = Theoretical mass (polymer + drug)

The production yield of microbeads prepared by ionic gelation technique was found to be 84.43 to 89.85 is shown in table 2. It was found that the production yield with polymer GG is low comparing with LBG and TKP due to its high viscosity decreasing its syringe ability and clogging of the orifice. Along with this agglomeration and sticking of the polymers to the walls of the beakers is also one of the reasons for the decrease of product yield with GG.

DRUG ENCAPSULATION EFFICIENCY

About 100 mg of microbeads was taken and triturated with HCl buffer pH 1.2 and transferred to 100 mL volumetric flask. The volume was made up to 100 ml and mixed well. The solution was then kept aside for 12 hours. It was then sonicated in ultrasonicator and then filtered through membrane filter (0.45µm) and estimated for drug content by measuring the absorbance at 250 nm. The drug entrapment efficiency was calculated using the formula.

Drug Encapsulation Efficiency =

$$\frac{\text{Estimated \% drug content}}{\text{Theoritical \% drug content}} \times 100$$

Drug Encapsulation Efficiency was found to be 96.25±0.23 to 99.75±0.18 as shown in the table 2. The drug entrapment efficiency from PTa formulation was found to be 99.75±0.18. It was found that the increasing the polymer concentration to 3% increased the entrapment efficiency due to high cross linkage between calcium and pectin ions.

PARTICLE SIZE ANALYSIS

Particle size of different batches of microbeads was determined by optical microscopy. The projected diameter of microbeads from each batch was determined using ocular micrometer and stage micrometer equipped with optical microscope. Analysis was carried out by observing the slide containing microbeads under the microscope. The average particle size of the microbeads was expressed as diameter. Mean particle size of all the formulations are in the range of 200±0.04 to 591.52±0.05. Microbeads formed with the polymers are in the preferred range of 1-1000 µm.

FLOW PROPERTY OF MICROBEADS

The flow properties of prepared microbeads were investigated by measuring the Angle of repose by using fixed funnel method. Depends upon these values, the flow properties of the microbeads can be assumed. The value of Angle of repose was calculated by using the following formula.

Angle of repose (θ) = $\tan^{-1}(H/r)$

H = cone height, r = radius of circular base formed by the microbeads on the ground. It was found to be all the values are within the range of 22º.20' to 26º.20' which shows all formulations exhibit good flow properties. The results are shown in the table 2.

SCANNING ELECTRON MICROSCOPY

Shape and surface morphology of microbeads was studied using scanning electron microscopy (SEM). The microbeads were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electron microscope (JEOL-JSM-6510, Japan). SEM photographs of formulation PTa was shown in the images, which indicated that the microbeads were discrete, uniform and spherical. The images were shown in the figures 10, 11.

BUOYANCY STUDIES

The time between the introduction of the FDDS into the medium and its buoyancy to the upper one third of the dissolution vessel (floating lag time) and the time for which the formulation constantly floated on the surface of the medium (floating duration) were measured simultaneously as a part of dissolution studies by visual observation. Here, the microbeads were placed in dis solution jar containing 900ml of 0.1N HCl buffer and 10ml of Tween80. The time required for the 2/3rd of the microbeads to rise to the surface and float was determined as floating lag time and total duration of time by which dosage form remain buoyant is called Total Floating Time (TFT). The best formulation (PTa) has shown less FLT of 6sec and TFT of more than 8hrs.It was shown in the figure 2, and the floating lag time of different formulations was given in the table 2.

IN VITRO **DRUG RELEASE STUDIES**

The *in vitro* release of drug from the prepared Valsartan microbeads was decided to carry out the dissolution in the dissolution medium of 1.2 Ph, 0.1 N HCl buffer for 8 hrs.

DISSOLUTION PARAMETERS

Apparatus	Electrolab ETC-11L, India (Rotating basket)
Dissolution medium	900 mL
RPM	75
Temperature	37 ± 0.5 ºC
Sample collection volume	5mL
Replacement	5mL of respective dissolution medium kept at 37 ± 0.5 ºC.
Sampling Interval	For every one hour up to 8hrs

Sample dilution: The samples were appropriately diluted and their absorbances were measured at 250 nm.

The affect of various polymers and its concentration were studied for the release profile of prepared microbeads of Valsartan. The release mainly dependent on theconcentratration and vis type of polymer, its cocity .The results are shown in Table 3, 4 and 5. The result indicates that PTa formulation, showed the amount of drug release 95.1±0.02 upto 8 hrs. Hence formulation (PTa) was chosen as a better formulation to retard the release for the drug Valsartan. The release was dependent on amount of polymer and copolymer. The amount of polymer (LMP) and copolymer (TKP) was selected for further retarding the release of the drug. The *in-vitro* release profile for all the prepared microbeads is shown in Figures 3, 4, and 5 and the results were given in the tables 3, 4, and 5 respectively. The formulation PLa shows 71.235±0.09 of drug release in 8 hrs where formulation PGd 42.98±0.09 release in 8hrs, there is a marked difference in the release profile of different formulations due to variation in the polymers and copolymers and their concentrations. The *in vitro* release profile of microbeads shows controlled release of Valsartan among the formulation, PTa showed slowest release rate of in zero order fashion.

FT-IR STUDIES

The Valsartan microbeads were subjected to FT-IR analysis by the following method, an approximately minimum quantity (less than 4mg) of sample was thoroughly blended with adequate quantity of IR grade KBr (less than 100mg) in mortar. The mix was then made into KBr pellets by hydraulic compression lever. The samples were analysed in a double beam

IR Spectrometer using KBr film as negative control (blank). The FT-IR studies were shown in the Figures 6, 7, 8, and 9.The interaction study between the drug (Valsartan) and Polymer (LBG, TKP) was evaluated using FT-IR spectrophotometer. By practical examination of Valsartan through FT-IR studies revealed characteristic absorption bands at particular frequency 3610 cm^{-1}(O-H), 900 cm^{-1} (C-H), 1198 cm^{-1} (C-N), 1142 cm^{-1} (C=O), 3313 cm^{-1}(aromatic N-H). The IR spectra of pure drug was compared with IR spectra of formulation (PTa),and combinations drug with polymers Pectin and TKP, drug with Pectin and LBG it was observed that all the characteristic peaks were observed in the combinations indicating no chemical interactions between drug and excipients.

RELEASE KINETICS

The analysis of drug release mechanism from a pharmaceutical dosage form is an important but complicated process and is practical evident in case of matrix systems. As a model independent approach, the dissolution data was fitted to four popular release models such as zero order, First order, Higuchi model and Korsmeyer –Peppas model (power law), which have been describe in the literature. The orders of drug release from prepared microbeads were known from zero order models and first order kinetics. The mechanisms of drug release were known by subjecting the dissolution data from the Higuchi and Korsmeyer –Peppas. The results are given in Table 6.

STABILITY STUDIES

Stability studies were conducted on Valsartan release from the microbeads formulation (M3C3) to assess their stability with respect to their physical appearance, drug content and drug release characteristics after storing at 40±2°C/75±5% RH for 3 months. The Stability study of the formulation (PTa) was performed for 3 months and the effect on the various parameters was studied and is reported below,

a) Physical Appearance
   Even after 3 months PTa formulation shows good physical appearance same as that of initially.
b) Drug Content
   After 3 months the formulation PTa under stability Study was assayed for the drug content and compared with the drug content of the initial formulation the results obtained were 99.51% initially and 96.68 %finally. The results were shown in Table 7.
c) *In vitro* Drug release Profile
   These release studies were performed after storage for 3 months 40±2ºc/75±5% RH, the results are shown in Table 8 and also represented in Figure 12. The *in vitro* release studies showed that there was no much difference in the drug release profiles indicating that the formulation (PTa) is stable.

STATISTICAL EVALUATION

In the development of new formulations of existing medicaments will be successful only if the drug release profiles of the prepared formulation are similar to the commercial formulation. This can be determined by subjecting the dissolution profiles of commercial and experimental products for model independent methods.

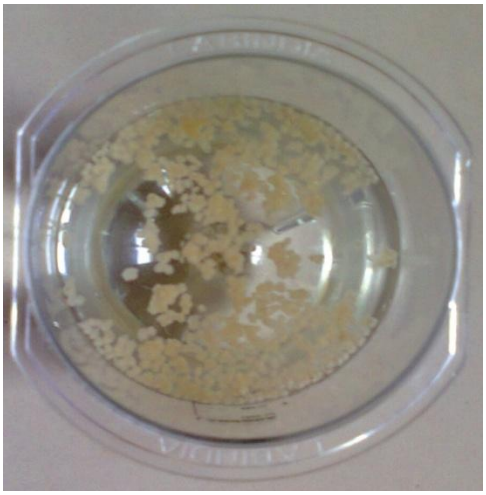
Table 2: Physico-chemical characterization of valsartan microbeads (Mean ± SD)

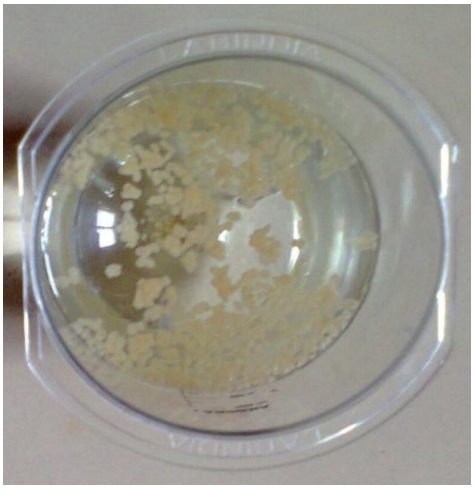
Formulation code	Angle of repose (θ)	Particle size(µm)	Percentage Yield (%)	Lag time (sec)	Total floating time (h)	Drug Content (%)
PLa	**22.38 ±0.01**	**200±0.04**	**88.93**	**22**	**>8**	**97.32±2.3**
PLb	**24.80±0.04**	**228.32±0.01**	**86.31**	**31**	**>8**	**98.56±2.0**
PLc	**24.60±0.04**	**238.17±0.02**	**84.43**	**32**	**>8**	**98.21±1.8**
PLd	**22.20 ±0.01**	**201.27±0.02**	**86.47**	**29**	**>8**	**95.91±1.5**
PTa	**32.08 ±0.08**	**200.32±0.03**	**89.85**	**6**	**>8**	**99.75±2.3**
PTb	**23.17 ±0.01**	**208.87±0.02**	**84.10**	**19**	**>8**	**98.25±1.8**

PTc	**24.34 ± 2.51**	**209.68±.04**	**86.68**	**16**	**>8**	**98.48±2.8**
PGa	**28 .46 ± 2.41**	**591.52±0.05**	**86.69**	**15**	**>8**	**97.69±2.4**
PGb	**31.49 ± 3.71**	**505.31±0.06**	**84.85**	**18**	**>8**	**97.35±1.7**
PGc	**24.60±0.01**	**528.24±0.08**	**84.60**	**16**	**>8**	**96.25±1.8**
PGd	**22.20 ±0.04**	**521.12±.07**	**84.65**	**24**	**>8**	**98.56±1.8**

Values are mean ± SD, n=3

(a)





(b) **(c)**

Fig 2: Images of Formulation PTa in the dissolution media of 0.1N HC pH 1.2

(a) Initially (b) After 3 hours (c) After 8 hours

Table 3: *In vitro* drug release data of formulations PL a to PL d (% CDR)

Values are mean ± SD, n=3

TIME (HRS)	PL a	PL b	PL c	PL d
0	0	0	0	0

1	3.611±0.02	4.29±0.51	29.58±0.15	36.35±0.21
2	4.08±0.06	7.24±0.23	37.51±0.02	38.24±0.02
3	4.71±0.15	7.73±0.12	38.846±0.12	50.15±0.12
4	6.155±0.07	8.225±0.07	40.07±0.07	55.38±0.15
5	13.631±0.09	12.32±0.23	41.19±0.23	57.59±0.1
6	15.26±0.13	13.73±0.08	41.97±0.08	63.65±0.18
7	15.57±0.15	14.03±0.01	48.27±0.01	69.51±0.03
8	20.675±0.22	25.13±0.03	55.41±0.03	71.235±0.09

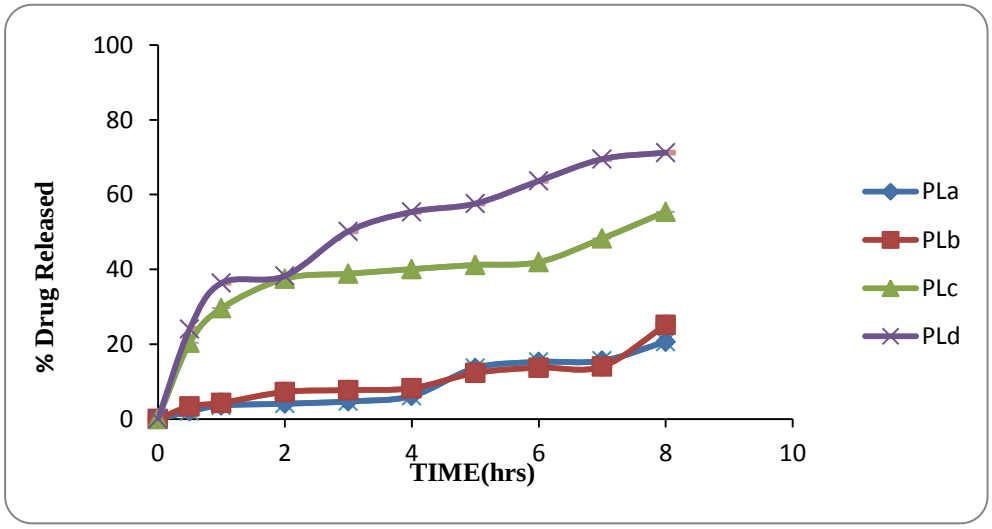


Fig 3: *In vitro* release profile of Formulation PL a, PL b, PL c, PL d

Table 4: *In vitro* drug release data of formulations PT a to PT c (%CDR)

Values are mean ± SD, n=3

TIME (HRS)	PT a	PT b	PT c
0	0	0	0
1	39.6±0.13	24.75±0.15	24.3±0.01
2	49.17±0.09	30.96±0.05	29.16±18
3	61.22±0.12	35.95±0.14	29.54±0.07
4	70.5±0.22	39.4±0.17	31.95±0.10
5	79.57±0.11	45.08±0.12	36.63±0.12
6	85.4±0.15	49.65±0.06	37.5±0.05
7	91.36±0.18	52.77±0.13	39.73±0.04
8	95.1±0..02	60.25±0.11	40.62±0.02

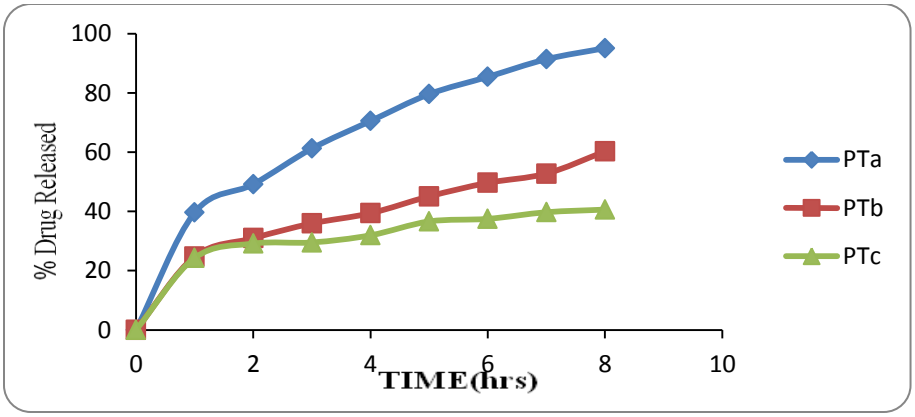


Figure 4: *In vitro* release profile of Formulation PT a, PT b, PT c

Table 5: *In vitro* drug release data of formulations PG a to PG d (%CDR)

TIME (HRS)	PG a	PG b	PG c	PG d
0	0	0	0	0
1	7.875±0.23	20.925±0.28	22.725±0.18	30.37±0.27
2	9.718±0.17	21.266±0.15	25.1±0.11	31.83±0.13
3	15.17±0.09	21.608±0.12	25.91±0.14	32.74±0.03
4	16.83±0.06	22.4±0.16	28.75±0.17	35.4±0.06
5	17.14±0.04	24.77±0.08	29.59±0.08	36.94±0.08
6	17.46±0.01	25.135±0.16	35.15±0.12	39.17±0.04
7	18.01±0.05	25.49±0.12	35.56±0.04	42.08±0.01
8	18.56±0.01	26.3±0.10	36.434±0.01	42.98±0.09

Values are mean ± SD, n=3

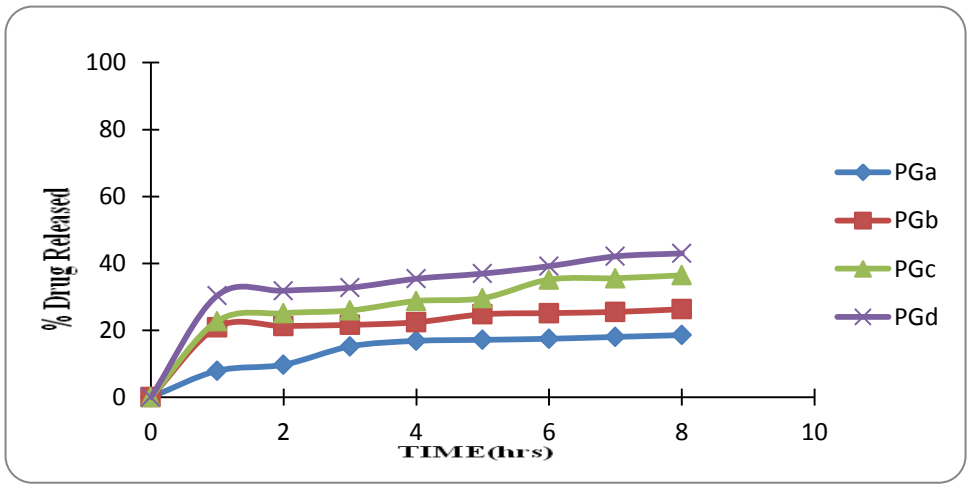


Fig 5: *In vitro* release profile of formulation PG a, PG b, PG c, PG d

FT-IR studies

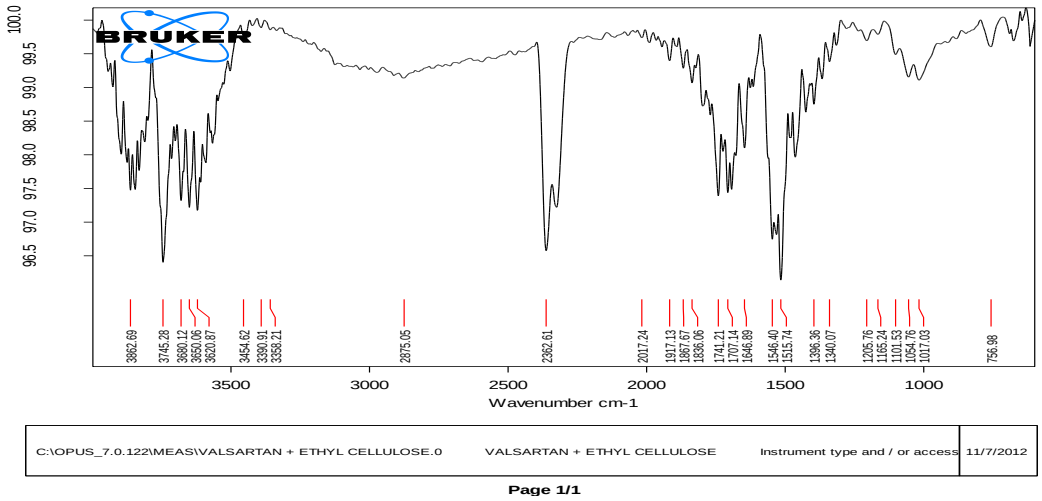


Fig 6: FT-IR spectrum of valsartan pure drug

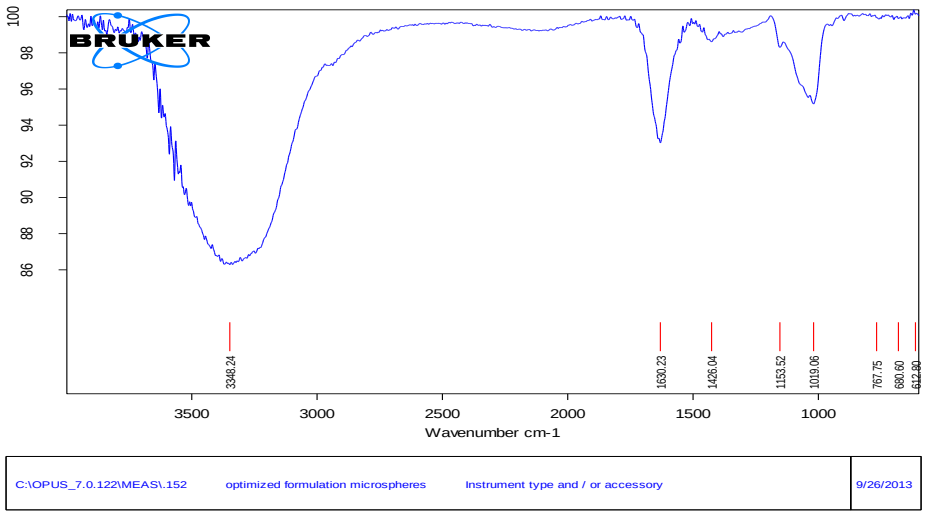


Fig 7: FT-IR spectrum of optimized formulation

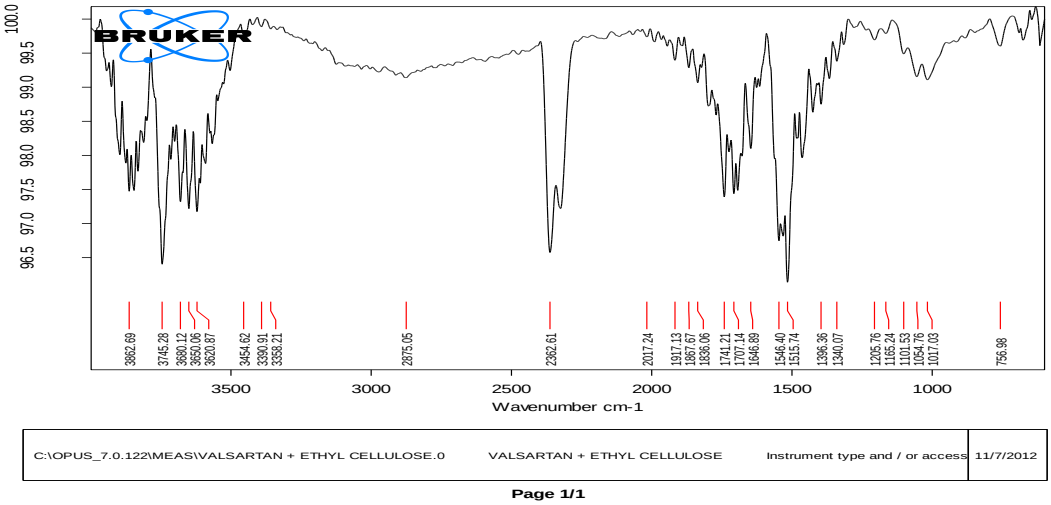


Fig 8: FT-IR spectrum of valsartan + Ethylcellulose

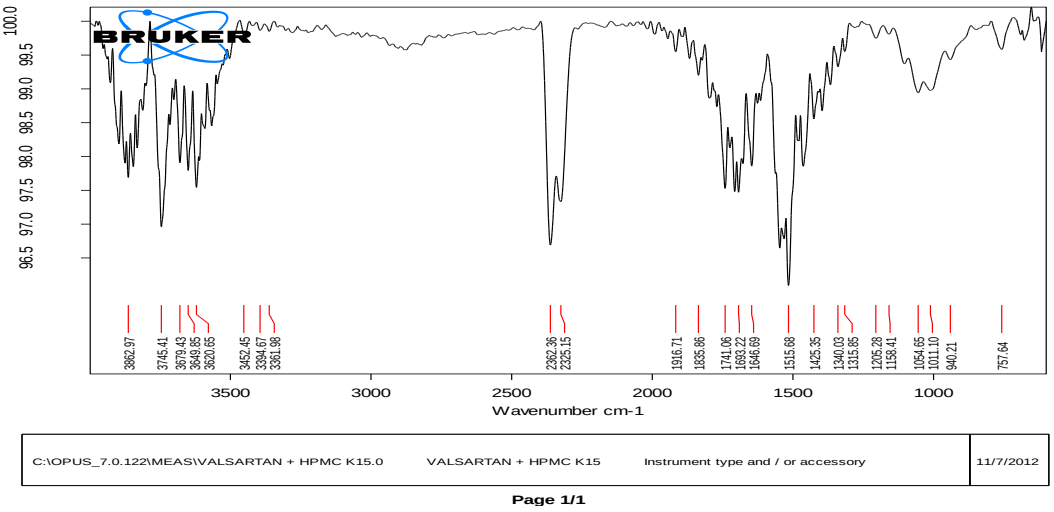


Fig 9: FT-IR spectrum of valsartan + HPMC K15

Table 6: Drug Release kinetics Data for Valsartan Gastroretentive Microbeads

Formulation Code	Zero order r^2	First order r^2	Higuchi r^2	Korsmeyer Peppas *n*	Korsmeyer Peppas K	D.E $_{5\%}$	MDT (Hrs)
PLa	0.938	0.981	0.961	0.912	0.407	74.14	6.18
PLb	0.985	0.943	0.962	0.717	0.595	74.42	10.13
PLc	0.947	0.971	0.958	0.242	1.473	72.61	11.29
PLd	0.953	0.94	0.964	0.352	1.527	71.40	7.08
PTa	0.995	0.971	0.996	0.949	1.579	67.86	16.14
PTb	0.994	0.969	0.979	0.914	1.372	70.06	12.50
PTc	0.964	0.982	0.971	0.950	1.377	69.83	13.24
PGa	0.876	0.963	0.946	0.437	0.909	70.95	8.35
PGb	0.908	0.956	0.961	0.119	1.3	70.76	15.92
PGc	0.935	0.989	0.988	0.240	1.331	67.68	16.14
PGd	0.948	0.963	0.942	0.173	1.458	74.61	10.29

Scanning electron microscopy analysis (SEM Pictures)

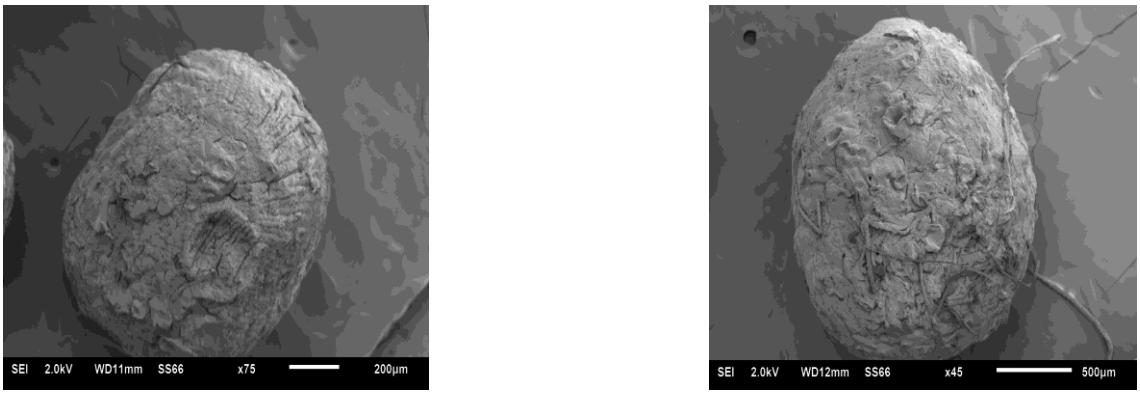
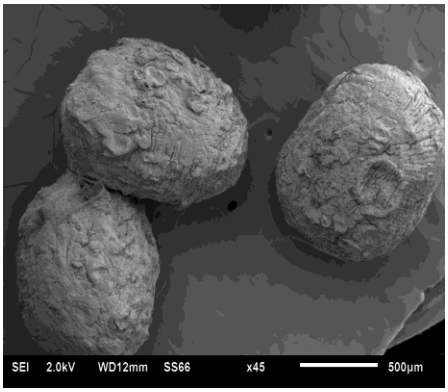


Fig 10: Surface morphology before placing in pH 1.2 dissolution media



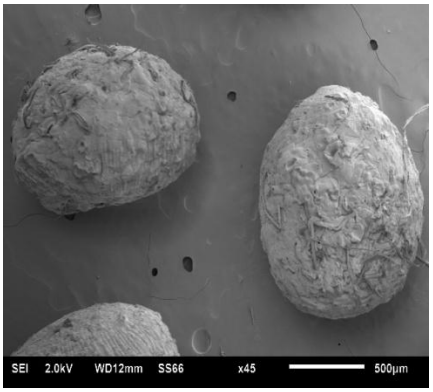


Fig 11: Surface morphology after placing in pH 1.2 dissolution media

Table 7: Drug content before and after 3 months storage

Formulation Code	% Drug Content	
	Initial	After 3 months
PTa	99.51	96.68

Table 8: Comparison of *In vitro* drug release of formulation initially and after 3 months storage

Formulation PTa	% Cumulative Drug Release	
Time (hrs)	Initial (1st day)	After 3 months
0	0	0
1	39.6±0.06	39.45±0.06
2	49.17±0.01	48.97±0.02
3	61.22±0.03	60.32±0.06
4	70.5±0.15	69.25±0.04
5	79.57±0.17	78.71±0.06
6	85.4±0.12	84.8±0.12
7	91.36±0.03	90.6±0.01
8	95.1±.012	94.9±0.05

Values are mean ± SD, n=3

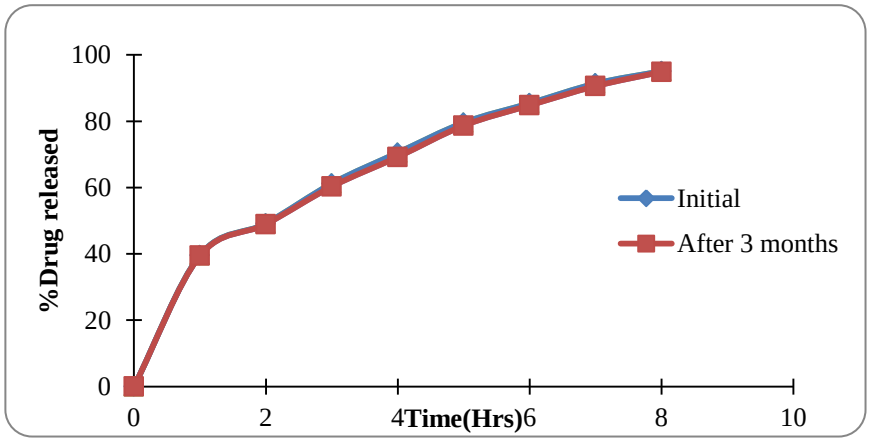


Fig 12: Comparison of *In vitro* drug release of formulation (PT a) initially and after 3 months storage

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