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RESEARCH ARTICLE

Verapamil Hydrochloride Systems Design for Pulsincap Drug Delivery: Development and Evaluation Studies

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ABSTRACT

Aim of the present work was to formulate and evaluate pulsatile drug delivery system to achieve time release of Verapamil HCl, based on pulsincap approach for the treatment of anti hypertensive drug. As fat production takes place in night time is more than day time. Pulsatile delivery system is capable of delivering drug when and where it required most. Time-delayed tablets, designed to release drug after a predictable lag time. The basic design consists of an insoluble hard gelatin capsule body filled with physical mixture of Verapamil HCl with HPMC and Guar gum, lactose as channeling agent and sealed with a Sodium alginate and xanthan gum plug. The Verapamil HCl pulsincaps were prepared by physical mixture method with lactose by varying drug to polymer ratio and evaluated for the micromeretic property, percentage yield, drug content, IR and *in vitro* release study. A hydrogel polymer Sodium alginate and Xanthan gum was used as plugs to maintain a suitable lag period. The *in vitro* release study were carried out using pH 1.2 buffer for a period of 2 h then 7.4 pH phosphate buffer for a period of 10 h. The cumulative % release for HPMC formulations were found to be in the range of 82.87% to 90.28% and for Guar gum were found to be75.28 to 98.08% at the end of 12 h. From the obtained result formulation GS3 showing 94.5% drug release at 12 h with 3h lag time was selected as an optimized formulation for designing pulsatile device. The programmable pulsatile release has been achieved from prepared formulation over a 12 h period, consistent with the demands of Pulsincap drug delivery.

KEY WORDS: Pulsatile drug delivery; Verapamil HCl; Xanthan gum; Guar gum; *In vitro* study.

INTRODUCTION:

Chronotherapeutics refer to a clinical practice of synchronizing drug delivery in a manner consistent with the body's circadian rhythm including disease states to produce maximum health benefit and minimum harm. A pulsatile dosage form, taken at bed time with a programmed start of drug release in the early morning hours, can prevent this. By timing drug administration, plasma peak is obtained, at an optimal time. Number of doses per day can be reduced. The chronotherapy of a medication may be accomplished by the judicious timing of conventionally formulated tablets and capsules. A pulsatile release profile is characterized by a time period of no release (lag time) followed by a rapid and complete release [1, 2]. Pulsincap is a novel drug delivery system capable of releasing its drug contents at either predetermined time or at specific site in the GI tract. The pulsincap system consist of a water- insoluble capsule body (exposing the body to formaldehyde vapour which may be produced by the addition of trioxymethylene tablets or potassium permanganate to formalin or any other method), Filled with the drug formulation and plugged with a swellable hydrogel at the open end. Upon contact with dissolution media or gastrointestinal fluid, the plug swells and comes out of the capsule after a lag time, followed by a rapid release of the contents. The lag time prior to the drug release can be controlled by the dimension and position of the plug [3-5]. Mastiholimath et al., [6] investigated on oral colon specific, pulsatile device of Theophylline consists of an insoluble hard gelatin capsule body, filled with Eudragit microcapsules of Theophylline and sealed with a hydrogel plug. The entire device was enteric coated to achieve colon-specific release. The Theophylline microcapsules prepared with EL-100 and ES-100 (1:2) by varying drug to polymer ratio showed uniform drug content and sustained drug release in upper GIT followed by drug dumping in colon. Sangalli et al., [7] prepared pulsatile release drug delivery system to target the drug in colon. The system is composed of a drug-containing core and a hydrophilic swellable polymeric coating capable of delaying drug release through slow interaction with aqueous fluids. They concluded that the different hydroxy propyl methylcellulose (HPMC) viscosity grades used retarded initial release and delivered drug to colon due to the capacity of hydrophilic layer. Abraham S et al., [8] formulated modified pulsincap drug delivery system of Diclofenac sodium using hydro gel polymers, HPMC, HPC, Sodium alginate and cellulose acetate phthalate, modified pulsincap that would ensure chronotherapeutics delivery of Diclofenac sodium in the colon for the relief of

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rheumatoid arthritis. They reported that the formulations investigated revealed suitable physic chemical properties and *in vitro* drug release. Verapamil Hydrochloride (VH) is a Calcium channel antagonist. It is a drug of choice in the treatment of angina pectoris and mild to moderate systemic hypertension. Dosage schedule of 3times daily, for prolonged antihypertensive therapy has been rated as quite cumbersome using conventional formulations. The short biological half life of 4-6 hrs and low dose 40-80 mg of VH coupled with the pharmacodynamic requirement of sustenance of blood pressure fall in hypertensive patients call for its once a day controlled release formulation. VH is a calcium ion influx inhibitor (slow channel blocker or calcium ion antagonist) which exerts its pharmacologic effects by modulating the influx of ionic calcium across the cell membrane of the arterial smooth muscle as well as in conductile and contractile myocardial cells. The aim of the present study is to prepare pulsincap drug delivery system of VH using hpmc, guar gum, xanthan gum, sodium alginate, lactose by physical mixture method. To evaluate prepared capsules for their micromeritic properties and interaction studies (FTIR), and *in vitro* dissolution studies [9,10].

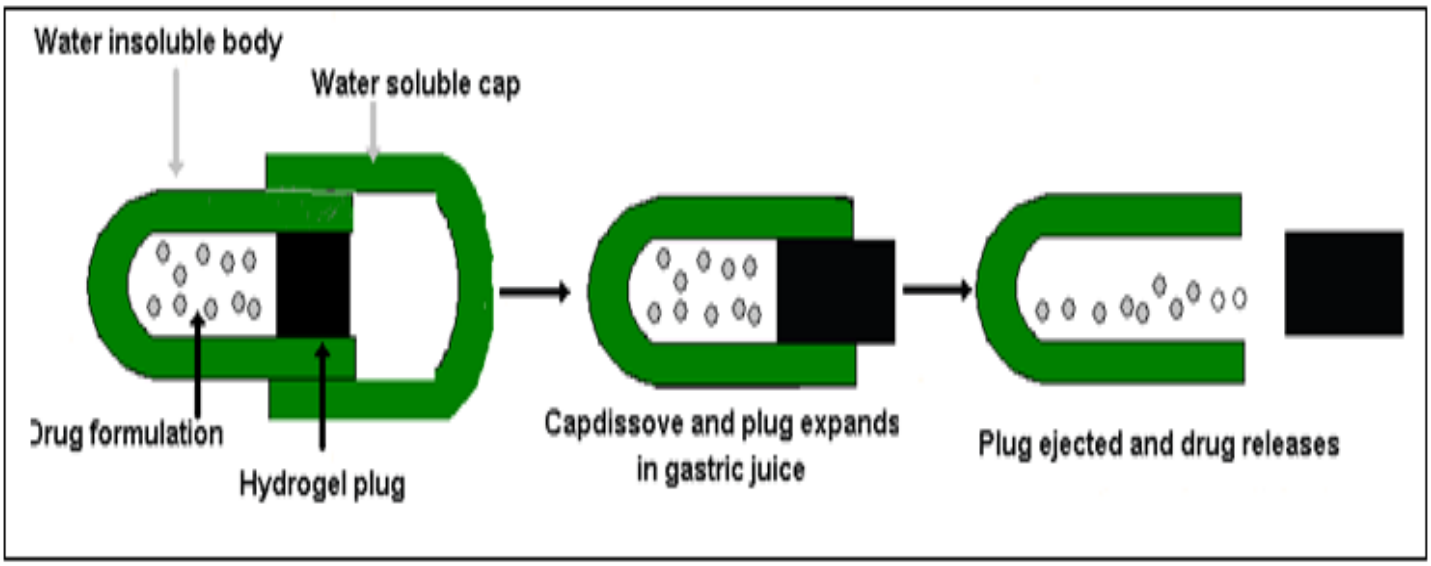


Figure No. 1: Drug Release Mechanism in Pulsincap formulation Systems

MATERIALS AND METHODS:

VH obtained as complimentary sample from Aurabindo pharma, Hyderabad. HPMC is procured from Shreeji Chemicals Mumbai. Guar Gum, Xanthan Gum, Sodium alginate, Lactose, Formaldehyde, Potassium dihydrogen orthophosphate and Sodium hydroxide was obtained from S.D Fine chemicals. Methanol was supplied by Qualigens fine chemicals, Mumbai. All other ingredient used was of analytical grade.

CALIBRATION CURVE IN SIMULATED GASTRIC FLUID:

Pipette out 0.2, 0.4, 0.6, 0.8, 1.0 ml of II stock solution (100 µg/ml) into a series of 10 ml volumetric flask and volume was adjusted to with pH 1.2 Hcl buffer solution to obtain 2, 4, 6, 8, and 10 µg/ml of solution. The absorbance of the resulting solutions was measured at 278 nm keeping pH 1.2 Hcl buffer as blank. The optical density values are recorded in table. Concentration versus optical density values are plotted and displayed in the in the concentration range of 2-10µg/ml.

DRUG-EXCIPIENT INTERACTION STUDIES [11]:

The compatibility between pure drug and polymers were detected by IR spectra obtained on Perkin Elmer 1600 series, (USA). The pellets were prepared on KBr–press. To prepare the pellets, a few mg of the physical mixture were ground together in a mortar with about 100 times quantity of KBr. The finely ground powder was introduced into a stainless steel die. The powder was then pressed in the die between polished stainless steel anvils at a pressure of about 10t/in2. The spectras were recorded over the wave number range of 4000 to 500 cm^{-1}.

PREPARATION OF MODIFIED PULSINCAP:

The drug and all the excipients as indicated in formulation chart were sifted through mesh # 40, weighed accurately, and then was mixed in a plastic bag for 5 minutes, followed by lubrication, which, was carried out in a plastic bag for 5 min by adding the weighed quantity of magnesium sterate and mixing. The Physical mixture equivalent to 120mg of drug was accurately weighed and was filled into the '0' size capsule [6].

Code	Quantities taken for 1 capsules (mg)		
	Drug	Polymer	Lactose
HS1	120	90	0.0
HS2	120	50	40
HS3	120	70	20
HX1	120	90	0.0
HX2	120	50	40
HX3	120	70	20
GS1	120	90	0.0
GS2	120	50	40
GS3	120	70	20
GX1	120	90	0.0
GX2	120	50	40
GX3	120	70	20

Table No. 1: Formulation details of VH pulisincap dosageform batches

FILLING OF CAPSULES:

Hard gelatin capsule size '2' with soluble cap and insoluble body was taken for filling. One particular ratio of drug-lactose physical mixture was filled into each of the 25 capsules. The various steps involved in capsule filling are: Step 1: From weighed capsules, cap and body was separated individual by hand. Step 2: 212 mg of the physical mixture (equivalent to 120 mg of the drug) was filled into each of 25 capsule body. Step 3: 60mg of the loading dose was filled above the physical mixture and was pressed tightly with glass plunger. Step 4: To the remaining volume, lactose was filled and pressed tightly with a glass plunger until a 2 mm empty space is left at the mouth of capsule body. Step 5: 20mg of Sodium alginate or Xanthan Gum was filled into the empty space and forms hydrogel plug [7].

PREPARATION OF CROSS-LINKED GELATIN CAPSULES:

FORMALDEHYDE TREATMENT:

Formalin treatment has been employed to modify the solubility of the gelatin capsules. Exposure to formalin vapors or treatment with aqueous formalin solution results in an unpredictable decrease in solubility of gelatin, owing to the cross linkage of the amino groups in the gelatin. The '0' sized hard gelatin capsules, about 100 in number were taken. Their bodies were separated from the caps. The bodies of the capsules were then placed on a wire mesh. 25ml of 15% v/v formaldehyde was taken into a desiccators and potassium permanganate was added to it to generate formalin vapors. The desiccators were closed tightly. The reaction was carried out for 12 hrs. After which the bodies were removed and dried at 500 C for 30 min to ensure completion of reaction between gelatin and formaldehyde vapour. The bodies were then dried at room temperature to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated caps and stored in a polythene bag [7].

EVALUATION OF FORMALDEHYDE TREATED EMPTY CAPSULES [12]:

Various physical and chemical tests were carried out simultaneously for formaldehyde treated and untreated capsules.

SOLUBILITY STUDIES OF THE TREATED CAPSULES:

The solubility tests were carried out for both normal capsules and formaldehyde treated capsules for 24hrs. Ten capsules were randomly selected. These capsules were then subjected to solubility studies at room temperatures in buffers of pH 1.2 and pH 7.4. 100ml of buffer solution was taken in a beaker. A single capsule was placed in the buffer solution and stirred for 24 hrs. The time at which the capsule dissolves or forms soft fluffy mass was noted.

QUALITATIVE TEST FOR FREE FORMALDEHYDE:

Formaldehyde treated bodies of the capsules were cut into small pieces and taken into a beaker containing distilled water. This was stirred for one hr. with a magnetic stirrer, to solubilize the free formaldehyde. The solution was then filtered into a 50ml volumetric flask, washed with distilled water and the volume made up to 50 ml with the washings. To 1ml of sample solution, 9 ml of water was

added. 1ml of the resulting solution was taken into a test tube, and mixed with 4ml of water and 5ml of acetone. The test was warmed in a water bath at 40^0 C and allowed to stand for 40 min. The solution was not more intensely colored then a reference solution prepared at the same time and in the same manner using 1ml of standard solution in place of the sample solution. The comparison should be made examining the tubes down their vertical axis [12].

PRE-FILLING PARAMETERS:

The flow properties of the physical mixture were studied by measuring the Carr's index and angle of repose of physical mixture.

ANGLE OF REPOSE [13]:

The angle of repose of powder blend was determined by the funnel method. The accurately weight powder blend were taken in the funnel. The height of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation. $\theta = Tan^{-1} (h/r)$. Where, h and r are the height and radius of the powder cone respectively.

BULK DENSITY AND TAPPED DENSITY[13]:

Physical mixture from each formula was introduced into a 100 ml measuring cylinder and the initial volume was observed. The cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2 Sec intervals. The tapping was continued until no further change in volume was noted. The bulk density, and tapped density were calculated using the following formula. Bulk density = W / V_O, Tapped density = W / V_F. Where, W = weight of the granules, V_O = initial volume of the granules, V_F = final volume of the granules.

HAUSNER'S RATIO [14]:

It indicates the flow properties of the powder and is measured by the ratio of tapped density to the bulk density. Hausner's Ratio = Tapped density/Bulk density

COMPRESSIBILITY INDEX (CARR'S INDEX) [14]:

Compressibility index is an important measure that can be obtained from the bulk and tapped densities. In theory, the less compressible a material the more flowable it is. A material having values of less than 20% has good flow property. CI = tapped density - bulk density/ tapped density x 100

POST –FILLING PARAMETERS:

The capsules were subjected to Drug content estimation and *In-vitro* release profile.

DRUG CONTENT [7]:

120 mg of microspheres were weighed and powdered. This was dissolved or extracted in methanol in 100 ml volumetric flask and made up to volume. The solution was shaken occasionally for 1h and filtered. From this 1ml of solution was diluted upto 100 ml with pH 7.4 buffer solution in 100 ml volumetric flask. The drug content was analyzed by measuring absorbance in a UV spectrophotometer at 278 nm using pH 7.4 phosphate buffer as blank. The studies were carried out in triplicate.

***IN-VITRO* RELEASE PROFILE [6]:**

Dissolution study was carried out to measure the release rate of thee drug from the dosage from. Dissolution medium: 900ml of pH 1.2 buffer for 2 h and followed by pH 7.4 buffer for 10 h. Apparatus: USP apparatus I (Basket type for capsules), Rotation speed: 75 rpm, Temperature: 37^0 C. sampling time: Every hour up to 12 h. The *In-vitro* release profile was carried by maintaining the above condition, the formulation was placed in 900 ml of pH 1.2 buffer for 2 h and in pH 7.4 for 10 h, 5ml of the sample was withdrawn at an interval of an hour and replaced with fresh dissolution media. The withdrawn samples were analyzed and the amount of the VH released was determined by UV absorption method at 278nm. The studies were carried out in triplicate. The *in vitro* dissolution data was studied for release kinetics by using dissolution software viz., PCP Disso V3.0.

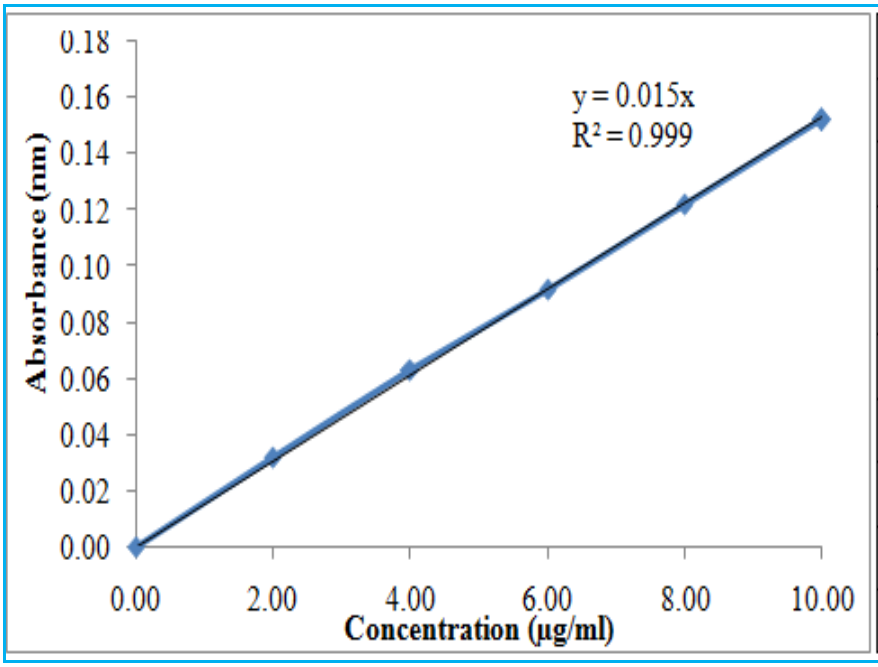
KINETICS OF DRUG RELEASE [15]:

The results of *in vitro* release profile obtained for all formulations were plotted in modes of data treatments as follows: Zero-order kinetic model (cumulative percent drug released versus time), First order kinetic model (log cumulative percent drug remaining versus time), Higuchi's model (cumulative percent drug released versus square root of time) and Peppa's model (log cumulative percent drug released versus log time).

STATISTICAL ANALYSIS [16]:

Values obtained from dissolution studies of formulations were compared with one-way anova at 95% confidence interval using Dunnett multiple comparison test. A significance level of $P < 0.05$ was used to denote statistically significance in all cases.

RESULTS AND DISCUSSION:



Absorption maxima	278 nm
Beers law limit(µg/ml)	2-10 µg/ml
Molar absorptivity(1 mole-1/cm)	1.39x105
Sandell's constant	0.0205
Slope	0.01539 to 0.01647
Y-intercept when X=0.0	-0.001061 to 0.005442
X-intercept when Y=0.0	-0.3515 to 0.06481
1/slope	62.78
r^2	0.9994
P value	< 0.0001

Figure No. 2: Calibration curve of VH in simulated gastric fluid and statistical data

FTIR spectral studies of VH, Guar Gum, Xanthan gum shows characteristic peaks of aromatic C-H stretching and N-H stretching at 3250 cm-1 and 3400 cm-1 respectively. The IR spectra of all the matrix forming polymers clearly revealed the presence of peaks associated with functional groups C=O, -OH, aliphatic C-H. This further supports the chemical identity of these polymers. Analysis of IR spectra of solid dispersions of VH with Compritol revealed the decrease in intensity of characteristic peaks of aromatic C-H stretching of methyl and methylene groups (3030 and 2860 cm-1), C-O stretch in methoxy group while the broad peak for the N-H stretch remained unchanged. These results thus indicate that there is no interaction between drug and polymers.

Code	Angle of Repose (θ) ±SD	Bulk Density (g/ml)±SD	Tapped Density (g/ml) ±SD	Carr's Index. (%)±SD	Hausner's ratio ±SD	% drug content
HS1	25.99±3.68	0.410±0.09	0.545±0.016	20.72±0.036	1.329±0.028	99.25
HS2	17.84±0.66	0.410±0.09	0.508±0.015	29.90±0.003	1.239±0.023	98.88
HS3	11.87±3.15	0.410±0.09	0.545±0.016	20.72±0.035	1.329±0.024	99.25
HX1	25.99±3.68	0.454±0.02	0.545±0.021	26.40±0.049	1.235±0.02	99.25
HX2	14.79±2.02	0.440±0.011	0.508±0.011	37.19±0.047	1.136±0.03	98.88
HX3	15.41±0.85	0.468±0.012	0.545±0.011	31.37±0.049	1.164±0.03	98.88
GS1	25.99±3.68	0.454±0.09	0.508±0.016	38.57±0.049	1.118±0.02	99.25
GS2	39.04±0.64	0.434±0.09	0.511±0.017	30.30±0.047	1.228±0.023	99.06
GS3	14.79±2.02	0.454±0.02	0.511±0.016	33.83±0.047	1.177±0.024	99.06
GX1	11.87±3.15	0.454±0.011	0.508±0.016	38.57±0.003	1.118±0.02	98.88
GX2	17.84±0.66	0.416±0.02	0.545±0.015	21.83±0.036	1.310±0.03	99.25
GX3	15.41±0.85	0.410±0.02	0.508±0.016	29.90±0.035	1.239±0.024	99.06

Table No. 2: Micromeretic properties of VH pulsincap formulations and drug content studies

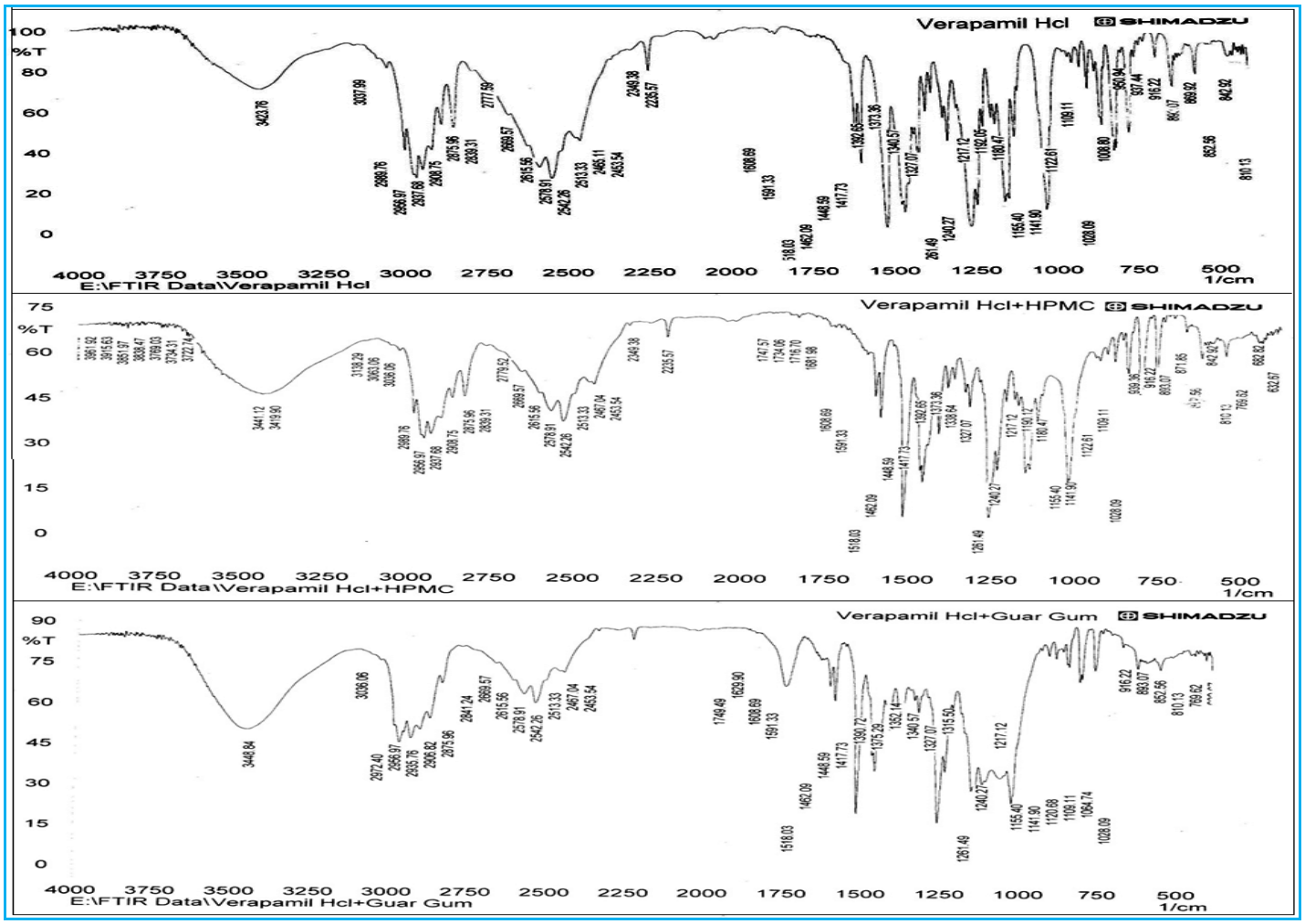


Figure No. 3: Drug-polymer interaction FTIR studies of VH, VH+HPMC and VH+Guar gum

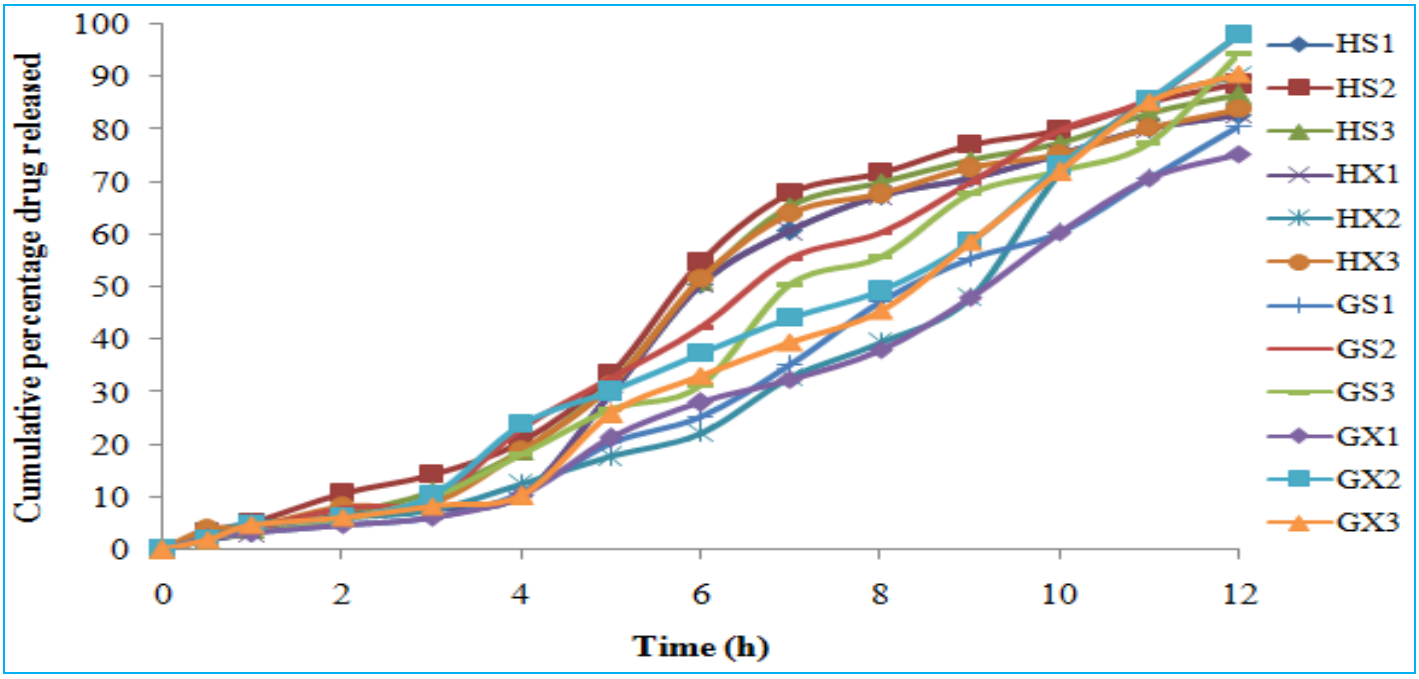


Figure No. 4: The *in vitro* drug release studies of VH Pulsincap formulations

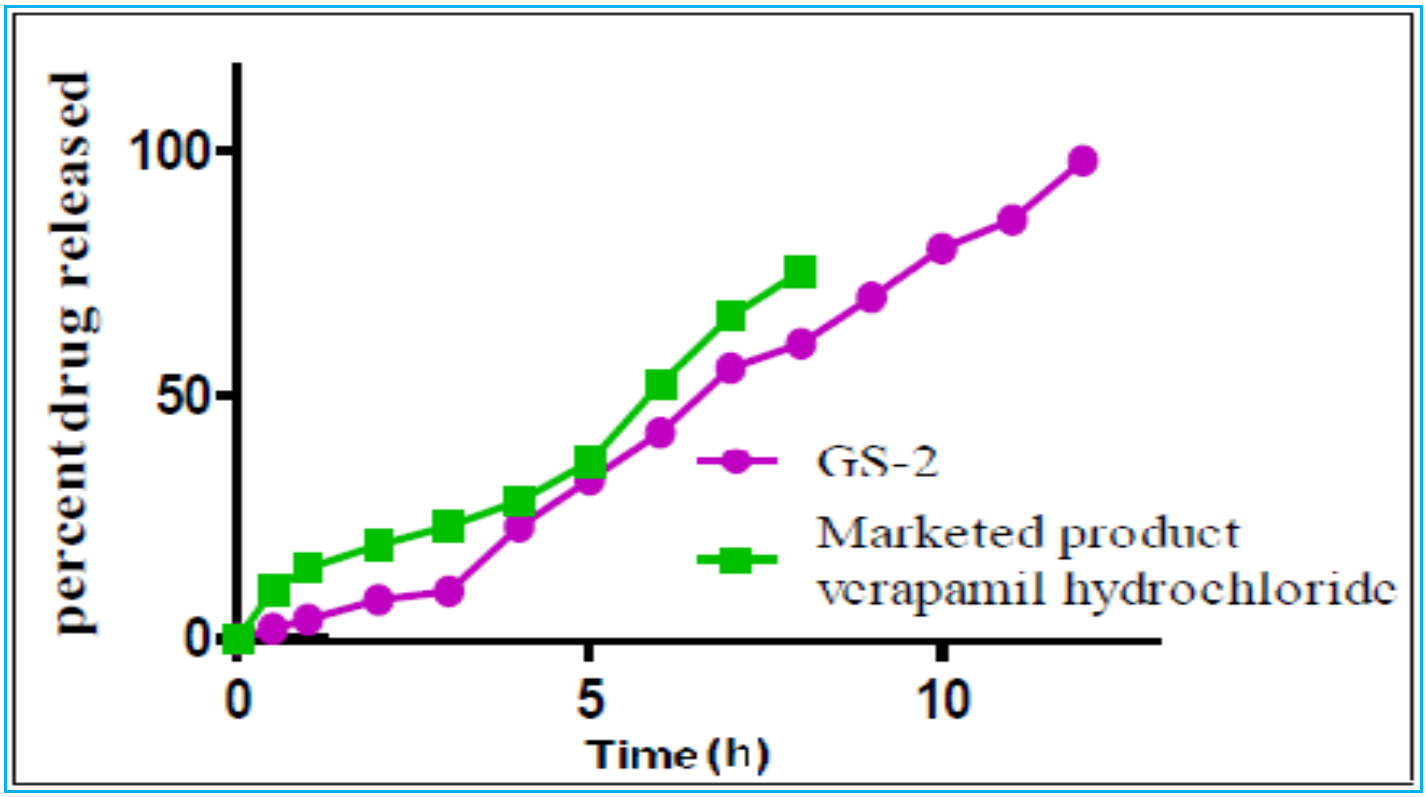


Figure No. 5: ***In vitro*** **release profiles of VH pulsincap Formulation GS2 and marketed product**

Code	Zero Order		First order		Matrix		Peppa's	
	R	K	R	K	R	K	R	n
HS-1	0.9665	7.329	0.9545	-0.132	0.9686	20.300	0.9686	1.177
HS-2	0.9788	7.986	0.9601	-0.158	0.8995	22.347	0.9851	1.159
HS-3	0.9757	15.42	0.9575	-0.147	0.8866	21.43	0.9853	1.327
HX-1	0.9665	7.329	0.9545	-0.132	0.8694	20.300	0.9686	1.052
HX-2	0.9788	7.986	0.9601	-0.158	0.8995	22.347	1.0906	1.052
HX-3	0.9757	15.42	0.9575	-0.147	0.8866	21.436	0.9853	1.156
GS-1	0.9665	7.329	0.9545	-0.132	0.8694	20.300	0.9686	1.253
GS-2	0.9788	7.986	0.9601	-0.158	0.8995	22.347	0.9851	1.280
GS-3	0.9757	15.42	0.9575	-0.147	0.8866	21.436	0.9853	1.277
GX-1	0.9665	7.329	0.9545	-0.132	0.8694	20.300	20.300	1.227
GX-2	0.9788	7.986	0.9601	-0.158	0.8995	20.300	0.9851	1.249
GX-3	0.9757	15.42	0.9575	-0.158	-0.1470	0.8866	0.9853	1.242

Table No. 3: Model fitting values and Peppas parameters of VH Pulsincap formulations

The prepared system contained HPMC and xanthan gum drug physical mixture, with lactose as channeling agent. Sodium alginate and xanthan gum were used as hydrogel plug. The effect of different concentrations of lactose and nature of hydrogel plug on release of drug and lag time was determined. The lag time is defined as the time until 10% of the drug has been released. The UV absorption maximum of drug was found at 278 nm. Which corroborated with the literature value of 269 nm, Standard calibration curve of VH was drawn by plotting absorbance v/s concentration as shown in figure 2. Standard calibration curve of VH obeyed beer's law in the range of 2-10 µg/ml. In FT-IR study of VH with polymers showed no significant variation in height, intensity and position of peaks, suggesting that drug and excipients were compatible. There is no interaction between drug and polymer. The spectras are reported in the figure 3. The results of micromeritic properties are presented in table 2. For compression of materials, it is required to possess good flow and compacting properties. Values obtained for angle of repose, Hausner's ratio and Carr's index showed good flow properties. The drug content was in the range of 98.88 % to 99.25 %, it was observed that drug content was uniform and reproducible. The SD value calculated for such formulation is very less which suggest that the results are reproducible and accurate in the method used to prepare the capsules. *In-vitro* drug release profiles of pulsatile device were found to have very good sustaining efficacy. During dissolution studies, it was observed that, the initial 2 h drug release was very slow; as the cap of formulation dissolved but hydrogel plug (Sodium alginate and xanthan gum) remained intact in gastric fluid and in pH 7.4 phosphate buffer the exposed polymer plug absorbed the surrounding fluid, swelled and released the drug through the swollen matrix. After complete wetting of the plug, it formed a soft mass, was then easily ejected out of the capsule body, exposing the physical mixture for simulated intestinal fluid (pH 7.4 phosphate buffer).

EFFECT OF LACTOSE ON DRUG RELEASE:

Study shows that about 10% of drug was released in 4 h by physical mixture of HPMC and guar gum without lactose, hence the lag time for these formulations is 4h, at the end of 12 h the release of drug is slow i.e 75.2%, 80.58%, 82.87% and 82% for GX1, GS1, HX1 and HS1 formulation. The reason may be small volume of dissolution fluid entering the capsule through the open end of body of hard the gelatin capsule. The progressive development of the gel phase and the associated swelling of the polymeric matrix retarded the drug release. In formulation at the end of 4 h from GX3, GS3, HX3 and HS3 formulations containing 80mg of lactose, release was 23.89%, 23.07%, 12.66% and 20.6% respectively, where as with 20mg of lactose the drug release at the end of 4 h was found to be 10.34%, 18.27%, 19.09% and18.77% in GX3, GS3, HX3 and HS3 respectively. At the end of 12 h GX2 and GS2 has shown 98% and 98.08% of drug release respectively which shows increase in drug release; however lag time of drug was reduced by increasing concentration of lactose.

EFFECT OF POLYMERS:

HPMC and guar gum were used as hydrophilic polymers. Further the study also shows that release of drug at the end of 12 h from formulation containing guar gum is greater than HPMC, the slow release of drug from HPMC is due to formation of viscous gel layer. The ability of hydrophilic polymer HPMC and guar gum to retard release of drug is in order of HPMC>Guar gum. Hence it was found that the ability of sustaining release of drug for plug is Xanthan gum >sodium alginate. The formulation GS2 showed maximum release of 98.08% at the end of 12 h, with23.07% of release at 4 h where as GS3 released 94.5% and 18.27% of drug at 12 h and 4 h respectively. Hence GS3 with lag time of 3 h was selected as best formulation. The dissolution profile of optimized formulation GS3 was compared with the formulation of VH available in the market (120 mg VH). To know the release rate kinetics of the drug from the dosage formulations, the *in vitro* drug release data were fitted with various models such as first order, zero order, and Higuchi release model. The data obtained were also put in Korsemeyer peppas model in order to find out '*n'* value, which describes the drug release mechanism. The release rate constants K, 'n' values of Peppas model and correlation coefficient 'r' values of are summarized in table 3. The dissolution pattern of all batches of microcapsules showed Zero-order release with highest 'r' (correlation coefficient) values, n value indicating swelling controlled drug release (n>0.89).Statistical analysis of data obtained shows $p<0.05$ is found to be significant.

CONCLUSION:

The results obtained from the above study of revealed the following conclusions. The FTIR spectral studies indicated that, there was no interaction between polymer and drug. Polymers used were compatible with VH. The result for micromeritic properties showed good flow property for physical mixture and the drug content of all formulation with low SD value indicating uniform distribution of drug within the various batches of capsule prepared with negligible loss during the formulation stage.

Increase in lactose concentration increases the drug release but decreases lag time. Among HPMC and Guar gum as hydrophilic polymer, HPMC shows good retarding ability. Ability of xanthan gum to sustain drug release is more than sodium alginate as hydrogel polymer. The dissolution pattern of all pulsincap formulation Zero-order release with highest 'r' (correlation coefficient) values. With n>0.89 showed swelling controlled mechanism for drug release. *In vitro* release data obtained was statistically analyzed by anova and a value of $p< 0.05$ was considered to be significant. The polymers like HPMC and guar gum can be used as hydrophilic polymers where as sodium alginate and xanthan gum are suitable for hydrogel plug.

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