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Effect of Sodium Alginate in Combination with Natural and Synthetic Polymers on the Release of Verapamil HCL from its Floating Microspheres

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Abstract:
The aim of the present research work is to formulate and evaluate the verapamil hydrochloride floating microspheres (VH FMs) by an ionotropic gelation method using different polymers like guar gum (GG), xanthan gum (XG) and HPMC K100M in the ratio of (1:1, 1:2, 1:3), using sodium alginate (SA) as a cross linking agent. The drug- excipients compatibility studies were carried by FT-IR and DSC studies. All the FMs were evaluated for micrometric studies, % yield, % drug content, % *in vitro* buoyancy, % swelling index, and *in vitro* dissolution studies. Among the 10 formulations, F8 is optimized one and it is further evaluated for zeta potential, SEM, DSC and accelerated stability studies up to 6 months as per ICH guidelines. Micrometric studies results indicates FMs have good flow properties. The F8 showed maximum release of 94% at 12th h. More extended release was observed with the increase in the concentration of polymers. The effect of sodium alginate in combination with natural and synthetic polymers in extending the release of verapamil from its gastro-retentive FMs was studied.

Key Words: Verapamil hydrochloride**,** sodium alginate, gastro retentive, floating microspheres, buoyancy.

INTRODUCTION:

Oral route is one of the most extensively utilized routes of administration of dosage forms. Drugs that have an absorption window in the stomach or upper small intestine, have low solubility and stability of alkaline pH were suitable to convert as gastro retentive drug delivery systems (GRDDS). GRDDS significantly prolong the time of drug release, and thereby decreasing the dosing frequency of drugs with a shorter elimination half-life ($t_{1/2}$ < 5h) and will enhance patient's compliance [1, 2].Various approaches for GRDDS includes: Floating drug delivery system (FDDS), bio adhesive systems, swelling, expanding systems and high density systems [3]. FDDS has a bulk density less than gastric fluids and thus remain buoyant in the stomach for an extended period of time, without affecting by gastric emptying rate. The drug release will be extended from the system, When the system is floating on the gastric fluids. Based on the mechanism of buoyancy, two different technologies for FDDS are effervescent and non-effervescent systems [1-3]. Effervescent systems contain carbonates (eg. Sodium bicarbonate) and / or organic acids (e.g.. Citric acid / tartaric acid) in their formulation to produce carbon dioxide (CO_2) gas when comes in contact with gastric fluids. The CO_2 gas entrapped in the matrix system reduces its density and makes the system buoyant [1-3]. The non-effervescent systems are based on the mechanism of swelling of the polymer or bio-adhesion to the mucosal layer in the GIT [1-3]. Floating microspheres (FMs) are hollow and their mechanism of buoyancy is by non-effervescence. Verapamil hydrochloride (VH) is a calcium channel blocker, used for the indications of arrhythmias is and hypertension. VH has a half-life of (about 2.8-7.4 h). More than 90% of VH is majorly absorbed from the stomach due to the presence of its therapeutic window in the stomach region. Due to its low oral bioavailability (about 35.1%), dosage forms that will retain in the stomach and prolongs the release of VH will enhance its oral bioavailability [4-6]. In this study an attempt was made to design VH FMs by the ionotropic gelation technique by utilizing various concentrations and combinations of natural polymers [xanthan gum (XG) and guar gum (GG)] with a synthetic polymer (HPMC K100M) and sodium alginate (SA) as a cross linking agent.

MATERIALS & METHODS:

***Materials*:** Verapamil Hydrochloride (VH) was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad. Sodium alginates (SA), xanthan gum (XG), guar gum (GG), HPMC K100M, calcium chloride were purchased from Hi Media Laboratories Mumbai and Sodium bicarbonate from Merck, Life science, Mumbai. All the chemicals used in the study were of analytical grade.

***Methods*:**

Drug-excipient compatibility by FT-IR studies: FT-IR spectra of drug and drug: polymer (1:1) physical mixtures were recorded out, in the region of 4000-400 cm^{-1} at a spectral resolution of 2 cm^{-1}, by the direct sampling method with isopropyl alcohol as solvent, using (Agilent technologies Cary 630 FT-IR, Japan) and the comparative FT-IR spectra were shown in (Fig.1) [7].

Standard calibration curve of VH in 0.1N HCL: 100 mg of VH was dissolved in 100 mL of 0.1 N HCl (stock solution-1000 µg/ mL) and then placed in a sonicator for 10 min, from this 10 mL of the solution was taken and the volume was adjusted to 100 mL with 0.1 N HCl (100 µg/mL). The above solution was subsequently diluted with 0.1N HCl to obtain the series of working dilutions containing 5, 10, 15, 20, 25 and µg/ mL of VH solution. The working dilutions were analyzed at 275 nm by using a double beam UV-Vis spectrophotometer (Agilent technologies Cary 60 UV-Vis, Japan). The standard calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis [8].

Preparation of VH FMs: By ionotropic gelation technique using different proportions of drug: polymer(s) [XG, GG and HPMC K100M] with SA as cross linking agent. SA was dissolved in distilled water at a concentration of 2 % w/v, the solution was stirred thoroughly using magnetic stirrer after the addition of drug and polymer(s). The gelation medium was prepared by dissolving calcium chloride in 2% glacial acetic acid. The homogenous alginate solution with drug and polymer(s) was extruded using 21guage syringe needle into the gelation medium. The gel microspheres formed were left in the solution with gentle stirring for 30 min at room temperature to improve mechanical strength. After that formed floating microspheres were collected and washed with distilled water twice, dried at room temperature for 24 h and stored in a desiccator.The formulation of F_1-F_{10} floating microsperes of $VHFM_3$ are shown in (Table 1) [9, 10].

Evaluation of microspheres:

Micrometric studies: All the formulations were evaluated for: Angle of repose (θ), Bulk density (BD), Tapped density (TD), Hausner's ratio (HR) and Carr's index (CI). The angle of repose was determined by the fixed funnel method (10). A 50 mL glass cylinder was weighed and filled with 30 mL of sample and weighed. The opening was secured with para film. The cylinder was gently handled and the powder was carefully leveled without compacting to determine the bulk volume. Tap volume was measured after 2000 taps on a tap density tester (Dolphin^{TM}). Each analysis was repeated thrice (n = 3). Values of BD and TD were used to calculate HR and CI [11-15]. The micrometrics were determined by the Eq. No(s): 1-5 which are given below

$\theta = \tan^{-1} (h / r)$ ***Eq. No. (1)***

BD = Wt. of blend / bulk volume ***Eq. No. (2)***

TD = Wt. of blend / tapped volume ***Eq. No. (3)***

$CI = (TD-BD)/ TD \times 100$ ***Eq. No. (4)***

$HR = TD / BD$ ***Eq. No. (5)***

The consolidated results of micrometric studies were tabulated in (Table 2) and the acceptable limits for flow characteristics were mentioned in (Table 3) [15].

Mean and Particle Size Distribution (PSD): Mean and PSD of microspheres was determined by using optical microscope with stage and ocular micrometer(s). From each batch about 100 VH FMs were spread on a clean slide and the size was compared with the ocular micrometer readings [16].

%yield: The prepared and dried microspheres were collected and weighed. % yield was determined by the Eq.No. (6); [16].

%yield = (Actual wt. / Theoretical wt.) X 100 Eq.No. (6)

Drug Entrapment Efficiency (DEE): Microspheres equivalent to 10 mg of the drug were triturated and transferred to 100 mL of 0.1N HCl. The solution was stirred for 8 hours at 500 rpm. Then the solution was filtered with 0.45 micron membrane filter. By making suitable dilutions the drug content was determined spectrophotometrically at 275 nm by using UV-Visible spectrophotometer (Agilent Technologies Cary 60 UV-Vis). The amount of drug loaded and entrapped in the microspheres was calculated by the Eq. No. (7); [16].

$$\%DEE = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100 \qquad \textit{Eq. No. (7)}$$

In vitro buoyancy studies: Weighed qty. of microspheres (100 mg) was spread over the surface of the dissolution medium (100 mL of 0.1 N HCl) that was agitated by a paddle rotated at 100 rpm. After agitation for a predetermined time interval, the time required for the majority of microspheres to rise to the surface and float was determined at the floating lag time (FLT) and the duration of the time the microspheres constantly floats on the dissolution medium was noted as the total floating time respectively (TFT).The microspheres that floated over the surface of the medium and those settled at the bottom of the flask were recovered separately. After drying, each fraction of the microspheres was weighed and their % buoyancy was calculated by the Eq. No. (8); [16] (Fig.2).

Buoyancy (%)

$= [Q_f / (Q_f + Q_s)] \times 100$ ***Eq. No. (8)***

Where, Q_F and Q_s are the weight of the floating and the settled microspheres respectively.

Swelling index (SI): SI of microspheres was determined by conducting *in vitro* dissolution by placing them in a basket (USP-I dissolution apparatus), in 900 mL of 0.1N HCl at temperature 37±0.5°C. At time points of 3rd h, each dissolution basket containing microspheres was withdrawn, blotted with tissue paper to remove the excess water and weighed on the analytical balance (Schimdzu, AX 120). The experiment was performed in triplicate (n=3). The % SI was calculated by the Eq. No. (9); [16].

% SI

$= [(W_W - D_W) / D_W] \times 100$ ***Eq. No. (9)***

Where, W_W and D_W are the wet weight and dry weight of the floating microspheres respectively.

In vitro dissolution studies: Were conducted by using the USP-II (paddle) dissolution apparatus (Disso 2000, Labindia Analy. Inst. Pvt. Ltd., India.)**,** each flask was filled with 900 mL of 0.1N HCl; speed of paddle was maintained at 50 rpm, the temperature was kept constant at $37^0C \pm 0.5^0C$. At time points 0, 1, 2, 3, 4, 6, 8, 10 and 12 h, 5 mL of dissolution media was withdrawn, filtered through 0.45µ membrane filter, suitably diluted and analyzed at 275 nm using a double beam UV-Vis spectrophotometer (Agilent technologies Cary 60 UV-Vis, Japan). Each sample withdrawn was replaced with an equal amount of fresh 0.1 N HCl, to keep the volume constant. *In vitro* dissolution profiles of VH FMs were shown in (Fig.3) [16].

In vitro drug release kinetics: The *in vitro* drug release data of all batches were fitted into zero order, first order and Higuchi and Korsemeyer-Peppas models to ascertain the drug release kinetics. The drug release from the hydrophilic matrix whether depends on drug's concentration or not was explained by zero and first order models. Higuchi model describes whether the drug release

is predominantly by diffusion or not. The Korsemeyer-Peppas model further explains the mechanism of diffusion. The respective models were defined by the Eq. No(s): 10-13 [17-19].

Zero order plot: $Q_t = Q_0 + K_0 t$ ***Eq. No. (10)***
First order plot: $Log\ Q_t = log\ Q_0 - K_1 t / 2.303$ ***Eq. No. (11)***
Higuchi plot: $Q_t = K_H\ t^{1/2}$ ***Eq. No. (12)***
Korsemeyer -peppas plot: $M_t / M\infty = K\ t^n$ ***Eq. No. (13)***

Where Q_t is the amount of drug dissolved at time, t; Q_0 is the initial amount of drug in the solution at time $t=0$, Q is the amount of drug remaining at time, t; $M_t/M_{\acute{a}}$ is the fraction of drug released at time, t and n is diffusion exponent. K_0, K_1, K_H and K refer to the rate constants of respective kinetic models. Drug release mechanisms based on n-values, for cylindrical shape, as per Korsmeyer - Peppas model, were tabulated in (Table 5). The consolidated drug release kinetic data of VH FMs were tabulated in (Table 6).

Evaluation studies on the optimized formulation:

Surface morphology: Shape and surface characteristics of the microspheres were determined by scanning electron microscope (JEOL JSM-6360, scanning microscope; Germany). Dry microspheres were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microspheres was taken by random scanning of the stub at an accelerating voltage of 25-15 KV and particle shape and surface morphology were studied. The SEM picture of the optimized VH FMs (F8) was shown in the (Fig.4) [20].

Zeta potential: The Zeta-potential of floating microsphere was measured by Zeta sizer (Malvern Zetasizer 3000HS, UK). To determine the zeta potential, floating microspheres samples were diluted with KCl (0.1 mM) and placed in electrophoretic cell where an electrical field of 15.2 V/cm was applied. Each sample was analyzed in triplicate [20]. (Fig.5).

In vivo x-ray imaging studies: Were carried according to the protocol (SVCP/IAEC/I-006/2016-17 dated on 06/12/2017) approved by the institutional animal ethical Committee (IAEC) of the Sree Vidyanikethan College of Pharmacy, Tirupati-517 102, Chittoor (Dist.) A.P.; India. The *in vivo* radiographic studies of the $BaSO_4$ loaded placebo of optimized VH FMs (F8) were conducted in young and healthy male albino rabbits weighing 2.0 kg to 2.2 kg. The animals were kept under standard laboratory conditions [temp. 25 ± 2°C]. Rabbits were kept for one week in animal house to acclimatize them and were fed a fixed standard diet. One healthy male albino rabbit, which had no symptoms or past history of gastro-intestinal disease was used to monitor the *in vivo* transit behavior of the $BaSO_4$ loaded placebo of optimized VH FMs (F8) . In order to standardize the conditions of gastrointestinal motility, the rabbit was fasted for 12 h prior to the commencement of experiment. The first radiographic image of the rabbit at 0^{th} h was taken to ensure the absence of radio-opaque material in the gastrointestinal tract. The $BaSO_4$ loaded placebo of VH FMs (F8) were placed in a hard gelatin capsule was administered through plastic tubing followed by flushing of 25–30 mL of water. During the study the rabbit was not allowed to eat, but water available with ad libitum. For radiographic imaging, the legs of the rabbit were tied over a piece of plywood (20 inch×20 inch), and location of the microspheres in the stomach was monitored by keeping the rabbit in front of X-ray machine (Allengers, Bharat Electricals, India, model No. E-080743). The distance between the source of X-rays and the object was kept same during the imaging process. Gastric radiography was done at the intervals of 0^{th}, 2^{nd},4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} h. In between the radiographic imaging, the animals were freed and allowed to move and carry out normal activities but were not allowed to take any food [21] (Fig.6).

Accelerated stability studies: Accelerated stability studies of the optimized VH FMs (F8), in the final pack (10 cc screw capped HDPE containers) up to 6 months were carried according to International Conference on Harmonization (ICH) guidelines [22]. 2 gm of the optimized VH FMs (F8) were packed, labelled and sealed in the containers and placed in a humidity chamber (NSW-175, Narang Scientific work, India) maintained at 45°C ± 2°C and 75% ± 5% RH. At the end of every month (1^{st}, 2^{nd}, 3^{rd} and 6^{th} month), the samples were withdrawn and evaluated. The consolidated results of accelerated stability studies were tabulated in (Table 7). Comparative *in vitro* dissolution profiles of initial and accelerated stability samples were shown in (Fig.9). The chemical stability of drug in the 6M-accelerated stability sample of the optimized VH FMs (F8); which will influence the *in vitro* and *in vivo* dissolution characteristics was investigated using FT-IR and DSC studies.

FT-IR studies: Spectra were recorded in the region of 400-4000 cm^{-1} at spectral resolution of 2 cm^{-1}, by the direct sampling method with isopropyl alcohol as solvent, using (Agilent technologies Cary 630 FTIR, Japan) (Fig.7).

DSC studies: The thermographs were recorded at the heating rate of 20 ^{0}C/min over a temperature range of 60^0 to 300^0C, using (SHIMADZU DSC-60). Highly pure indium sample is used for the calibration of the instrument. Peak transition and enthalpy of fusion were determined by TA60 integration software (Fig.8).

RESULTS & DISCUSSION:

Drug-excipient compatibility / FT-IR studies: The major IR peaks observed in pure VH were (3030 and 2860) CH stretching of methyl and methylene groups, (2838) C-H stretching vibrations of the methoxy groups, (2800-2300) N-H stretching vibrations of the protonated amine, (2237) C=N of stretching vibrations of the saturated alkyl nitrile, (1597, 1518 and 1462) C-H stretch of benzene ring, (1258) strong CO stretching vibrations of the aromatic ethers (Fig.1). The FT-IR spectra of the pure VH and physical mixture of drug and polymers were analysed to check for any interaction between drug and polymers. The

characteristic peaks of VH were appeared in the spectra without any significant change. The IR spectrum did not show presence of any additional peaks for new functional groups indicating no chemical interaction between VH & the used polymer(s). IR spectrums of drug & polymer(s) showed all prominent peaks of VH which was comparable with standard pure VH IR graph [23].

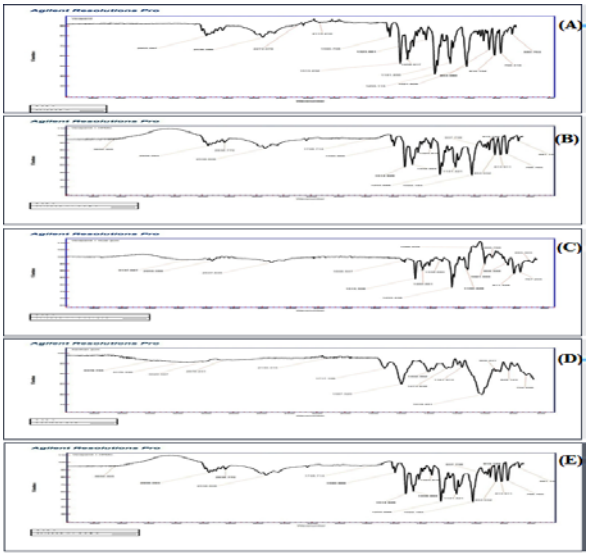

Fig.1. FT-IR spectrum of A) Verapamil HCl (VH); B) VH + Sodium alginate; C) VH + Guargum; D) VH + Xanthan gum and E) VH + HPMC K100M

Standard calibration curve: Is defined by a straight line equation, y = 0.025x + 0.002, following linearity with a regression (r^2 = 0.999). This obeyed the Beer-Lambert's law in the concentration range of 1-10 µg/mL. Lower relative standard deviation (RSD) values ensured reproducibility of the method. As the excipients used in the study were not interfering and good % recovery indicates this method was suitable for the estimation of VH content and *in vitro* dissolution studies of formulations [23].

Evaluation of microspheres:

Mean and particle size distribution (PSD): The mean particle size of all the VH FMs are ranging from 145.3-178.6 µm. The batch F8 is having the lowest mean particle size of 145.3 µm. The particle size distribution of all the batches are narrow, indicating almost uniform particle sizes. (Table 2)

Micrometric studies: The angle of repose of all the VH FMs are ranging between 28°.06' to 29°.69', CI and HR were found to be in the range of 12.23 to 15.60% and 1.11 to 1.18 respectively, indicating excellent flow properties of FMs. (Table 2)

Yield: The % yield is ranging from 80-88%, the batch F8 is having the highest % yield of 88%. (Table 4)

In vitro buoyancy studies: % floating of all the batches ranges from 63-89%, the batch F8 is having the highest % floating of 89%. The total floating time of all the batches is up to 12 h. (Table 4)

Table 1. Formulation table of VH FMs

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Verapamil HCl	100	100	100	100	100	100	100	100	100	100
Sodium alginate	100	50	100	150	50	100	150	50	100	150
Guar gum	-	50	100	150	-	-	-	-	-	-
HPMC K100M	-	-	-	-	50	100	150	-	-	-
Xanthan gum	-	-	-	-	-	-	-	50	100	150
$NaHCO_3$	100	100	100	100	100	100	100	100	100	100
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
D:P ratio	**1:1**	**1:1**	**1:2**	**1:3**	**1:1**	**1:2**	**1:3**	**1:1**	**1:2**	**1:3**

Table 2. Results of micrometric studies of VH FMs

F. Code	Mean particle size (µm)	AR (θ)	BD (g/cc)	TD (g/cc)	CI (%)	HR ( )
F1	155.3	28.06±0.31	0.45±0.045	0.52 ± 0.09	15.60±0.2	1.15±0.02
F2	162.5	27.58±0.15	0.45±0.045	0.50 ± 0.07	12.23±0.6	1.11±0.04
F3	156.3	28.44± 0.11	0.44±0.044	0.50 ± 0.09	12.58±0.8	1.13±0.08
F4	152.3	28.36± 0.13	0.45±0.045	0.52 ± 0.04	15.19±0.1	1.15±0.06
F5	178.6	28.52± 0.19	0.44±0.044	0.52± 0.01	15.48±0.6	1.18±0.08
F6	164.2	29.32± 0.19	0.45±0.045	0.51 ± 0.04	13.48±0.8	1.13±0.09
F7	153.8	29.69± 0.19	0.51±0.045	0.59 ± 0.04	14.48±0.8	1.15±0.09
F8	145.3	28.06±0.41	0.45±0.041	0.52 ± 0.10	15.60±0.21	1.15±0.04
F9	153.4	28.52±0.15	0.44±0.041	0.52± 0.11	15.48±0.54	1.18±0.12
F10	162.3	28.52±0.15	0.43±0.041	0.51± 0.11	15.48±0.54	1.18±0.12

Table 3. Acceptable limits for flow characteristics

Flow Character	AR (°)	CI (%)	HR
Excellent	25–30	≤ 10	1.00-1.11
Good	31–35	11-15	1.12-1.18
Fair	36–40	16-20	1.19-1.25
Passable	41–45	21-25	1.26-1.34
Poor	46–55	26-31	1.35-1.45
Very Poor	56–65	32-37	1.46-1.59
Very, very Poor	>66	> 38	> 1.60

Table 4. Results of evaluation of VH FMs

F.Code	% Yield (%)	Floating properties		SI at 3rd h (%)	DEE (%)
		%Floating (%)	TFT (h)		
F1	80±1.13	63±1.15	Up to 12 h	30.91±1.00	62±1.18
F2	83±1.19	67±1.19	Up to 12 h	32.33±1.03	72±1.26
F3	85±1.22	75±1.24	Up to 12 h	33.32±1.01	79±1.34
F4	86±1.25	79±1.28	Up to 12 h	34.66±1.15	56±1.21
F5	82±1.16	85±1.45	Up to 12 h	38.55±1.16	67±1.17
F6	80±1.13	80±1.52	Up to 12 h	41.32±1.17	72±1.26
F7	87±1.34	70±1.28	Up to 12 h	45.18±1.12	80±1.36
F8	88±1.32	89±1.35	Up to 12 h	35.11±1.10	84±1.42
F9	80±1.13	84±1.48	Up to 12 h	36.12±1.06	82±1.40
F10	86±1.25	82±1.45	Up to 12 h	37.25±1.09	82±1.40

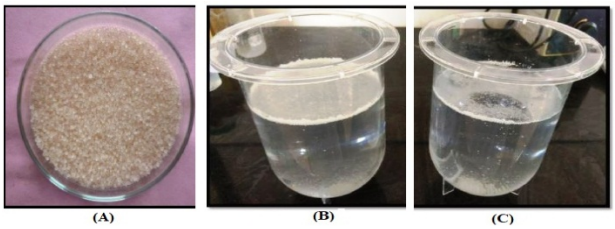

Fig.2. Images of optimized VH FMs (F8) A) Dried microspheres; B) Before and C) After test for buoyancy

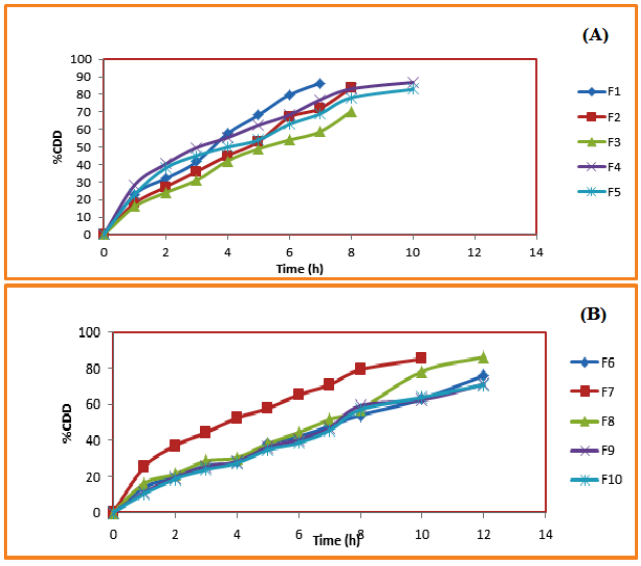

Fig.3. *In vitro* dissolution profiles of VH FMs A) F1-F5 and B) F6-F1

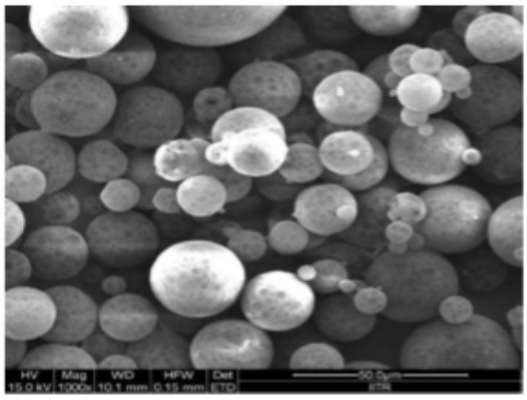

Fig.4. SEM of optimized VH FMs (F8)

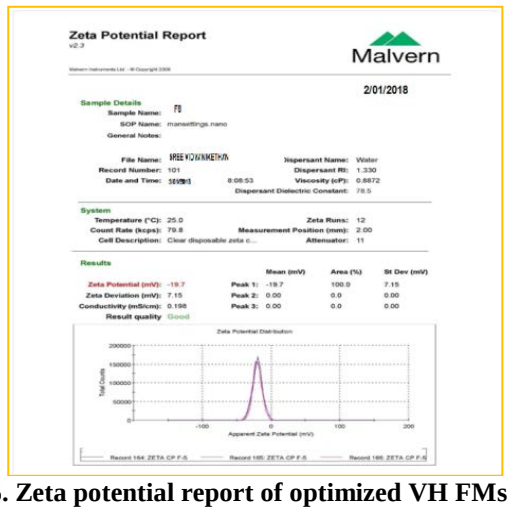

Fig.5. Zeta potential report of optimized VH FMs (F8)

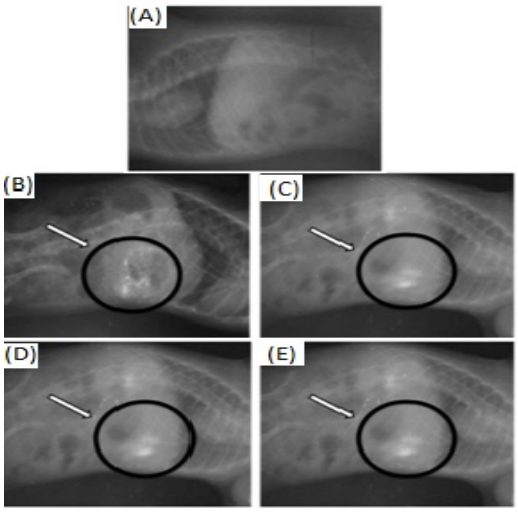

Fig.6. X-ray images of $BaSO_4$ loaded placebo of optimized VH FMs (F8) in a rabbit model at A) 0th h, B) 2nd h, C) 6th h, D) 8th h and E) 12th

Swelling index (%SI): % SI of all the batches after 3rd h ranges from 30.91-45.18%. Where the highest and lowest swelling was observed with the formulation F1 and F7 after 3rd h, respectively.The swelling index was significantly increased with the batches (F5-F7) with HPMC, since this polymer absorbs 0.1 N HCl due to its more hydrophilic nature. When natural and synthetic gums were combined with SA, the SI of the combined hydrophilic matrix was more, when compared to the formulation with SA alone. Due to the combination of two different polymers; the swelling of combined matrix was depending on the hydration of both the polymers at the particular pH of the dissolution medium [24]**.** Among the used three polymers in combination with SA, their effect on increasing the SI of combined hydrophilic matrix is in the order: HPMC > XG > GG. (Table 4)

Drug Entrapment Efficiency (%DEE): %DEE of all the batches ranges from 56-84%. The batch with SA alone is the one with lowest %DEE. As the conc. of the combination of polymers increases the DEE increases [24]. Among the used three polymers in combination with SA, their effect on increasing DEE of combined hydrophilic matrix is in the order: HPMC > XG > GG. (Table 4)

In vitro dissolution studies: As the conc. of SA increases, there is an increased viscosity of the gel matrix and decrease in the effective diffusion coefficient of the drug [25]. Other factors that may contribute to differences in drug release profiles include; differences in water penetration rate, water absorption capacity, swelling and drug:polymer ratio [26]. Among all factors, drug : polymer ratio is an important factor affecting the rate of drug release from the combined hydrophilic matrix, which has to be optimized [26]. The pH independent, zero order release profile of the bio pharmaceutics classification system (BCS) class-II drug (high permeability and low solubility) like VH can be attained from the hydrophilic matrix systems, by the combination of SA with synthetic polymer (HPMC K100M) or with natural polymers (XG and GG) [27]. The combined matrix when exposed to gastric fluids; the hydrophilic polymer (HPMC/ XG/ GG) hydrates first to form a gel layer at the surface of the microspheres, while the SA due its lesser hydration rate in acidic pH remains insoluble. The resulting combined matrix system acts as a barrier for diffusion of poorly soluble drugs like VH and extends its release [27]. Except the formulations in combination of SA with XG (F8-F10), others cannot able to extend the release up to 12 h. Among the used three polymers in combination with SA, their effect on extending the VH release is in the order: XG > GG > HPMC. Among all the VH FMs, F8 (1:1 ratio of SA:XG) extends the release of VH up to 12 h with a better zero order release profile (r^2 =0.999) (Table 5). Hence, among all the VH FMs (F8), is selected as optimized batch.

***Drug release kinetics*:** The optimized VH FMs, F8 (1:1 ratio of SA:XG) batch's release profile is best fitted to the zero order kinetics (as zero order, r^2 = 0.999), indicating the drug release from the matrix does not depends on its conc. The drug release process is not predominantly by diffusion (as Higuchi, r^2 = 0.921); and the mechanism of diffusion is by super case-II transport, i.e. a combination of both diffusion and erosion (as Korsemeyer- Peppas, n = 1.135). (Table 6)

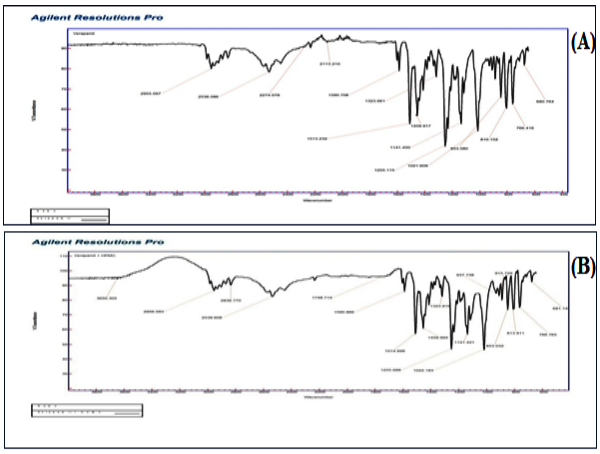

Fig.7.Comparative FT-IR spectra of A) VH (pure drug) and B) 6M-Accelerated stability sample of optimized VH FMs (F8)

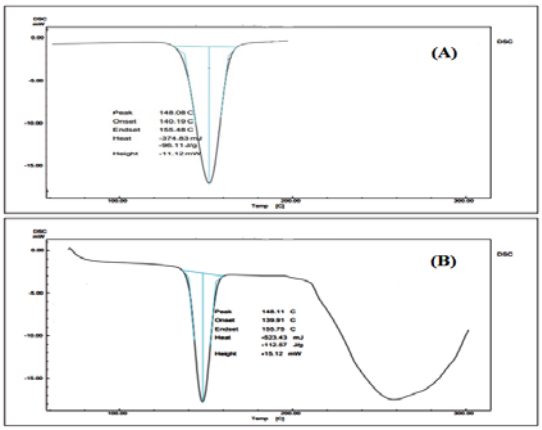

Fig.8. Comparative DSC thermographs of A) VH and B) 6M-Accelerated stability sample of optimized VH FMs (F8)

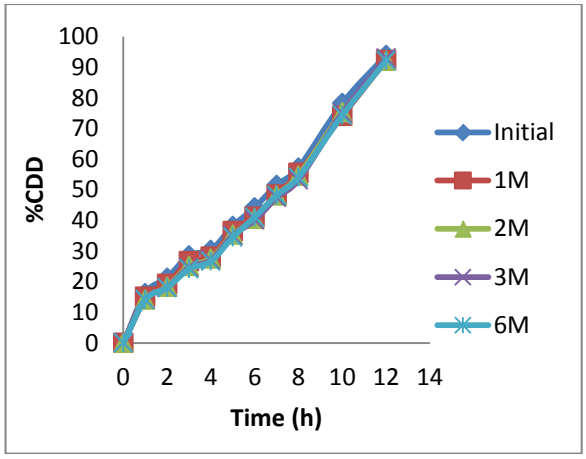

Fig.9. *In vitro* dissolution profiles of initial & 1, 2, 3 & 6M-accelerated stability samples of optimized VH FMs (F8)

Table 5. Drug release mechanisms for spherical shape in Korsmeyer-Peppas model

Diffusion exponent (n)	Mechanism
0.45	Fickian diffusion
0.45 < n <0.89	Anomalous (Non- Fickian) diffusion
0.89	Case II transport
n > 0.89	Super Case II transport

Table 6. Results of drug release kinetics of VH FMs

F. Code	Zero order	First order	Higuchi	Korsmeyer-Peppas	
	r^2	r^2	r^2	r^2	n
F1	0.984	0.964	0.956	0.975	1.315
F2	0.99	0.943	0.947	0.99	1.220
F3	0.980	0.980	0.963	0.992	1.182
F4	0.905	0.985	0.996	0.994	1.450
F5	0.925	0.981	0.996	0.988	1.378
F6	0.987	0.955	0.952	0.980	1.096
F7	0.929	0.988	0.996	0.998	1.398
F8	0.999	0.913	0.921	0.959	1.135
F9	0.976	0.982	0.951	0.99	1.044
F10	0.983	0.979	0.943	0.99	1.010

Table 7: Results of accelerated stability studies of optimized VH FMs (F8)

Time Interval	Floating properties		SI (%)	DEE (%)
	% Floating (%)	TFT (h)		
Initial	80.00±1.35	Up to 12 h	37.32±1.02	84.00±1.42
1 Month	79.12±1.12	Up to 12 h	38.22±1.11	83.03±1.03
2 Month	81.05±1.21	Up to 12 h	38.23±1.04	83.15±1.11
3 Month	78.13±1.02	Up to 12 h	39.16±1.13	83.13±1.13
6 Month	81.03±1.11	Up to 12 h	40.05±1.07	82.06±1.04

Evaluation studies on the optimized VH FMs (F8):

Surface morphology:The surface of the optimized VH MFs (F8) is almost spherical as indicated in SEM pictograh in Fig.4.

Zeta potential: The zeta potential of the optimized VH MFs (F8) is -19.7 mV as indicated by the report in Fig.5. Which is indicating the good stability of the prepared dispersion.

In vivo x-ray imaging studies: X-ray images of a rabbit taken at 0^{th}, 2^{nd}, 6^{th}, 8^{th} and 12^{th} h after the administration of $BaSO_4$ loaded placebo of optimized VH FMs (F8), indicates the optimized VH FMs are strong enough in withstanding repetitive gastric contractions and able to retain in gastric region up to 12 h. (Fig.6)

Accelerated stability studies: As there were no significant differences in floating characteristics (% floating and TFT); SI at 3^{rd} h and % DEE of initial & accelerated stability samples of optimized VH FMs (F8), it passes the test for stability (Table 7, Fig.9). Comparative FT-IR spectra (Fig.7) and DSC thermographs (Fig.8), reveals there is no significant change in the functional groups of the VH due to interaction with polymers and other excipients.

CONCLUSION:

In the view of above findings, among all factors which effects the drug release profiles from the combined hydrophilic matrix, drug:polymer ratio is an important factor, which has to be optimized. Among the natural gums (XG, GG) and synthetic polymer (HPMC K100M) used in combination with SA; XG in combination of SA in the ratio 1:1 respectively forms a better matrix for the extending the release of VH in gastric pH up to 12 h. The VH FMs (F8, 1:1 ratio of SA:XG) extends the release of VH up to 12 h with a better zero order release profile (r^2 = 0.999), % Floating of 89±1.35 and TFT up to 12 h. Hence it is an optimized formulation. A combination hydrophilic matrix design of this kind can serve as an alternative strategy for extending the release of other BCS class II drugs (Low Solubility, High Permeability) by gastric retention in acidic pH.

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