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DESIGN AND DEVELOPMENT OF CARBOXYMETHYL CHITOSAN-HPMC MATRICES OF VERAPAMIL HCL CAPSULE

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ABSTRACT

Aim of the study was to find out the potential of HPMC-based polymeric matrices formulation such as carriers for gastrointestinal tract targeted drug therapy of verapamil hydrochloride capsule formulation. Prepared single-unit hydrodynamically balanced (HBS) capsule formulations by the encapsulation using hard gelatin capsules technique properly mix all the physical mixtures of various drug polymers such as HPMC, K4M and Carboxymethyl Chitosan. At the time of In-vitro study in 0.1M HCL shows good floating time (5-10 hr) and drug release profile were carried out using 0.1M HCL as a dissolution medium. Finally prepared HPMC-based polymeric matrices formulation was very helpful for gastrointestinal tract delivery.

Keywords: Carboxy-methyl Chitosan, HPMC K4M, Verapamil Hydrochloride, Hydrodynamic Balance System

INTRODUCTION

Solubility of verapamil hydrochloride (VHCL) and absorption decreases with increase in pH. Drug retention time in gastrointestinal tract and rate of drug released is very important **[1]**. An approach to improve the retention time of VHCL in

stomach and control the rate of release from the dosage form is to engineer a polymer matrix capable of swelling and erosion in the acidic environment of the gastrointestinal tract to form a swelled yet erodible hydrodynamically balanced mass **[2]**. From such an *in-situ*-forming hydrogel, it is expected that the swelling of the polymer matrix will produce low density mass and continuous erosion causes a constant delivery the drug with constant rate from the matrix **[3]**.

In the present investigation, pH independent swellable and erodible HPMC K4M and pH dependent swellable carboxymethylated chitosan were used to engineer the polymer matrix. Chitosan is polysaccharide in nature and similar in structure to cellulose, consisting of both units of glucosamine and N-acetylglucosamine. Chitosan is commonly obtained through deacetylation of chitin derived from exoskeleton of crustaceans. Although Chitosan is very useful polymer with low toxicity, its limited solubility at a particular physiological pH is a major barrier for pharmaceutical and cosmetic applications. Polymer chitosan is a weak base at a 6.2 to 7.0 pKa value due to D-glucosamine residue **[4]**. Polymer chitosan is insoluble in neutral and alkaline pH and soluble in water at a particular pH below 6.5 or in acidic solutions.

MATERIALS AND METHODS

Materials

Verapamil Hydrochloride (VHCL) was obtained as sample from Dr. Reddy's Laboratories, India. Chitosan [degree of Deacetylation (DD) > 75%] was purchased from Sigma Aldrich, Brookfield. HPMC K4M was procured from Colorcon, India. Isopropyl alcohol (IPA) was purchased from CDH, India. HPLC grade water was used during all the experimental work.

Methods

Preparation of Carboxymethyl Chitosan

It was prepared using various components like chitosan (5 g), NaOH (40 g), and 100 ml solvent was added into a 500 ml flask to swell and alkalize for 60 min at 70ºC. 15g Monochloroacetic acid was dissolved in 20 ml isopropanol at 70ºC with continuous stirring. The entire mixture was neutralized using 10% acetic acid solution, and poured into a 70% ethanol. Solid residue is formed and is filtered and washed with 90% ethanol to diesel and dewater, followed by dry using hot air oven at 50ºC for 12 hr **[5]**. Composition of different reaction mixtures is shown in **(Table 1)**.

Table 1: Samples of carboxymethyl chitosan prepared with different reaction mixtures

S. No.	Type of polymer	Solvent system
1	Carboxymethyl Chitosan A	Water
2	Carboxymethyl Chitosan B	Water: IPA (1:1) ratio

CHARACTERIZATION

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of chitosan were compared with spectra of carboxymethyl chitosan. Spectra of both polymers were recorded using the Nicolet FTIR Spectrophotometer **[6]**.

Degree of substitution

Carboxy methyl cellulose degree of substitution was determined with the help of potentiometric titration. 0.2 g of both samples of carboxymethyl chitosan A/B was dissolved in 40 ml distilled water and adjust the pH below 2 using 0.1N hydrochloric acid. Then, the carboxymethyl chitosan solution was titrated using 0.1M NaOH solutions and pH values of both solutions were recorded **[7]**. The value of degree of substitution can be calculated using the formula

$$\mathbf{DS} = \frac{161 \times A}{m_{\mathbf{CMC}}\mathbf{H} - 58 \times A}$$

$$A = V_{\mathbf{NaOH}} \times c_{\mathbf{NaOH}}$$

$$DS = \frac{161 \times A}{mCMC\ H - 58 \times A}$$

$$A = V_{NaOH} \times C_{NaOH}$$

Where

V_{NaOH} = Means volume of aqueous sodium hydroxide.

C_{NaOH} = Means molarity of sodium hydroxide solution.

m_{CMCH} = is the weight of carboxymethyl chitosan (g) and 161 and 58 are the molecular weights of glucosamine and carboxymethyl groups respectively.

Preparation of hydrodynamically balanced (HBS) Capsule formulations

Single unit verapamil hydrochloride loaded HBS capsules were prepared with a sufficient amount of polymers using double cone blender for 15min followed by encapsulating the hard gelatin capsules shell **[8]**. The composition of hydrodynamically balanced capsule formulations was shown in **(Table 2)**.

Table 2: Composition of Various HBS formulations

Formulation Code	Chitosan (mg)	Carboxymethyl chitosan A (mg)	Carboxymethyl chitosan B (mg)	HPMC K4M (mg)	Verapamil Hydrochloride(mg)
D	-	-	-	200	80
D1	25	-	-	200	80
D2	-	25	-	200	80
D3	-	-	25	200	80
D4	50	-	-	200	80
D5	-	50	-	200	80
D6	-	-	50	200	80

Drug Contents of HBS Capsules

The drug contents in hydrodynamic capsules were determined from each capsule formulation. Average weight of powder was added in 0.1M hydrochloric acid with continous stirring at 37 ± 0.5^0C **[9]**. Solution was filtered through a 0.45μ membrane filter and measured the absorbance using spectrophotometericaly at 278 nm.

***In-vitro* drug release**

Measured the *in-vitro* drug release rate of verapamil loaded hydrodynamically balanced capsules formulation was performed at 50 rpm. Drug release was performed using 900mL 0.1M hydrochloride (pH 1.2) at 37 ± 0.5^0C. I withdrew the sample at different time intervals and maintained the sink condition using 0.1M hydrochloride. Then analyze the drug content at 278 nm **[10]**.

Mechanism of Drug Release

These are the various kinetic methods are used for the interpretation of drug release profile. Zero order release kinetics is very useful for a uniform rate of drug release from matrix system. The drug concentration is not depend during a given time period **[11]**. Zero-order kinetics is very helpful for provides the highest therapeutic value with least side effects.

RESULTS AND DISCUSSION

Chitosan is a cationic co-polymer consist both glucosamine and N-acetyl glucosamine a unit. It is obtained from deacetylation derivative of natural chitin polysaccharide. It is obtained from deacetylation derivative of natural chitin polysaccharide **[12]**. Chitin is one of the most abundant carbohydrates. Recently developed chemically modified chitosan to improve oral drug delivery. The direct alkylation method was utilized in which monochloroacetic acid was used to prepare N, O-carboxymethyl chitosan. Chitosan polymer is activated by immersing it in alkaline solution before continuing with the carboxymethylation of chitosan reaction using water or isopropyl alcohol **[4]**. During carboxymethylation reaction only the amine groups will be activated. The structure of chitosan and N-Ortho carboxymethyl chitosan was shown in **(Figure 1)**.

Percentage yield of carboxymethyl chitosan

In present study, the % yield of carboxymethyl chitosan A and B was found to be 15.21% and 97.16% respectively. The relative amount of water and isopropyl alcohol is the most important factor for calculating the yield of carboxymethyl chitosan **[4]**. The production percentage yield of chitosan with isopropanol is very low, which increases the production rate of

chitosan with water. The reason is the previously formed carboxymethyl chitosan jelly with water which covers the chitosan particle and protect from the course reaction **[13]**. The relative amount of water and isopropanol is 1:1 and 1:4 respectively, very useful in the production of quantitative yields. Percentage yield shown in **(Table 3)**. Increase the ratio of water and isopropanol so reduce the carboxymethylation fraction increases the insolubility at higher pH **[4]**.

FTIR of carboxymethyl chitosan

Chitosan and carboxymethyl chitosan A and B comparison study were shown in **(Figure 2)**. FTIR spectra of pure chitosan exhibited characteristic peaks at 1645, 1558 and 1375 cm^{-1} correspond with the C=O, N-H bending and C-N stretching respectively. The peaks at 2983 and 2876 were attributed to vibration absorbance of C-H. Broad peaks in between the region of 3500-3000 cm^{-1} were stretching vibrations of free hydroxyl groups overlapped with stretching of N-H bonds in amino overlapping **[6]**.

There were differences between the chitosan and carboxymethyl chitosan A and B spectra. The most visible difference was new peaks at 1408 A and 1406 cm^{-1} B respectively. These peaks could be attributed to the carboxymethyl groups. Carboxymethyl chitosan A and B were shown in **(Figure 3 & 4)**. The broader bends centered at 3408-09 cm^{-1} showed the more hydrophilic character of carboxymethyl chitosan A and B than Chitosan **[13]**. Comparison with the chitosan peaks at 1636-37 and 1406-08 cm^{-1} corresponding to the carboxyl group overlapping the NH and CH_2COOH group peaks respectively, suggesting carboxymethylation of amino and hydroxyl groups of chitosan.

Degree of substitution of carboxymethyl chitosan A and B

Degree of substitution values of carboxymethyl chitosan A prepared in water as solvent was 0.98. Whereas the ratio of water: IPA (1:1) showed 1.13 degrees of substitution both values are shown in **(Table 4)**.

There is no relation between degree of substitution and chitosan deacetylation value. It is directly depend on concentrated sodium hydroxide concentration **[4]**. Concentrated sodium hydroxide percentage is helpful in determining the degree of substitution. In the present investigation, 40% NaOH was used. Scientist Tokura represents degree of substitution value of carboxymethyl chitosan from 20 to 40% increased with NaOH concentration **[14]**.

Increased the concentration of NaOH from 40 to 50%, when increase the value of degree

of substitution from 0.15 to 0.63 and 0.43 to 0.68 respectively. Whereas excessive concentration of sodium hydroxide affect the carboxymethylation reaction rate, so drop the degree of substitution value from 0.57 to 0.46 respectively **[7]**. Low concentration of NaOH rigid the crystallinity of chitosan structure and reduces the degree of substitution. Further, the relative amount of amine and acid is most important cause for determine degree of substitution **[15]**. The ratio of amine and acid is used at low concentration, so the degree of substitution is 1:1 and at higher concentration it is 1:4. In the present study, the ratio of amine and acid was 1:3.

Preparation of HBS formulations

The various number of HBS formulations were prepared by physically blending chitosan/carboxymethyl chitosan A and B with HPMC K4M and model hydrophilic drug Verapamil HCl (solubility of 525 mg/ml in 0.1 M HCl at 37^0C). It possesses a very stable structure due to strong hydrogen bonds, because of this region; the solubility of HBS capsule formulation was observed only acidic solutions below pH 6.5. In acidic solutions, such as 0.1 M HCl, chitosan swells and erodes simultaneously. Plain chitosan based matrices exhibited burst release of very soluble drugs. Since HPMC is a nonionic polymer and drug solubility is pH dependent, the matrices show pH independent drug release characteristics **[9]**. Example release rate of drug may be disturbed by changing media. Further, HPMC matrices are responsible for disintegration of the drugs. This characteristic of matrix has shown the faster dissolution rate of the drug from the surface, while the polymers produce a barrier-forming gel layer. Moreover, HPMC also possesses good mucoadhesive properties. The mucoadhesion of HPMC is pH independent and is responsible for the formation of physical bonds **[16]**. It also possesses a different number of hydroxyl groups that are responsible for adhesion.

Both carboxymethyl chitosan A and B are carboxymethylated products of Chitosan and hence they are anionic in nature. The combination of HPMC and anionic polymer matrices has been responsible for the development of pH independent drug release profiles. Due to creation of an insoluble mass that serves as a barrier to drug diffusion, the insertion of anionic polymers in the matrix can both slow the rate of drug release in acidic circumstances and affect the rate of drug release in basic environments **[14]**. Aside from regulating the pH of the microenvironment, anionic polymers may also have an impact on the speed at which drugs are released.

Characterization of HBS formulations

HBS formulations were evaluated in the form of *in-vitro* drug release studies using 0.1 M HCl at pH 1.2.

***In-vitro* Studies**

HBS formulation exhibited instant drug release as there was no lag time. The HBS formulation containing only HPMC, K4M and drug (Formulation D) remained buoyant for up to 540 min as the gelled irregular mass. This could be explained as, when can HPMC based carrier system is exposed to the dissolution medium, rapid swelling of the carrier system occurs due to fast disturbance of hydrogen bonding among the polymeric chains, resulting the formation of thick gel layer **[10]**. The reason for the formation of an irregular mass could be recognized to the high diffusional driving force exerted by the hydrophilic drug verapamil HCl. The high diffusional driving force caused degradation of gel matrix, cause irregular shape of the hydrogel.

HBS capsule formulations D1 and D4 shows swelling properties and degradation of polymeric matrices in **(Table 5)**, resulting in a shorter flotation period (300 min) compared to HPMC K4M matrices. Rapid erosion of hydrogel could be attributed to the presence of protonated chitosan (NH_3^+) and ionized drug (Verapamil H^+) in the hydrogel form **[17]**. As both the species (drug and polymer) have the ability to be protonated in the hydrogel form, increased magnitude of osmotic and electrical forces the caused increased access of, dissolution medium into the hydrogel structure in the has resulted in increase the hydrogel density.

HBS capsule formulations D_3, D_6 exhibited rapid swelling and prolonged buoyancy (600 min). It was observed after that dissolution of hard gelatin capsule, a cylindrical shape hydrogel capsule structure was formed. It is postulated that polymers show a high viscosity, which allowed to degradation at a rate equation to the movement between glassy and rubbery polymer **[9]**. This might have balanced the erosive and diffusive forces, leading to form a stable low density cylindrical hydrogel capsule.

***In-vitro* drug release**

In-vitro drug release study was conducted in triplicate using 0.1 M HCl (pH 1.2) as the dissolution medium. The rate of release profile was shown in **(Figure 5)**.

Drug release studies from HPMC K4M matrices

There was a drug from formulation D with more than 53% drug was released at last one hour. After that, collect the drug sample at different time intervals, like 2, 4 and 8 hrs. Although gel formation was rapid, but it

seems that high diffusional driving force coupled with increased magnitude of osmotic and electrical forces (generated due to ionization of a drug, Verapamil H+) weakened the diffusive barrier, leading to rapid drug release initially **[18]**. As the concentration of drugs reduced in the hydrogel matrix, the degree of osmotic and electrical forces also went down, leading to sustained drug release thereafter. The surface area of the resulting drug-polymer matrices, which could not be enough to cover the full matrix surface, could be another explanation for the initial rapid drug release **[19]**.

Drug release studies from HPMC K4M-Chitosan matrices

Formulation D1, D4 remained buoyant for 300 min and then sank as an irregular hydrogel mass. So, in this case, the drug release profile was determined by the duration of floating only **[10]**. In the case of formulation D1, at the end of 1 hr about 37% of the drug was released. However, compared to HPMC K4M matrices (Formulation D), it was significantly reduced ($p<0.05$). Increasing the Chitosan concentration in the matrix (Formulation D4), drug release was even more sustained, with 17% drug was released at last one hr ($p= 0.0001$ compared to D1). This could be attributed to the synergistic increase in viscosity within the hydrogel capsule.

Drug release studies from HPMC K4M-Carboxymethyl Chitosan matrices

Monochloroacetic acid and chitosan were combined in an alkaline medium to create carboxymethyl chitosan A and B. Because of carboxymethylation, the opposing ionizable group's carboxyl and amine could coexist in the same polysaccharide chains **[4]**. These conversion methods bring particular qualities include high viscosity, large hydrodynamic volume with least toxicity, and biocompatibility in addition to carboxymethylation chitosan solubility. They all contribute to the allure of carboxymethyl chitosan as a potential medication delivery system.

Carboxymethyl chitosan prepared in present study (N, O carboxymethyl chitosan) is a zwitterionic polymer as it contains primary amine ($R\text{-}NH_3^+$) group, secondary amine derivative was derived from carboxymethyl group on primary amine ($R\text{-}NH_2^+\text{-}CH_2COO^-$) and $R\text{-}OCH_2COO^-$ group. At the present study on dissociation behavior of carboxymethyl chitosan in aqueous medium reported that –COOH group in carboxymethyl chitosan has the pKa of around 3.0, whereas, ammonium group in carboxymethyl chitosan has pKa of around

6.4. These values were obtained from carboxymethyl chitosan (degree of substitution of about 1.09) prepared from chitosan with 79% degree of deacetylation. In present study, chitosan used was more than 75% deacetylated and the DS of prepared carboxymethyl chitosan ranged from 0.98 A to 1.13 B respectively.

HBS capsule formulations D2, D3, D5 and D6 were composed of HPMC K4M, Carboxymethyl Chitosan and Verapamil HCl. From HBS formulation D_2, about 28% drug was released at 1 hr. collect the drug sample at different time interval 2, 4, 8 and 10 and maintain the sink condtition finally 92% of drug was released in a sustained manner at the end of 10 hr. from same formulation D_3 about 18% drug was released at the completion of 1 hr. after that drug was release in sustained manner **[10]**. This release profile is quite different from the release of drug from formulation D_1 ($p<0.0001$, compared to D_2 and D_3). This could be attributed to the strengthening of the diffusion barrier by carboxymethyl chitosan due to combined effect of protonated amine group and COO^- group due to synergistic increase in viscosity **[9]**.

Increase the concentration of carboxymethyl chitosan A (D_5) about 39% drug was released at the 1 hr. thereafter release rate was retarded ($p=0.0001$ compared to D_2) with finally after 10 hr about 97% drug was released. It is to be noted that in both the cases (D_2, D_5) initial drug release was rapid which was followed by slow extended release. This could be attributed to the low degree of substitution of carboxymethyl chitosan A (DS= 0.98) which might have shows slow wetting property of carboxymethyl chitosan A in the hydrogel capsule **[20]**. Once the carboxymethyl chitosan A becomes fully wetted, it reinforced the HPMC hydrogel structure due to synergistic increase in viscosity as evidenced by the slow extended drug release.

In this case the formulation D_3, D_6, carboxymethyl chitosan B was combined with HPMC, K4M and drug. The deacetylation of carboxymethyl chitosan B was found to be 1.13. From both the formulations, drug release was significantly retarded comparison of formulations D_2, D_5. This could be attributed to the higher deacetylation value of carboxymethyl chitosan B **[21]**. work is done on drug release profile of (Doxorubicin) from carboxymethyl chitosan nanoparticles reported that carboxymethyl chitosan with high deacetylation extended the drug release for prolonged period.

Release Kinetics

Obtained the release kinetics data using various kinetics models and results were shows in **(Table 6)**. From kinetic data, it was observed that all formulations are following zero order kinetic mechanism reaction **[22]**. The n values range from 0.28 to 0.62. Formulations D_1, D_2, D_4 and D_5 followed fickian diffusion, whereas, formulations D_3, D_6 followed non-fickian release kinetics mechanism **[23]**.

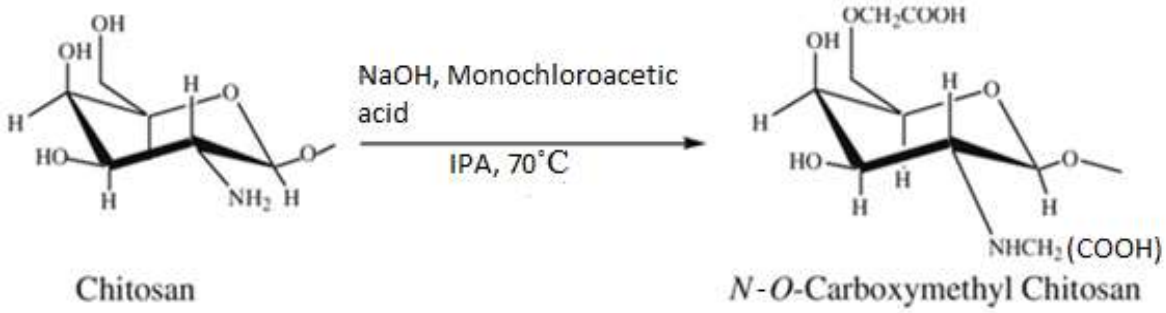

Figure 1: Conversion of Chitosan into N, O-Carboxymethyl Chitosan

Table 3: Percentage yield of carboxymethyl chitosan A and B

S. No.	Type	Total yield (%)	% yield (water soluble fraction)
1	Carboxymethyl chitosan A	106	25.21
2	Carboxymethyl chitosan B	130	97.16

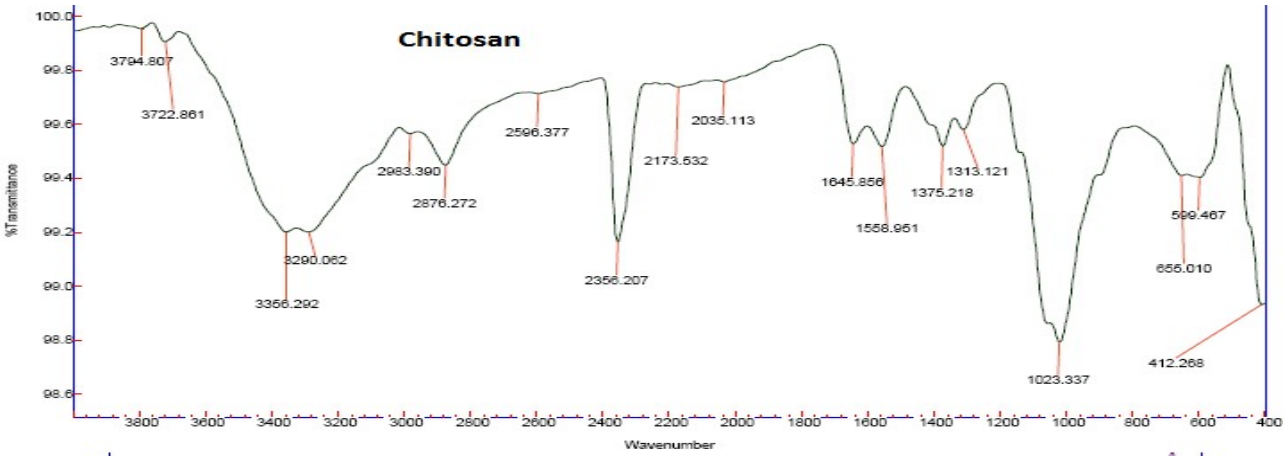

Figure 2: FTIR spectra of Chitosan

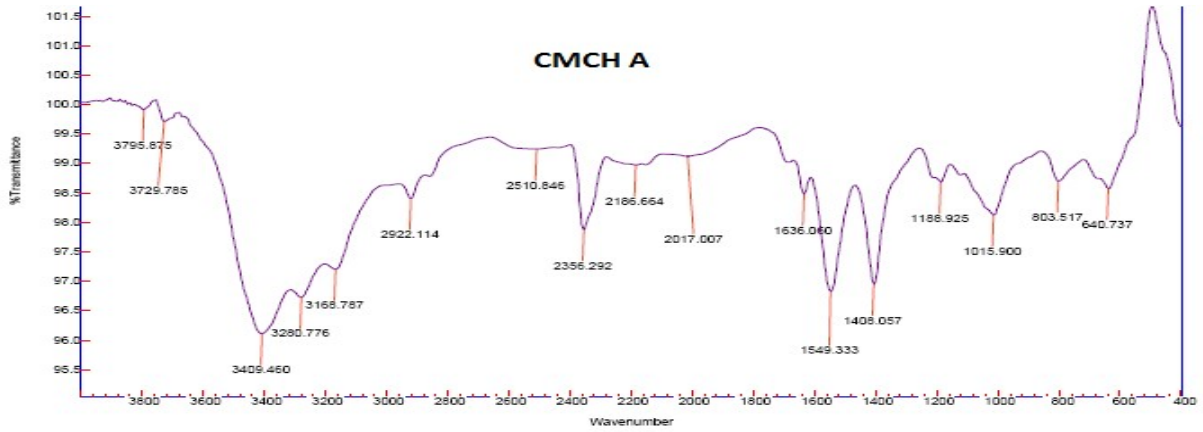

Figure 3: FTIR spectra of Carboxymethyl Chitosan mixture A

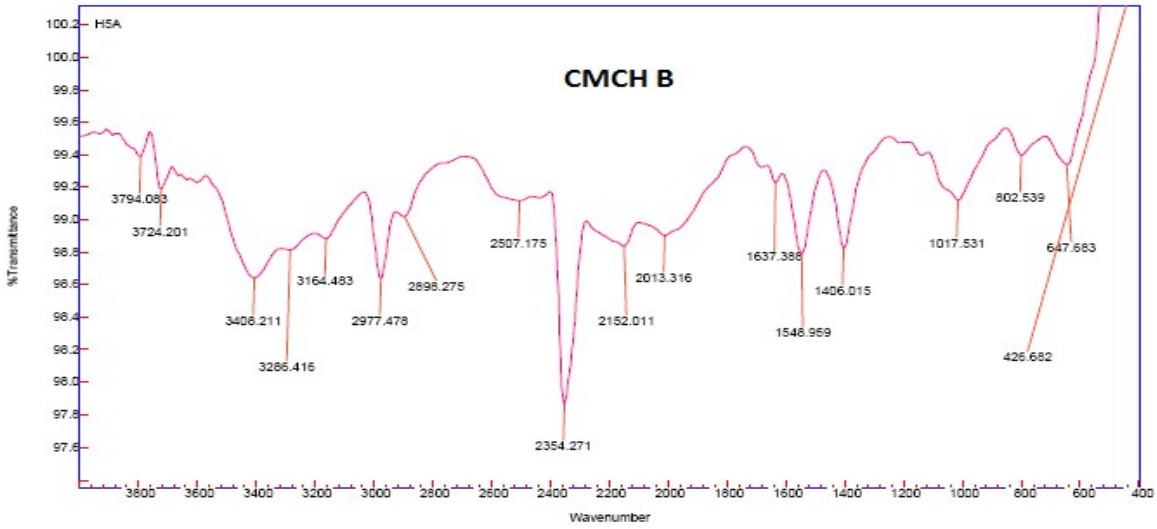

Figure 4: FTIR spectra of Carboxymethyl Chitosan mixture B

Table 4: Represent physical mixture degree of substitution of carboxymethyl chitosan A and B

Type of physical mixture	Degree of substitution	Reported Degree of substitution values
Carboxymethyl chitosan A	0.98	0.52-1.44
Carboxymethyl chitosan B	1.13	

Table 5: Duration of Buoyancy of various HBS formulations

Formulation code	Duration of buoyancy (Min.)
D1	300
D2	600
D3	600
D4	300
D5	600
D6	600

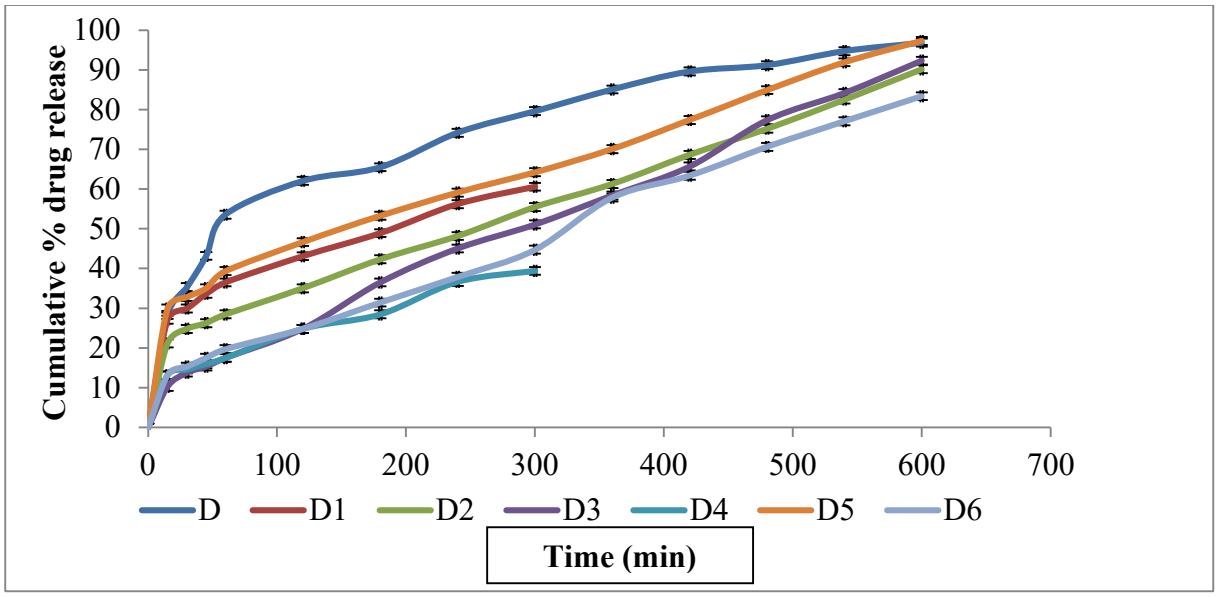

Figure 5: In vitro Drug release from various HBS capsule formulations

Table 6: Drug release kinetics from various HBS formulations

Formulation Code	Drug release kinetic				
	Zero order	First order	Higuchi	Korsmeyer-Peppas	n value
D	0.8131	0.9847	0.9645	0.9456	0.33
D1	0.9839	0.9484	0.936	0.9810	0.28
D2	0.9992	0.9642	0.976	0.9539	0.40
D3	0.9979	0.9233	0.968	0.9796	0.62
D4	0.9901	0.9647	0.982	0.9545	0.39
D5	0.9957	0.9589	0.972	0.9585	0.33
D6	0.9957	0.9587	0.958	0.9526	0.53

CONCLUSION

The goal of the current study was to learn more about the applicability of chitosan, carboxymethyl chitosan, and HPMC polymer a carrier for single unit sustained release formulation to the stomach. HBS capsules based on HPMC, K4M polymer and their combination with carboxymethyl chitosan carrier shown outstanding in vitro drug release (5 to 10 hr) and were found to be capable of supporting the release of verapamil hydrochloride. Taking into account the experimental findings of the current study, it can be said that HPMC, K4M in combination with carboxymethyl chitosan A or B can be used as a potential carrier for specific sustained release of hydrophilic drugs.

ABBREVATIONS

VHCL: Verapamil Hydrochloride, DD: Degree of Deacetylation, HBS: Hydrodynamically balanced System, DS: Degree of Substitution, CMCH: Carboxymethyl Chitosan, IPA: Isopropyl Alcohol, HPMC: Hydroxy Propyl Methyl Cellulose, FTIR: Fourier Transform Infrared,

HCL: Hydrochloric Acid, NaOH: Sodium Hydroxide.

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AUTHOR'S CONTRIBUTIONS

In the present research, Dharmendra Singh and Mukesh Kumar analyzed the data related to sustained release formulation and was the most important contribution in making the manuscript. Charu Saxena, Prashant Kumar and Prasanjit Paul performed the various approaches for preparation of verapamil loaded matrix. Anoop Kumar elaborated the various kinetics models in the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The author has declared that no conflicts of interest exist.

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