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Chitosan Based Biodegradable Hydrogel Microspheres for Controlled Release of an Anti HIV Drug

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ABSTRACT

Biodegradable hydrogel microspheres ofChitosan (CS) and hydroxypropyl cellulose (HPC)were prepared by emulsion- crosslinking method employing glutaraldehyde (GA) as a crosslinker. Valganociclovir hydrochloride (VHCl), an anti HIV drug was encapsulated into IPN microspheres. Microspheres were characterized by Fourier transform infrared spectroscopy (FTIR) to confirm the formation of crosslinking and absence of chemical interactions between drug, polymer and crosslinking agent. Scanning electron microscopy (SEM) was performed to study surface morphology of the microspheres and it showed that microspheres have smooth surface. Microspheres with the average particle sizes ranging from 297µm to 412µm were produced. X-ray diffraction (X-RD) studies were performed to understand the crystalline nature of drug after encapsulation into IPN microspheres. An in vitro release study was performed in phosphate buffer solution pH-7.4 at 37°C. The release rates were fitted to an emperical equation to understand the diffusion parameters, which indicate non-Fickian or anomalous trend release of VHCL.

Key words: *Chitosan; Hydroxypropyl cellulose; interpenetrating polymer networks; Microspheres; Valganociclovir hydrochloride.*

1. INTRODUCTION

Controlled drug delivery technology represents the more rapidly advancing area in recent years due to the involvement of multidisciplinary scientists, who are contributing to the human health care related problems. The drug delivery systems offer numerous advantages as compared to conventional dosage forms, such as improved efficiency, reduced toxicity, and improved patient compliance and convenience [1]. In the recent past, carbohydrate and biodegradable polymers have been extensively used to develop the controlled release (CR) formulations [2-3] to decrease the release rates of drugs having short plasma life. Among the various polymers employed, hydrophilic biopolymers are quite suitable in oral applications [4] due to their inherent advantages over the synthetic polymers. Chitosan, the deacetylated derivative of chitin, is one of the most abundant naturally occurring polysaccharides. Recently, it has attracted much interest in the biomedical industry because of its excellent biodegradability, biocompatibility, antimicrobial activity and accelerated wound healing properties [5-7]. Even though several industry, Chitosan (CS) has been one of the most widely used polymers due to its reduced toxicity and better patient compliance [8] and used in drug delivery applications [9-11]. However chitosan has some disadvantages because it is only soluble in acetic acid and low mechanical properties. Therefore, it is difficult for controlled drug-release behavior because of the various pHs of the internal organs of the human body. In order to improve their properties the chitosan blended with other hydrophilic polymers. The drug release was also enhanced by adding hydrophilic polymers with chitosan. Hydroxypropyl cellulose (HPC) is an alkyl- substituted hydrophilic cellulose derivative. HPC has many advantages such as excellent film forming properties, degradability, and biocompatibility [12-13]. HPC has been a focus of research because of desirable properties and its industrial applications [14-16]. HPC shows phase transition behaviour in aqueous solution and in some solvents [17]. The modified HPC used in the preparation of HPC hydrogel [18], and HPC blends studied with PVA, PAA etc based on hydrogen bonding [19].

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Valganociclovir hydrochloride (VHCl), a hydrochloride salt of the L-valyl ester of ganciclovir that exists as a mixture of two diastereomers. VHCl is used for the treatment of cytomegalovirus retinitis in patients with AIDS. It is chemically known as 2-[(2-amino-6-oxo-6, 9-dihydro- 3H-purin-9-yl) methoxy]- 3-hydroxypropyl (2S)-2-amino-3-methylbutanoate [20]. In continuation of our research work on the development of controlled release devices [21-23], utilizing carbohydrate polymers, presently we are aiming to prepare hydrogel microspheres, consisting of CS and HPC loaded Valganociclovir hydrochloride (VHCl). The prepared microspheres have not presented in the literature for drug delivery applications. In order to investigate the release of VHCl from the microspheres, VHCl loaded hydrogel microspheres were prepared by varying blending ratio, VHCl content and amount of cross linking agent. In vitro release studies have been performed by dissolution experiments. Release data have been discussed in terms of Fickian equation and diffusion parameters.

2. EXPERIMENTAL

2.1. Materials

High molecular weight chitosan (degree of deacetylation 85%, viscosity and 800-2000 cPs) and Hydroxypropyl cellulose with a molecular weight (15,000) were purchased from Aldrich Chemical Company, Milwaukee, WI, USA. Valganociclovir hydrochloride drug was received as gift sample from Apotech laboratory, Bangalore, India. Analytical reagent grade glutaraldehyde solution 25% (v/v), n-hexane and light liquid paraffin were all purchased from s.d.fine chemicals, Mumbai, India. Span-80 was purchased from Loba Chemicals, Mumbai, India. All the chemicals were used without further purification.

2.2. Preparation of hydrogel microsphere

The hydrogel microspheres were prepared by emulsion-crosslinking method. CS was dissolved in 2% aqueous acetic acid solution by continuously stirring to form a homogeneous solution and HPC was dissolved in distilled water. The required ratios (Table-1) of two polymer solutions were mixed and stirred until to obtained homogeneous solution. Then, VHCl (10. 15, 20 wt %) was dissolved in the above polymer solution. This solution was added slowly to a light liquid paraffin (100g, w/w) containing 1% Tween-80 under constant stirring at 400 rpm speed for 10 min. To this w/o emulsion, GA containing 1N HCl was added slowly and stirring was continued for 3h. The harden microspheres were separated by filtration and washed with n-hexane. Finally, the microspheres were washed with distilled water to remove the unreacted GA. The microspheres were vacuum dried at 40^{o}C for 24h and stored in desicator until further use. Totally, eight formulations were prepared and assigned formulation codes are given in Table.1.

2.3. Drug content

Microspheres of known weight (~10 mg) were crushed using an agate mortar and extracted with 50 ml of water and then sonicated using a probe sonicator (UP 400 s, dr. hielscher, GmβH, Germany) for 15 min to break the microspheres. The whole solution was centrifuged (R-8C DX Remi, India) to remove the polymeric debris. The polymeric debris was washed twice to extract the drug completely. The clear supernatant solution was analyzed by UV spectrophotometer (LABINDIA, UV3000^{+}) at the λ_{max} value of 255nm. Encapsulation efficiency was calculated as:

$$\%\ \text{Drug loading} = \left(\frac{\text{Amount of drug in } MPs}{\text{Amount of } MPs}\right) \text{x} 100 \quad (1)$$

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) \text{x} 100 \quad (2)$$

These data for various formulations are given in Table 1.

2.4. Particle size measurements

Particle size and distributions were measured using a laser light scattering technique (Mastersizer-200, Malvern, UK). Particle size was measured using the dry sample adopter. These data are also included in table 1.

2.5. Fourier transform infrared (FTIR) spectral studies

FTIR spectral data were taken on a Perkin Elmer instrument (model Impact 410, Wisconsin, MI, USA) to confirm the formation of IPN structure and also to find the chemical stability of the drug in the microspheres. FTIR spectra of the placebo CS, placebo HPC, placebo microspheres, drug-loaded microspheres and pristine VHCl drug were obtained. Samples were crushed with KBr to get pellets at 600 kg/cm2 pressure. Spectral scanning was done in the range between 4000 and 400 cm^{-1}.

2.6. X-ray diffraction (X-RD) studies

Crystallinity of VHCl drug after the encapsulation was evaluated by X-ray diffraction (X-RD) measurements recorded for pristine VHCl drug, placebo microspheres and drug-loaded microspheres using X-ray diffractometer (x-Pert, Phillips, UK). Scanning was done up to 2θ of 50^{o}C.

Table 1: Results of % of encapsulation efficiency, mean particle size and water uptake of different formulations.

Sample code	Chitosan (w/w %)	HPC (w/w %)	GA (ml)	Drug (w/w %)	Mean particle size (μm)	% Encapsulation efficiency	% water uptake
CH-1	60	40	3	10	348±25	73.24±17	325
CH-2	60	40	3	15	362±07	76.27±19	372
CH-3	60	40	3	20	387±09	78.31±34	396
CH-4	60	40	2	15	394±31	79.05±26	402
CH-5	60	40	4	15	318±28	70.19±05	317
CH-6	80	20	3	15	347±16	71.16±23	384
CH-7	40	60	3	15	412±43	80.13±47	412
CH-8	100	-	3	15	397±05	67.03±21	297

2.7. Scanning electron microscopic (SEM) studies

SEM images of microspheres prepared by crosslinking with 5ml of glutaraldehyde and loaded with VHCl. Microspheres were sputtered with gold to make them conducting and placed on a copper stub. Scanning was done using JEOL model JSM-840A, Japan instrument available at Indian Institute of Science, Bangalore, India. The thickness of the gold layer accomplished by gold sputtering was about 15 nm.

2.8. Swelling studies

Equilibrium water uptake of the crosslinked microspheres loaded with the drug was determined by measuring the extent of swelling of the matrix in water. To ensure complete equilibration, samples were allowed to swell for 24h. The excess surface adhered liquid drops were removed by blotting with soft tissue papers and the swollen microspheres were weighed to an accuracy of 0.0001g using an electronic microbalance (Adam, AFP-210L). The microspheres were then dried in an oven at 60^0C for 5h until there was no change in the dried mass of the samples. The % equilibrium water uptake was calculated as:

$$\%\,\text{Water uptake} = \left(\frac{\text{Wt of swollen } MPs\ (w_1) - \text{Wt of dry } MPs\ (w_2)}{\text{Wt of dry } MPs\ (w_2)} \right) \times 100 \quad (3)$$

2.9. In vitro release

In vitro release studies have been carried out by performing the dissolution experiments using a tablet dissolution tester (LABINDIA, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37^0C under 100 rpm rotor speed. Drug release from the microspheres was studied in an intestinalfluid (pH-7.4 phosphate buffer solution). At regular intervals of time, sample aliquots were withdrawn and analyzed by UV spectrophotometer (LABINDIA, UV3000$^+$) at the fixed max value of 255 nm.

3. RESULTS AND DISCUSSION

3.1. Fourier transform infrared spectroscopy

FTIR spectra of (a) plain CS, (b) plain HPC, (c) placebo microspheres, (d) drug loaded microspheres, and (e) plain VHCl were shown in Fig.1. The FTIR spectroscopy was used to investigate the effect of crosslinking and chemical stability of the drug after encapsulation into the matrix. In case of plain CS, a broad peak at 3427cm^{-1} is attributed to –N-H stretching vibration. The peak at 2937 cm^{-1} represents the –C-H stretching vibration. The 1647, 1587 and 1382 cm^{-1} indicates the amide symmetric and bending vibrations. The HPC showed a broad peak at 3454 cm^{-1} due to –O-H stretching vibrations. The peak at 2928 cm^{-1} shows the aliphatic C-H stretching vibration. In case of placebo microspheres, all the peaks of CS and HPC were observed in addition to a new band observed at 1632 cm^{-1}, which confirmed the –C=N stretching vibration of imine group of Schiff base. The peak at 1021cm^{-1} is due to the presence of an acetal group, which is confirmed due to the reaction of GA with hydroxyl groups of HPC. Thus, FTIR confirms the crosslinking reaction between CS and HPC.

FTIR spectral data were also used to confirm the chemical stability of VHCl in the microspheres. The FTIR spectra of plain drug VHCl (Fig.2-e) shows the characterisitc absorption peaks at 3426, 2966,1745,1693 and 1537cm^{-1} indicated the –OH,-NH_2,-CH, –C=O and –C-N stretching vibrations. All these drug peaks that were observed in VHCl drug loaded microspheres, indicating the stability of VHCl after encapsulation into the polymer matrix.

3.2. X-ray diffraction (X-RD)

X-RD analysis can provide a clue about crystallinity of the drugs in cross-linked microspheres. XRD patterns recorded for (a) plain

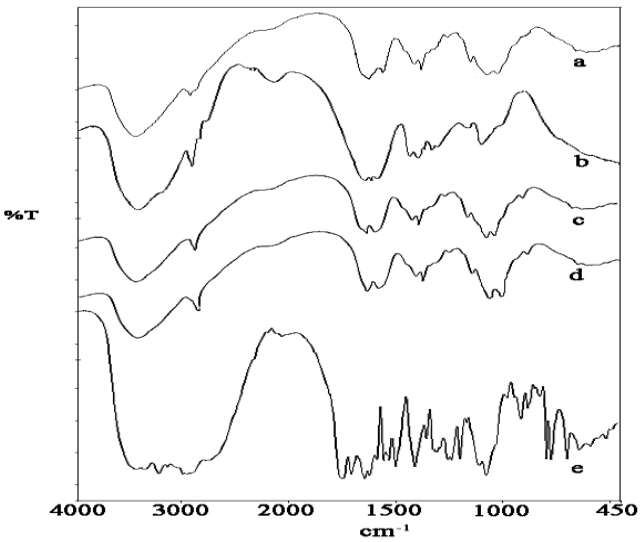

Figure 1. FTIR spectra of (a) plain CS, (b) plain HPC, (c) placebo microspheres, (d) drug loaded microspheres, and (e) plain VHC drug.

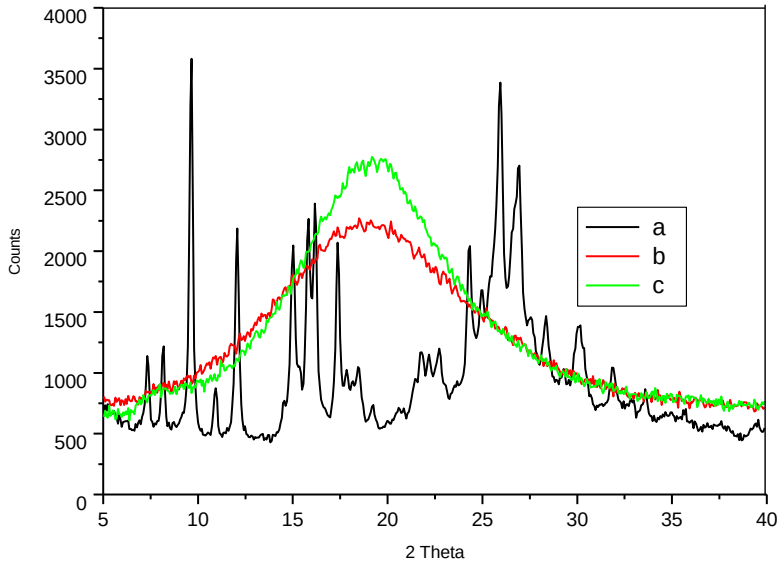

Figure 2. X-RD spectra of (a) plain VHCl drug, (b) placebo microspheres and (c) drug loaded microspheres.

VHCl, (b) pristine microspheres, and (c) drug loaded microspheres are presented in Fig. 2. Here,VHCl peaks observed at 2θ of 9.6,12, 15.8 and 26.9^0 are due to the crystalline nature of VHCl. These peaks are not found in the drug loaded microspheres. This indicates that drug particles are dispersed at molecular level in the polymer matrices since no indication about the crystalline nature of the drugs was observed in the drug-loaded microspheres.

3.3. Scanning electron microscopy

Scanning electron micrograph of (a) group of microspheres and (b) a single microsphere are shown in Fig. 3. Microspheres are spherical without forming agglomeration and their surfaces are slightly rough. However, polymeric debris seen around some particles could be due to the method of particle production (i.e., simultaneous particle production and formation of the blend matrix). Microspheres produced by blending of different polymers did not show and effect on the surface properties.

3.4. Microscopic study

Particle size was also measured alternatively by optical microscopy. These results along with % encapsulation efficiency, % drug-loading and mean particle size for different formulations are presented in Table 1. The size of particles depends on the amount of drug present, % HPC content and extent of GA employed. Particles are generally

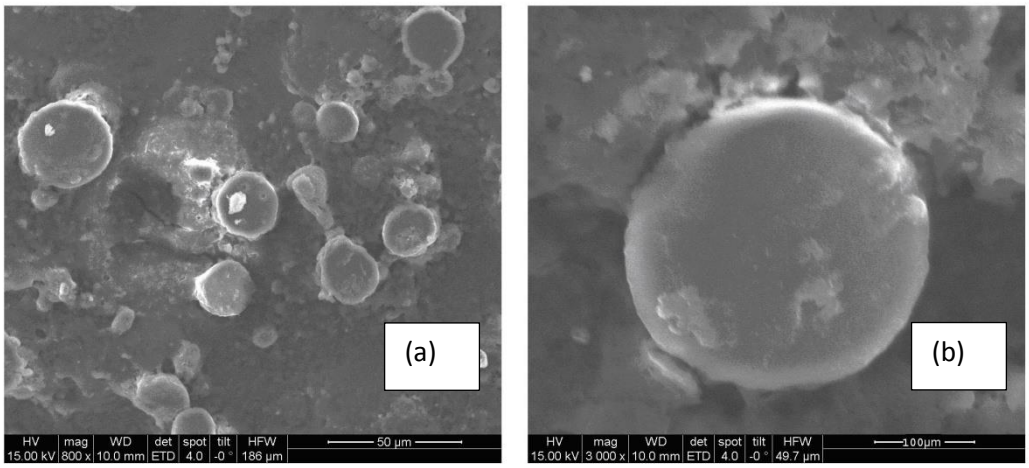

Figure 3.Scanning electron micrograph of (a) group of microspheres, and (b) a single microsphere.

spherical in shape with sizes ranging from 318 to 412 μm. Particle size of the pristine CS is higher than those of CH microsphere. By increasing the HPC content of the microspheres, size of the microspheres increased from 347 to 412μm for 15% VHCl-loaded microspheres. This can be explained on the basis of hydrodynamic viscosity concept, i.e., as the amount of HPC in microspheres increase, interfacial viscosity of the polymer droplets in the emulsion also increases. On the other hand, with increasing amount of HPC, the number of free sites available for cross-linking is less so that size of the microspheres will also increase with increasing HPC content of the microspheres. For instance, as the amount of HPC increases from 20% to 60%, the particle size has increased from 347 to 412 μm. For all the formulations, with increasing amount of drug in the microspheres, particle size also increased. For formulations containing 40% HPC and microspheres loaded with different amounts of drug, particle size has increased from 348 to 387μm; a similar trend was also observed for all other formulations (see Table 1). This is attributed to the fact that drug molecules might have occupied the free volume spaces within the matrix, thereby hindering the inward shrinkage of the polymer matrix [24]. However, the extent of cross-linking has shown an effect on particle size (see data in Table 1). For microspheres containing 40 wt% HPC and 15 wt% VHCl with increasing amount of GA from 2 to 4 ml, the particle size has decreased from 394 to 318 μm. This is attributed to the fact that with increasing amount of GA in the IPN matrix, the shrinkage of particles has taken place, thereby reducing their size [25].

3.5. Encapsulation efficiency

Three different concentrations of VHCl i.e., 10, 15 and 20 wt% were loaded during the crosslinking of the microspheres. Results of % encapsulation efficiency included in Table-1 show increasing trends with increasing drug loading. Encapsulation efficiency of 67.03% was observed for pristine CS microspheres, but for the remaining formulations, it was ranged from 70.19 % to 80.13 %. Notice that % of encapsulation efficiency increased with increasing amount of HPC in the microspheres. For the microspheres containing 20, 40 and 60 wt% HPC and 15 wt % VHCl with 3ml GA, encapsulation efficiencies were 71.16%, 76. 27%, and 80.13%, respectively. For microspheres crosslinked with 2, 3 and 4 ml of GA, encapsulation efficiencies was respectively, 79.05%, 76.27% and 70.19%. Such a decreasing trend is due to an increase in crosslink density, because the microspheres will become rigid, thereby reducing the free volume spaces within the polymer matrix and hence, a reduction in encapsulation efficiency is observed.

3.6. Swelling studies

The % equilibrium water uptake of the crosslinked microspheres presented in Table-1 indicate that, as the amount of GA in the matrices increased from 2 to 4, the equilibrium water uptake decreased significantly from 402 to 317%. Such a reduction in water uptake capacity is due to the formation of a rigid network structure at higher concentration of crosslinking. Hence, the crosslinking of microspheres has a great influence on the equilibrium water uptake as well as the release rates. Notice that formulations containing higher amount of HPC showed higher swelling rates than formulations containing lesser amount of HPC. Thus formulation CH-7 (60%, w/w, HPC) exhibited a higher swelling than formulation CH-2 (40%, w/w, HPC), similarly formulation CH-2 exhibited a greater swelling than formulation CH-6 (20%, w/w, HPC), due to hydrophilic nature of

HPC, there by leading to higher water uptake capacity.

3.7. Drug release kinetics

Drug release kinetics was analysed by plotting the cumulative release data vs time by fitting these data to exponential equation of the type [26].

$$(M_t/M_\infty)=kt^n \quad (4)$$

Here M_t and M_∞ represent the fractional drug release at time t, k is a constant characteristic of the drug-polymer system and n is an empirical parameter characterizing the release mechanism. Using the least square procedure, we have the values of n and k for all the formulations and these values are given in Table-2. If n= 0.5, the drug diffuses and release from the polymer matrix following a Fickian diffusion. For n > 0.5, anomalous or non-Fickian drug diffusion occurs. If n = 1, a completely non-Fickian or case-II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to an anomalous type diffusive transport. The values of k and n have shown a dependence on the extent of crosslinking, % drug loading and HPC content of the matrix. Values of n (0.612 to 0.597) for microspheres prepared by the varying amount of HPC in the microspheres of 20, 40, and 60 wt% by keeping VHCl (15%) and GA (3ml) constant, showed the non-Fickian type release. The VHCl loaded microspheres exhibited the n values ranged from 0.528 to 0.695 (see Table-2), indicating non-Fickian type mechanism. This may be due to the reduction in the regions of low microviscosity and closure of microcavities in the swollen state of the polymer. Similar findings have been observed elsewhere, where in the effect of different polymer ratios on dissolution kinetics was studied [24]. On the other hand, the values of k are smaller for drug loaded microspheres, suggesting their lesser interactions compared to microspheres containing varying amounts of HPC.

Table 2: Release kinetics parameters of different formulations.

Sample code	k	n	r
CH-1	0.198	0.528	0.941
CH-2	0.149	0.559	0.976
CH-3	0.126	0.604	0.979
CH-4	0.021	0.695	0.963
CH-5	0.181	0.679	0.963
CH-6	0.115	0.612	0.982
CH-7	0.180	0.597	0.942
CH-8	0.121	0.502	0.949

3.8. Effect of drug loading

Fig.4. Shows the release profiles of drug loaded microspheres at different amount of drug loadings. Release data showed that formulations containing the higher amount of drug (20 wt %) displayed the fast and higher release rates than those formulations containing smaller amount of VHCl. A prolonged release was observed for the formulation containing a lower amount of VHCl. Notice that the release rate becomes quite slower at the lower amount of drug in the matrix, due to availability of more free void spaces through which a lesser number of drug molecules will transport.

3.9. Effect of crosslinking

The % cumulative release vs time curves for varying amounts of GA, i.e., 2, 3 and 4 ml at a fixed amount of drug (15 wt%) are displayed in fig. 5

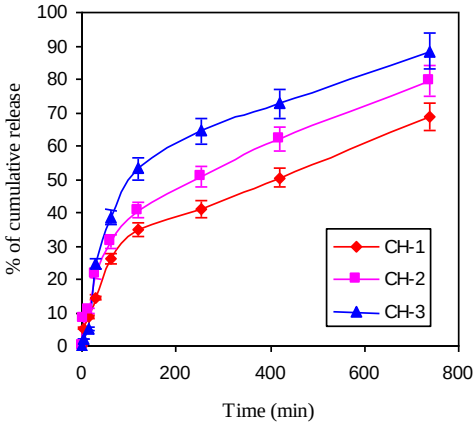

Figure 4. Percentage of cumulative release of VHCl through microspheres containing different amounts of VHCl. CH-1(10 wt %), CH-2 (15 wt%), and CH-3 (20 wt%).

The cumulative release is somewhat smaller when lower amount of GA was used probably because at higher concentration of GA, the polymeric chains would become rigid due to the contraction of microvoids, thus decreasing the % cumulative release of VHCl through the polymeric matrices. As expected, the release becomes slower at higher amount of GA, but becomes faster at lower amount of GA.

3.10. Effect of HPC

The effect of HPC content was studied at a constant loading of 15wt% VHCl with different amounts of HPC (20%, 40% and 60 wt %) are displayed in Fig.6. The % cumulative release is quite fast and larger at higher amount of HPC (60 wt %), where as the release is quite slower at lower amount of

HPC (20 wt %) (CH-7> CH-2> CH-6). All the formulations showed higher release rates than purechitosan, because with an increasing of HPC content, swelling of the matrix also increased due to more hydrophilic nature of HPC.

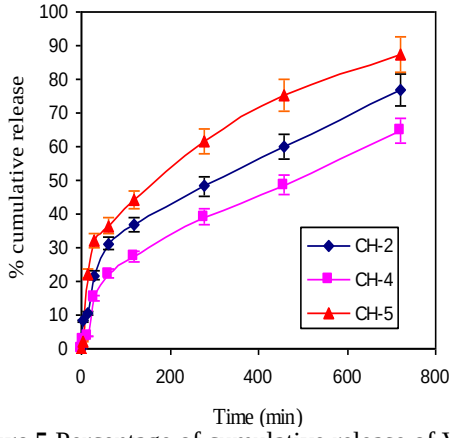

Figure 5. Percentage of cumulative release of VHCl through microspheres containing different amounts of crosslinking agent.CH- 2 (3ml), CH- 4 (2ml), and CH- 5 (4ml).

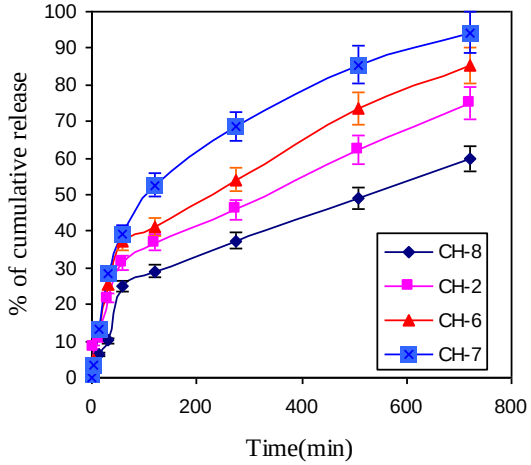

Figure 6. Percentage of cumulative release of VHCl through microspheres containing different amounts of HPC, CH- 8 (0 wt %), CH- 2 (40 wt %), CH- 6 (20 wt %) and CH- 7 (60 wt %).

4. CONCLUSIONS

The present study describes the preparation of hydrogel microspheres of chitosan and hydroxypropyl cellulose to study the controlled release of valganociclovir hydrochloride using water-in-oil emulsification method. The microspheres exhibited encapsulation efficiency up to a maximum of 80%. FTIR confirmed the formation of crosslinking as well as chemical stability of VHCl in the microspheres. SEM micrographs exhibited a spherical morphology of the prepared microspheres. The microspheres with the average particle size ranging from 318µm to 412µm. Swelling kinetics was dependent on the extent of matrix drug loading, crosslinking and HPC content of the matrix. The release mechanism showed a non- Fickian behavior. The microspheres of this study could be used as controlled release devices for the delivery of VHCl. By observing all the results the hydrogel microspheres were a quite promising for controlled release of anti-HIV drug. The prolong release rates of VHCl were observed upto 12h. The hydrogel microspheres can lead to a successful application for localized drug delivery.

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