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Preparation and characterization of microspheres for controlled release of anti HIV drug

P. Sudhakar[1], S. N. Bhagyamma[2], S. Siraj[1], K. V. Sekharnath[1], K. Chowdoji Rao[3], M. C. S. Subha[1*]

[1]Department of Chemistry, S.K University, Anantapuramu, A.P., India. [2]Department of Biochemistry, Govt. Medical college, Anantapuramu, A.P., India.
[3]Department of Polymer Science & Tech, S.K University, Anantapuramu, A.P., India.

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ABSTRACT

A series of blend microspheres were developed from gelatin and hydroxypropyl cellulose (HPC) by emulsion crosslinking method employing glutaraldehyde (GA) as a crosslinker. Valganociclovir hydrochloride (VHCL), an anti HIV drug, was loaded in to these microspheres via insitu method. These 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. Further the microspheres were characterized by scanning electron microscopy to study the surface morphology of the microspheres and observed that the microspheres have smooth surface with spherical structure and no phase separation. The microspheres with the average particle sizes ranging from 614.5µm to 693.4µm were obtained. X-ray diffraction (X-RD) studies were performed to understand the crystalline nature of drug and its uniform distribution into blend microspheres. An in vitro release study was performed in phosphate buffer solution (pH-7.4) at 37^0C. The release rates were fitted to an emperical equation to understand the diffusion parameters, which indicate non-Fickian or anomalous trend release of VHCL. Further the results indicated that the release of drug was found for more than 12 h.

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 (Uhrich *et al*., 1999). In particular gelatine microspheres (GMS) are widely accepted as an efficient drug carrier for various administration routes including nasal, gastrointestinal and rectal (Brime *et al*., 2000; Leucuta *et al*., 1997; Wang *et al*., 2001; Nakase *et al*., 2002). Encapsulation of drug molecules in particulate carriers as a method of controlled delivery of molecules has been studied extensively. In recent years, a number of different particulate systems, such as microspheres and microcapsules, have been proposed and used in topical formulations as drug carrier vehicles (Jansen and Maibatch., 2001). Natural polymers, such as gelatin, have been widely used for the preparation of particulate drug delivery systems. Generally it is soluble in hot water and on cooling it forms gel solution. Use of gelatin in pharmaceutical and biomedical field is very attractive since it is a nontoxic, biodegradable, inexpensive, nonimmunogenic material and has a very high potential for use with a variety of medicinal agents. It has also been used as wound dressing material (Choi *et al*., 1999; Ulubayram *et al*., 2001; Drave *et al*., 1998; Takahaski *et al*., 1993; Neumann *et al*., 1981), as hemostatic and wound healing agent (Petersen *et al*., 1984; Sung *et al*., 1999), as sealant for vascular prosthesis (Jonas *et al*., 1988; Chakfe *et al*., 1993), and in drug delivery systems such as hard and soft capsule (Digenis *et al*., 1994; Li *et al*., 1998), hydrogel (Tabata *et al*., 1998), or microsphere (Cortesi *et al*., 1998; Narayani & Rao., 1994; Lou & Groves., 1995). Another important natural polymer, Hydroxypropyl cellulose (HPC) belongs to the group of cellulose ethers which has been used in paper industry as glue and sizing material.

** Corresponding Author*
Subha Chinnasubbarao Marata, Department of Chemistry, Sri Krishnadevaraya University, Anantapur, INDIA.
Email: mcssubha3@gmail.com

The material is soluble in water as well as in polar organic solvents (Hofenk-de Graaff *et al*., 1981), HPC shows phase transition behaviour in aqueous solution and in some solvents (Cai *et al*., 2003; Nymweya *et al*., 2000; Suto *et al*., 1999; Aden *et al*., 1984; Immaneni *et al*., 1997; Jiang *et al*., 1999). It also has many advantages such as excellent film forming properties, degradability, biocompatibility etc. (Khutoryanskiy *et al*., 2004; Nurkeeva *et al*., 2001), HPC has been a focus of research because of these unusual and desirable properties, and it has been improved as prospective material in many industrial applications (Adrados *et al*., 2001; Fujii *et al*., 2001; Robitaille *et al*., 1991). Most studies mainly focused on modified HPC, HPC hydrogel, crosslinked HPC nanoparticles and HPC blends with PVA, PAANa, CH, PVME, PVP etc. Valganciclovir hydrochloride (VHCl), a hydrochloride salt of the L-valyl ester of ganciclovir that exists as a mixture of two diastereomers. Ganciclovir is a synthetic guanine derivative active against cytomegalovirus (CMV). Valganciclovir hydrochloride is a white to off-white crystalline powder with a molecular formula of $C_{14}H_{22}N_6O_5$. HCl. The chemical name for valganciclovir hydrochloride is L-Valine, 2[(2-amino-1, 6-dihydro-6-oxo-9H- purin-9-yl) methoxy]-3-hydroxypropylesterhydrochloride and VHCl is a polar hydrophilic compound with a solubility of 70 mg/mL in water at 25^0C. Valganciclovir, also called valcyte or valgan, is being used in treatment for an AIDS-related complication called CMV-retinitis. This is caused by a virus called cytomegalovirus (CMV) which infects the eye. If left untreated, CMV retinitis can cause people with HIV/AIDS (PHAs) to go blind. Polymer blending constitutes a most useful method for the improvement or modification of the physicochemical properties of polymeric materials. Some of the polymer blends exhibit unusual properties, which are different from the constituent homo polymers. An important property of a polymer blend is the miscibility of its components, because it affects the mechanical properties, the morphology, permeability and degradation (Paul *et al*., 1978; Olabisi *et al*., 1979). In this study we prepared the blend microspheres of Gelatine/HPC by simple water-in-oil emulsion technique with high water swell ability and high mechanical properties. Further these GC-HPC blend matrix microspheres were crosslinked with glutaraldehyde and are used for controlled release of Anti HIV drug at pH-7.4. Alternately, dried at room temperature for 24 h and stored in a desiccator until for further experimentation. Totally, eight formulations were prepared and used for drug release studies. These microspheres were characterized by Differential scanning calorimetry (DSC), Scanning electron microscopy (SEM), and X-ray diffractometry (X-RD) and these results are presented here.

EXPERIMENTAL

Materials and methods

Materials

Gelatin and Hydroxypropyl cellulose were purchased from Aldrich Chemical Company, Milwaukee, WI, USA. Glutaraldehyde, petroleum ether, paraffin oil and Tween-80 were all purchased from S.d fine Chemicals, Mumbai, India. All the chemicals used were analar grade and were used as received. Double distilled water was used throughout the study.

Preparation of Gelatin and Hydroxypropyl cellulose microspheres

The microspheres were prepared by emulsion-crosslinking method. Gelatin was dissolved in hot water and HPC was dissolved in distilled water. The required ratios of two polymer solutions were mixed and stirred until homogeneous solution was obtained. Then, VHCL (10, 20, 30 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 3 h. 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 vaccum dried at 40^0C for 24 h and stored in a desiccator until further use. Totally, eight formulations were prepared and the assigned formulation codes are given in table.1.

Characterization of blend microspheres

FT-IR spectroscopy (FTIR) studies

Fourier transforms infrared spectroscopy (FTIR) spectral measurements of pristine microspheres, drug loaded microspheres and pure Anti HIV drug were recorded using Perkin Elmer (model Impact 410, Wisconsin, MI, and USA) spectrophotometer. The microspheres were finely grounded with KBr to prepare the pellets under a hydraulic pressure of 600 dynes/m^2 and spectra were scanned between 4000 to 500 cm^{-1}.

Differential scanning calorimetric (DSC) studies

DSC analysis of pristine microspheres, drug loaded microspheres and pure Anti HIV drug were recorded on a TA instrument (Model: STA, Q_{600} USA). A sample weight of 10±2 mg was used in each experiment. The samples were heated from 30 to 400 ^{o}C at a heating rate of 10 ^{o}C/min in nitrogen atmosphere (flow rate 100 mL/min).

X-ray diffraction (X-RD) studies

XRD measurements of plain drug, drug-loaded microspheres and plain microspheres were recorded with a Rigaku Geigerflex Diffractometer equipped with Ni-filtered Cu K α radiation (λ= 0.1548 nm). The dried microspheres of uniform thickness were mounted on sample holder. The samples were scanned at a rate of 5^0/minute from 0^0 to 50^0 of 2θ.

Scanning electron microscopic (SEM) studies

SEM images of microspheres were recorded using a JSM 6400 SEM (JEOL Ltd., Akishima, Tokyo, Japan) at 50x magnification. Working distance of 9.0 mm was maintained and

the acceleration voltage used was 20 kV, with the secondary electron image (SEI) as a detector.

Swelling studies

Equilibrium swelling studies of microspheres were performed in water at room temperature. The weight of the dried microspheres measured directly and its weight is Wd then the dried microspheres were suspended in glass vessels containing 50 ml of water at 37^{0}C. After 24 hr the swollen microspheres were taken out and immediately weighed after removal of excess of water by using a blotter. The procedure was repeated until the microspheres reached constant weight (equilibrium water uptake). The swelling ratio of microspheres were calculated from the following equation:

$$\% SR = (Ws - Wd/Wd)X\ 100 \text{----------(1)}$$

Here Wd and Ws were the weight of dry and swollen microspheres, respectively.

Estimation of drug loading and encapsulation efficiency

The loading efficiency of Anti HIV drug in the microspheres were determined spectrophotometrically. About ~10 mg of the drug-loaded microspheres were placed in 30 mL of buffer solution and stirred vigorously for 48 h to extract drug from the microspheres. The solution was filtered and assayed by UV spectrophotometer (Lab India, Mumbai, India) at fixed λ_{max} value of 255 nm. The results of % drug loading and % encapsulation efficiency were calculated by following Eqs.

$$\% \text{ Drug loading} = \left(\frac{\text{Amount of drug in MGs}}{\text{Amount of MGs}}\right) x100 \quad \text{...(2)}$$

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) x100 \quad \text{--- (3)}$$

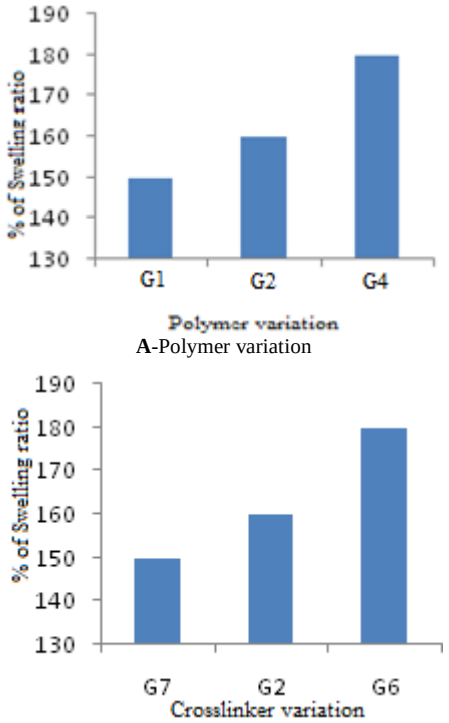
In vitro release studies

In vitro release studies were carried out using Tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37 $\pm$ 0.5 ^{o}C at constant speed of 100 rpm. Drug release from the microspheres were carried out in pH 7.4 phosphate buffer solutions. At regular intervals of time, sample aliquots were withdrawn and analyzed using UV spectrophotometer (Lab India, Mumbai, India) at the fixed λ_{max} value of 255 nm. After each sample collection, the same amount of fresh medium at the same temperature was added to the release medium to maintain the sink condition. All measurements were carried out in triplicate, and values were plotted with standard deviation errors.

RESULTS AND DISCUSSION

Swelling studies

The Gelatin-HPC blend microspheres swelling properties were influenced by the amount of Hydroxypropyl cellulose (Fig 1.a) and crosslinker (GA) (Fig 1.b). As the amount of HPC increases the swelling ratio of microspheres increase, it may be due to the enhancement of hydrophilic polymer chains with increase in HPC concentration. In the case of GA crosslinker variation, the swelling ratio decreases with the increase of crosslinker, it may be due to the formation of polymeric chains which become rigid network as a result of contraction of microvoids. The Various formulations and swelling ratios are shown in the Fig 1.



A-Polymer variation

B-B Crosslinking agent variation

Fig. 1: Variation of % swelling ratio with (a) Concentration of polymer and (b) Crosslinking agent.

Structural Characterization

Fourier transforms infrared (FTIR) study

FTIR spectral analysis were carried out for pure gelatin (a), pure HPC (b), Gelatin/HPC blend microspheres (c) and drug loaded blend microspheres (d) and shows in Fig. 2A & 2B. This study confirms the cross linking of Gelatin/HPC blend microspheres by GA as well as to confirm the absence of chemical reaction between drug and polymers.

The FTIR spectra of pure gelatin (a) revealed the presence of characteristic functional group at 3443cm^{-1} for amino group. In the spectrum of gelatin the other notable peaks observed were at 2917 cm^{-1} (C-H stretching of alkenes), 2823 cm^{-1} (C-H stretching of alkenes) and 1612 cm^{-1} (amide, CO and CN stretching). Other peaks observed were at 1152 cm^{-1}, 1030 cm^{-1} due to, C-O stretching of carboxylic acid and C-N stretching of amines respectively.

The broad peak in the pure HPC spectrum (b) at 3600-3100 cm^{-1}, with a maximum at 3460 cm^{-1}, was assigned to stretching vibrations of the –OH groups. (c) The appearance of a

sharp peak in Fig.2A(c) appears at 1610 cm^{-1} confirms the formation of ether linkages due to cross linking reaction. when the drug was incorporated into the cross-linked Gelatin/HPC blend microspheres, additional peaks have appeared due to the presence of VHCL in the matrix.

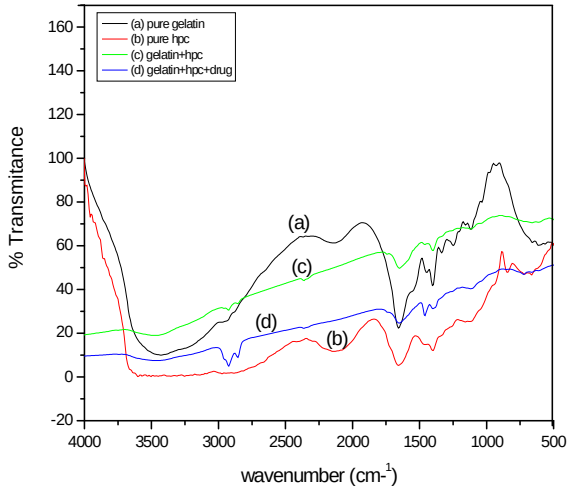


Fig. 2A: FT-IR spectra of pure gelatine (a), pure hydroxypropyl cellulose (b), Gelatin + Hydroxy propyl cellulose blend microspheres(c) and drug loaded blend microspheres (d).

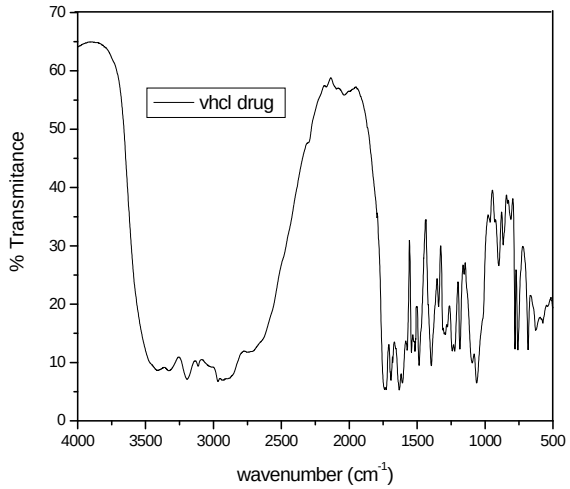


Fig 2B: FT-IR spectra of Valganciclovir hydrochloride (pure drug).

The plain drug VHCL (Fig 2B) shows the characteristic absorption peaks at 3426, 2966, 1745, 1693 and 1537 cm^{-1} indicated the –OH, -NH_2, -CH, -C=O and –C-N stretching vibrations. The characteristic bands of VHCL such as –C=O stretching and –C-N stretching vibrations appeared at 1693 cm^{-1} and 1537 cm^{-1} respectively, in the drug loaded matrix (Fig.2A.d) without any change. This indicates that VHCL did not undergo any chemical changes while forming the microspheres.

Differential scanning calorimetric (DSC) studies

DSC thermograms of pure VHCl drug (a), plain Gelatin-HPC blended microspheres (b) and VHCl-loaded Gelatin-HPC microspheres (c) are displayed in Fig 3. VHCl shows a sharp peak at 152^0C due to polymorphism and melting, but in case of VHCl loaded microspheres, no characteristic peak was observed that 152^0C (Fig 3-C) suggesting that VHCl is molecularly dispersion in the polymer matrix.

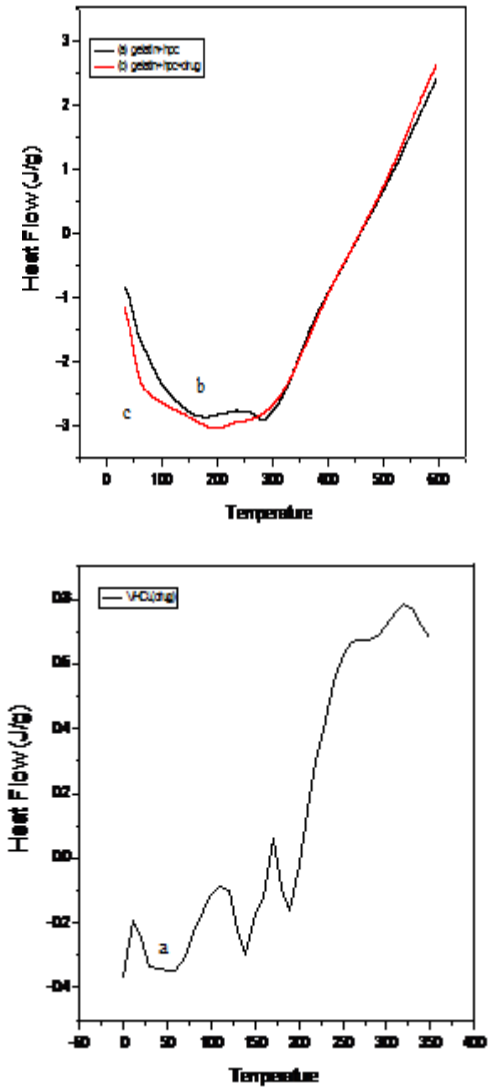


Fig. 3: DSC thermograms of (a) plain VHCl (b) plain Gelatin-HPC blend microspheres and (c) VHCl loaded Gelatin-HPC blend microspheres.

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 placebo VHCl (a), VHCl loaded microspheres (b), and pure microspheres (c) are presented in Fig 4. Here, VHCl peaks observed at 2θ of 6^0, 12^0, 16^0, 20^0, 25^0 and 27^0 are due to the crystalline nature of VHCl.

These peaks are not found in the VHCl 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.

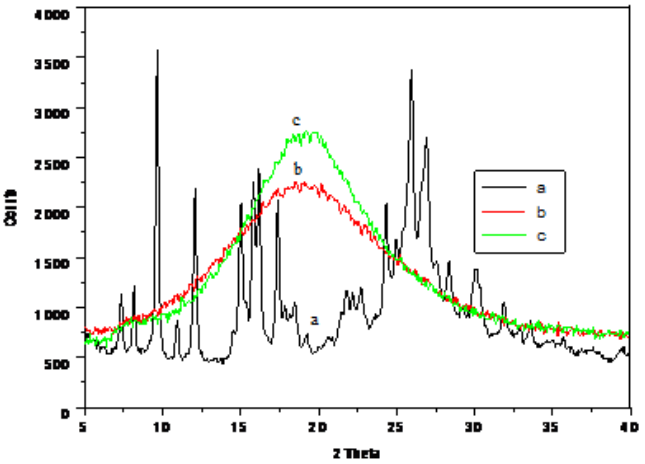


Fig 4: X-RD spectra of (a) plain VHCl drug, (b) drug loaded gelatin/hpc microspheres and (c) pure gelatin/hpc microspheres.

Scanning Electron Microscopic (SEM) studies

SEM micrograms of Gelatin and Hydroxypropyl cellulose blend microspheres(a) and drug loaded blend microspheres (b) are displayed in Fig 5.

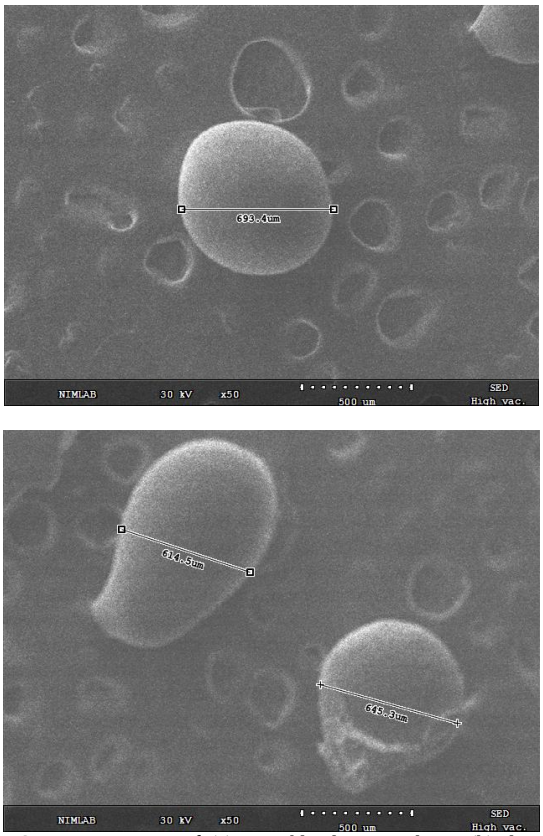


Fig. 5: SEM micrograms of (a) pure blend microspheres (b) drug loaded blend microspheres.

The surfaces of some of the microspheres were quite smooth and no pores were observed. Scanning electron microscopy has been used to confirm the formation of spherical structures of the microspheres. The microspheres were coated with gold colour and subjected to SEM, which showed that the microspheres are spherical in shape. We found particle size of microspheres ranging from 693.4µm, 614.5µm and 645.3µm.

Release studies

Release Kinetics Parameters of Different Formulations

Drug release kinetics was analyzed by plotting cumulative release data versus time and fitting these data to the exponential equation of the type (Ritger *et al*., 1987).

$$Mt / M\alpha = kt^n \quad \text{---------- (4)}$$

Here, Mt / Mα represents 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 squares procedure, we have estimated the values of n and k for all the eight formulations, and these values are given in Table 1. If n = 0.5, the drug diffuses and releases from the polymer matrix following a Fickian diffusion. If n > 0.5, an anomalous or non-Fickian type drug diffusion occurs. If n = 1, a completely non-Fickian or case II release kinetics are operative. The intermediary values ranging between 0.1 and 0.5 are attributed to the Fickian diffusion type transport. The values of k and n have shown a dependence on the extent of cross-linking, % drug loading, and HPC content of the matrix. Values of n for microspheres prepared by varying the amount of HPC in the microspheres of different percentages of blend microspheres by keeping VHCL and glutaraldehyde, ranged from 47-62%, leading to the drug diffuse and release from the polymer matrix following almost non-Fickian (or) case-II type diffusion.

Polymer variation

Effect of HPC content on encapsulation efficiency and invitro release of VHCL was investigated. In vitro release profiles of VHCL from formulations prepared by taking different amounts of HPC and 20% of VHCL are shown in Fig 6. Faster release rates were observed for formulations prepared with higher amount of HPC (30%) than those formulations prepared with lower amount of HPC i.e., (10%).

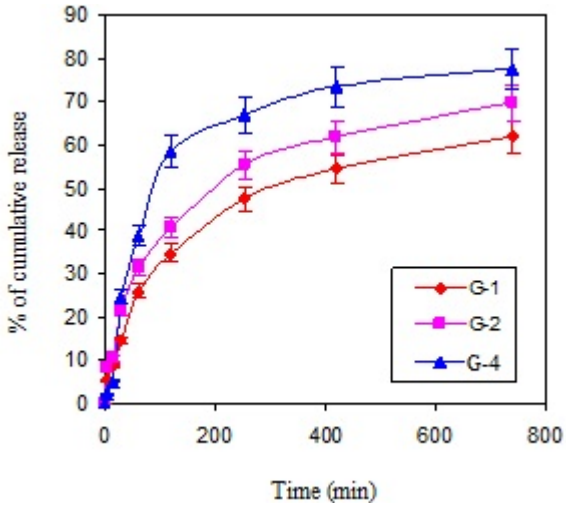


Fig 6: % of cumulative release of VHCL at different percentages of Gelatin-HPC content. Symbols (G-1, 10%, G-2, 20%, G-4, 30%).

Table 1: The results of % encapsulation efficiencies(EE) and release kinetics parameters of different formulations at pH - 7.4 are incorporated in table.

S. No	Gelatin (%)	HPC (%)	Drug (%)	Glutaraldehyde (mL)	% E.E±S.D.	k	n	r
G1	90	10	0.2	5	49.39±16	0.878	0.216	0.966
G2	80	20	0.2	5	51.24±67	0.847	1.023	0.954
G3	80	20	0.1	5	54.72±21	0.812	0.978	0.981
G4	70	30	0.2	5	58.64±38	0.832	1.062	0.967
G5	80	20	0.3	5	62.45±08	0.816	1.029	0.982
G6	80	20	0.2	2.5	56.92±21	0.725	0.781	0.964
G7	80	20	0.2	7.5	47.58±45	0.703	0.736	0.986
G8	100	00	0.2	5	48.28±04	0.893	0.228	0.962

% E.E = Encapsulation Efficiency , S.D = Standard devision.

Effect of Drug Loading Content

Fig 7. Shows the release profiles of VHCL loaded Gelatin-HPC blend microspheres at different amounts of drug loadings. Release data showed that formulations containing the highest amount of drug (30%) displayed fast and higher release rates than those formulations containing a small amount of VHCL. A prolonged release was observed for the formulation containing lower amount of VHCL. In other words, with a decreasing amount of drug in the matrix, it is noticed that the release rate becomes quite slower at the lower amount of drug in the matrix, and this is due to the availability of more free void spaces through which lesser number of drug molecules will transport. For all formulations the VHCL release was observed upto 720 min in a controlled manner.

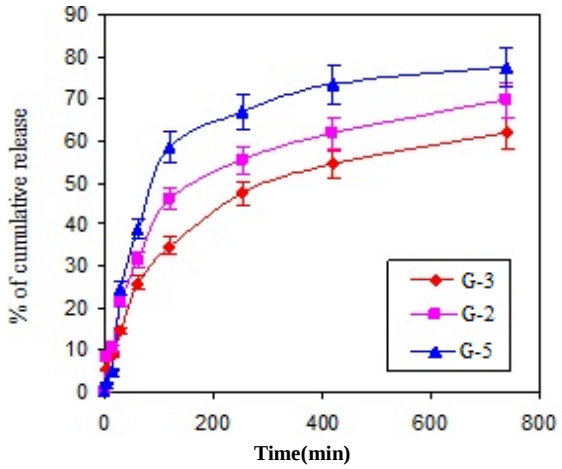


Fig 7: % of cumulative release of VHCL at different amounts of drug content: (G-3, 10%, G-2, 20%, G-5, 30%).

Effect of cross-Linking Agent

The % cumulative release data vs time plots for varying amounts of GA i.e., 2.5, 5.0 and 7.5 mL at the fixed amount of the drug (20%) are displayed in Fig 8. The % cumulative release is quite fast and large at the lower amount of GA (i.e., 2.5 mL), where as the release is quite slower at higher amount of GA (i.e., 7.5 mL). The cumulative release is somewhat smaller when lower amount of GA was used. Glutaraldehyde is expected to produce cross-links between gelatin molecules and thereby to slow down the rate of drug release from microspheres. At higher concentration of GA, polymeric chains become rigid due to the contraction of micro voids, thus decreasing % 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.

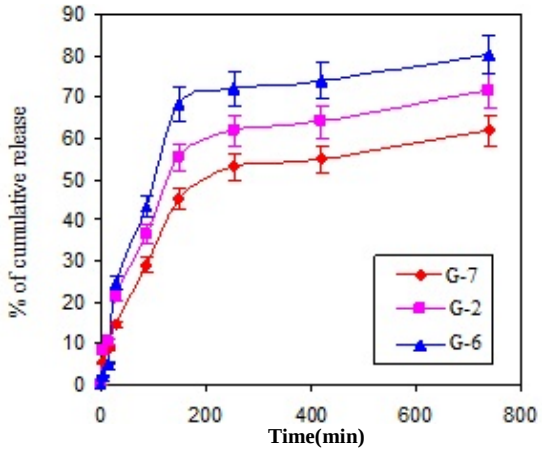


Fig. 8: % of cumulative release of drug through GC/HPC blend microspheres containing different amounts of cross-linking agent (G-7, 7.5 mL, G-2, 5 mL, and G-6, 2.5 mL).

CONCLUSION

Crosslinked gelatin(GC)-hydroxypropyl cellulose (HPC) blend microspheres were prepared by water-oil emulsion technique. VHCL was successfully loaded into these microspheres and encapsulation efficiency was found to be 47-62%, depending upon the blend composition, crosslinking agent used and the amount of drug loaded. The FTIR analysis were carried out to confirm the crosslinking of Gelatin-hydroxypropylcellulose blend microspheres. Scanning electron micrographs of the microspheres showed the formation of non-uniform spherical microspheres. X-RD and DSC studies on the microspheres indicated a molecular level dispersion studies performed in pH 7.4 buffer medium have shown that release of VHCL is dependent upon the amount of drug loaded, polymer composition and crosslinking. VHCL is released in a sustained and controlled release manner from the blend microspheres up to 12 h.

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