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Controlled Release of Verapamil Hydrochloride, an Antihypertensive Drug from the Interpenetrating Blend Microparticles of Gelatin and Gellan Gum

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ABSTRACT

Carbohydrate polymers are extensively used in the recent years in biomedical and pharmaceutical applications due to their biocompatibility and biodegradability. Among such natural polymers, gelatin (GL) and gellan gum (GG) have been chosen in the present work because of their pharmaceutical applications. This work reports on the development of novel interpenetrating polymer network (IPN) microparticles of GL and GG using maleic anhydride as crosslinker. Verapamil hydrochloride, an antihypertensive drug, was successfully encapsulated into these IPN microparticles. Various formulations of microparticles were prepared by varying the ratio of GL and GG and % of drug loading. These microparticles were characterized by Fourier transform infrared spectroscopy to understand the formation of IPN structure and to confirm the possible chemical interactions between drug, polymer, and cross-linking agent. Scanning electron microscopy was used to study the surface morphology of the microparticles which showed slight rough surfaces. Differential scanning calorimetry and X-ray diffraction studies were performed to understand the crystalline nature of the drug after encapsulation into IPN microparticles and distribution of the drug into the microparticles. Drug encapsulation of up to 90% was achieved as measured by the ultraviolet method. Both equilibrium and dynamic swelling experiments were performed in water. In vitro release studies indicated a dependence of release rate on both the extent of crosslinking and the amount of GL used to produce microparticles, but the slow release was extended up to 25 h. Cumulative release data were fitted to an empirical equation to compute diffusional exponent (n), which indicated non-Fickian trend for drug release. The studies of IPN microparticle system could be useful drug carrier for the encapsulation of fragile drugs and provide new opportunities in the field of bioencapsulation.

Key words: *Interpenetrating polymer networks, Verapamil hydrochloride, Encapsulation, Gelatin, Gellan gum.*

1. INTRODUCTION

Microspheres refer to the microparticulate polymer-based systems with an average particle size 1-1000 µm. Microsphere carrier system for drug delivery applications offers advantages such as limited fluctuation of drug plasma profile within a therapeutic range, reduction in side effects, decreased dosing frequency, and improved patient compliance [1-3]. Over the past few decades, microspheres made from naturally occurring biodegradable polymers have attracted considerable attention [4,5]. Among naturally occurring biodegradable polymers, gelatin (GL) and gellan gum (GG) are widely used for the designing and development of various drug delivery systems.

GL is a carbohydrate polymer and natural protein derived from collagen, which is a fibrous material that occurs in the skin, bones, and connective tissues of animals. It is insoluble in water, but it can be solubilized by hydrolysis. The raw materials used for its manufacturing process are obtained from bovine bones or porcine skins. The reaction can be carried out at an acid pH level, yielding Type A GL (which is primarily produced from skins), and at the basic pH level giving Type B GL (primarily produced from bovine bones). GL is a heterogeneous product that is a mixture of molecular species, such as α-, β-, and γ-peptides. The proportions and molecular weights are dependent on the nature of the chemical processes. GL is biocompatible, biodegradable, edible, and soluble at body temperature but undergoes gelation process at temperature just above ambient [6], which makes it an ideal material in pharmaceutical applications. On the other hand, GG is a water-soluble linear anionic

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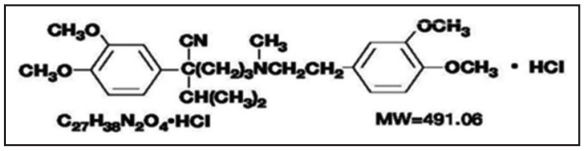
polysaccharide obtained as a fermentation product by a pure culture of pseudomonas elodea [7-9]. GG consists of a linear structure of repeating saccharide units of glucose, glucuronic acid, and rhamnose in a molar ratio of 2:2:1 [10,11]. The physical gelation ability of GG makes it suitable as structuring and gelling agent in foods and toothpastes, binders, and as a sustained release matrix [11].

Both GL and GG have the ability to undergo ionotropic gelation in aqueous solution in the presence of multivalent cations such as Ca^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, and Al^{3+} [11-13]. In pharmaceutical and biomedical research, the gel-forming properties of GL and GG in the presence of multivalent cations have been exploited for sustained release of drug molecules [14-19]. The mechanical stability of the ionotropically cross-linked gel systems is provided by multivalent cations. For the application as drug release systems in the physiological environment, extracellular concentrations of monovalent cations (such as sodium ions) exceed the concentration of divalent ones (such as calcium) [20]. Therefore, these ionotropically cross-linked gels tend to lose their mechanical stability over the long term due to diffusion, leading to exchange of multivalent cations for monovalent ones in the physiological fluid [21]. To avoid these problems, chemical modification of both GL and GG is proposed preferring covalent rather than ionotropic cross linking [9,20,22]. Thus, polysaccharide backbone carrying unsaturated substituents such as maleate semi-esters of polysaccharide could result in stronger gels than those ionotropically cross-linked gels [20]. In this context, the aim of the present investigation was to design microspheres made of GL/GG unsaturated esters for prolonged drug release through maleic anhydride-induced esterification. Verapamil hydrochloride (VPLHCl) was used as a model drug to evaluate the sustained drug release potential of these new microspheres.

VPLHCl (Scheme 1), the drug selected in the present work is a calcium channel blocker used for the treatment of hypertension, angina pectoris, cardiac arrhythmia, and most recently, cluster headaches [23,24]. It is completely absorbed (90%) from the gastrointestinal tract after oral administration but has a very low bioavailability of 20±8%. The low bioavailability is owing to the rapid biotransformation in the liver with a biological half-life of 4.0±1.5 h.



Scheme 1: Chemical structure of verapamil hydrochloride.

The short biological half-life and poor availability of drug favor development of controlled release formulations [25-27]. The chemical structure of VPLHCl is given in Scheme 1.

In continuation of our drug delivery studies [28-31], here, we are presenting the results based on the development of blend polymeric systems for the control release of the VPLHCl drug which after oral administration could prolong the gastric residence time and increase the drug bioavailability.

2. EXPERIMENTAL

2.1. Materials

VPLHCl and GL were purchased from Sigma-Aldrich (St. Louise, USA). GG was purchased from Sisco Research Laboratories, Taloja, Maharashtra, maleic anhydride (MA), acetone, tween-80, and n-hexane all are of Analar grade and were purchased from SD fine (India). Double-distilled water was collected in the laboratory and used throughout this research work. All the chemicals were used as received without further purification.

2.2. Preparation of Microparticles

The interpenetrating polymer network (IPN) blend microparticles were prepared using different ratios of GL and GG by varying the VPL concentration as well as the MA as the cross-linking agent by an emulsion crosslinking method (Table 1). 2% (w/v) of GL and 2% (w/v) GG were prepared by dissolving GL and GG in double-distilled deionized water separately and stirring the solution continuously until a homogeneous solution was attained.GG was dispersed in this GL solution and stirred overnight to obtain a homogeneous solution. VPLHCl was then dissolved in the above polymer blend solution, to which a light liquid paraffin (100 g, w/w) containing 2%, (w/w) tween-80 was added slowly under constant stirring at 400 rpm speed for about 15 min. To this w/o emulsion, MA in 0.5 ml of 1N HCl was added under continuous stirring for 4 h. The hardened microparticles were separated by filtration and washed repeatedly with n-hexane to remove light liquid paraffin oil, followed by washing with water to remove the unreacted MA. The details of the preparation of different formulation parameters of IPN blend microparticles are given in Table 1. The schematic representation of the GG and GL IPN is shown in Scheme 2.

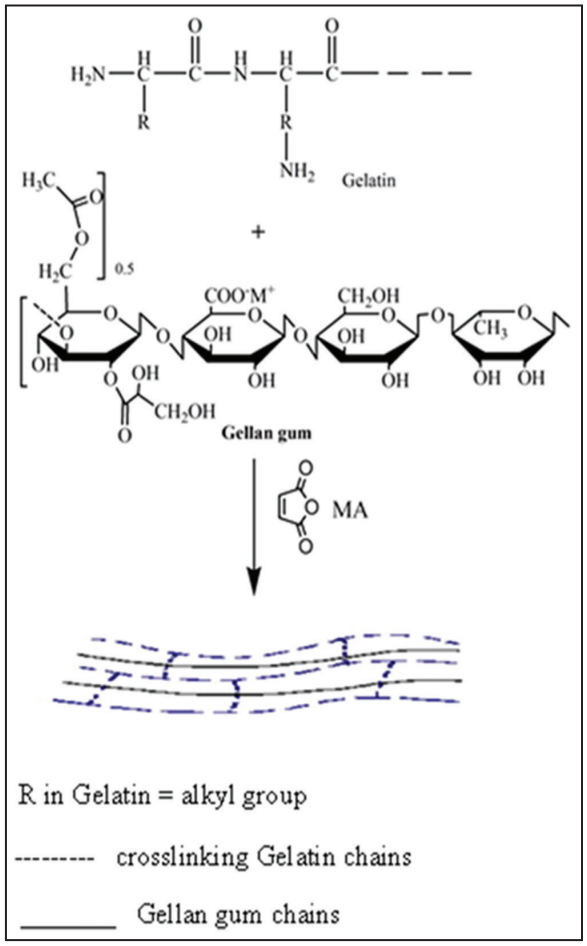
2.3. Drug Content

Estimation of drug concentration from the IPN microparticles was done as per the method described elsewhere [32,33]. Beads of known weight (10 mg) were grounded to get the powder using an agate mortar, extracted with 50 ml of distilled water, stirred for 24 h, and sonicated up to 60 min (UP 400s, Dr. Hielscher, GmBH, Germany). The solution was centrifuged (Joun, MR23i, France) to remove polymeric debris

Table 1: Formulation parameters of IPN blend microparticles.

Formulation codes	GL (% w/w)	GG (% w/w)	VPLHCl (% w/w)	MA (% w/w)	% swelling	% EE
S1	90	10	05	5	62.71	68±1.04
S2	80	20	05	5	59.70	65±0.92
S3	70	30	05	5	54.03	62±2.14
S4	80	20	7.5	5	61.67	69±1.78
S5	80	20	10	5	64.08	73±0.96
S_0	80	20	-	5	69.67	-

GL: Gelatin, GG: Gellan gum, VPLHCl: Verapamil hydrochloride, MA: Maleic anhydride, EE: Encapsulation efficiency



Scheme 2: Schematic representation of the synthesis of IPN polymers

and washed twice to extract the drug completely. The clear solution was analyzed by ultraviolet (UV) spectrophotometer (Lab India) at the fixed λ_{max} of 263 nm. The percent drug loading and encapsulation efficiency (EE) were calculated with the help of the equation 1 and 2, respectively, and presented in Table 1.

$$\% \text{ Of drug loading} = \frac{\text{Weight of drug in microparticles}}{\text{Weight of microparticles}} \times 100 \quad (1)$$

$$\% \text{ Of encapsulation efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100 \quad (2)$$

2.4. Swelling Experiments

Equilibrium swelling of IPN microparticles was determined gravimetrically by measuring the extent of swelling in double-distilled water. To ensure attainment of complete equilibrium, the samples were allowed to swell for 24 h, and excess surface-adhered liquid droplets were removed by blotting with a soft tissue paper. The swollen particles were weighed to an accuracy of ±0.01 mg on an electronic microbalance (Mettler, model AT120, Greifensee, Switzerland). The microparticles were then dried in an oven at 6°C for 5 h until no further weight gain of the dried samples was observed and percent equilibrium swelling was calculated using equation 3.

$$\% \text{ Of swelling} = \frac{\text{Ws-Wd}}{\text{Wd}} \times 100 \quad (3)$$

Where Ws and Wd are, respectively, the weights of swollen and dry microparticles. Experiments performed in triplicate were reproducible within ±3% standard errors, and the average values were considered in data analysis and graphical display. The percent swelling results are also included in Table 1.

2.5. In Vitro Drug Release Experiments

In vitro drug release from the different formulations of blend microparticles was investigated in pH 7.4 phosphate buffer media which acts as a simulated intestinal fluid. These experiments were performed using the fully automated dissolution tester coupled with UV system (Lab India, Mumbai, India) equipped with eight baskets at the stirring speed of 100 rpm. A weighed quantity of each sample equivalent to 250 mg VPL was placed in 500 ml (aqueous solubility of VPL is 1 in 100 parts) of pH 7.4 phosphate buffer maintained at 37°C. The instrument automatically measures the concentration of the drug released at the particular time intervals by UV spectrophotometer coupled with flow through cells attached with the

instrument and then replaces the solution back into the dissolution bowl. The VPL concentration was determined spectrophotometrically at the λ_{max} value of 251 nm. These studies were performed in triplicate for each sample, and the average values were used during data analysis.

2.6. Characterization

2.6.1. Fourier transform infrared (FTIR) spectral measurements

FTIR spectra were obtained using Nicolet (Bomem model: MB3000, Canada) instrument to confirm the formation of full IPN structure as well as to confirm any chemical interactions of MA and VPLHCl with the polymer matrix. FTIR spectra of GL, GG, placebo IPN microparticles, drug-loaded microparticles, and pristine VPL were all taken by grinding each sample separately with KBr and making pellets under a hydraulic pressure of 600 kg/cm^2. Spectral scanning was done in the range 4000-500 cm^{-1}.

2.6.2. Differential scanning calorimetry (DSC)

DSC (Model-SDT Q600, USA) was performed on placebo IPN microparticles, pristine VPL, and VPL-loaded microparticles by heating about the 8-10 mg of sample from 25°C to 400°C at the heating rate of 10°C/min in a nitrogen atmosphere at the flow rate of 20 ml/min.

2.6.3. X-ray diffraction (X-RD)

X-RD patterns of VPLHCl drug and drug-loaded GL/GG blend microparticles were recorded using Shimadzu Lab X-RD-6000x diffractometer (Japan), with the help of Nickel-filtered Cu Ka radiation (λ=0.54 nm). Dried samples were mounted on a sample holder, and the patterns were recorded in the range of 10-60° at the speed of 5/min to know the crystallinity.

2.6.4. Scanning electron microscopy (SEM)

SEM images were taken using a JEOL model JSM-840A, Japan, instrument (STIC, Cochin University, Kochi, India). The microparticles were sputtered with gold to make them conducting and placed on a copper stub. The thickness of the gold layer accomplished by gold sputtering was about 10 nm. The SEM images were recorded at the required magnification at room temperature. The acceleration voltage used was 10 kv with scanning electron images as a detector.

3. RESULTS AND DISCUSSION

3.1. FTIR Spectral Study

In an effort to investigate the possible chemical interaction of the drug and MA with the blend microparticles matrices, we have analyzed (a) pure anti-VPLHCl drug, (b) placebo blend microparticles, and (c) drug-loaded blend microparticles using FTIR and are shown in Figure 1. The characteristic FTIR absorption peak of VPLHCl (Figure 1a) showed the C-H stretching vibration of the methoxy group at

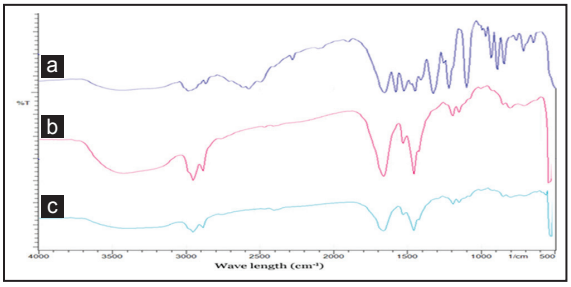


Figure 1: Fourier transform infrared spectra of verapamil hydrochloride (a), gelatin (GL)/Gellan gum (GG) blend microparticles (b), and drug-loaded GL/GG blend microparticles (c).

2840 cm^{-1}, the N-H stretching of the protonated amine group in the range 2800-2300 cm^{-1}, and a strong absorption band due to C-O stretching of aromatic ester group at 1262 cm^{-1}. In case of the placebo microspheres (Figure 1b), all the characteristic bands of both GL and GG are observed in addition to a new band appearing at 1650 cm^{-1}, this may be due to the presence of ester groups as a result of unsaturated esterification of GL/gellan -OH groups of GL and GG could perform nucleophilic attack on ester linkage. This process also leads to a ring opening of the anhydride group of maleic anhydride with the generation of a C=O group [34].

Inthe FTIR spectrum (Figure 1c) VPLHCl-loaded microspheres of GL/GG, various characteristic peaks of microspheres of GL/GG unsaturated esters without drug had appeared clearly without any significant shifting of peaks. Thus, FTIR data confirm the successful crosslinking of both GL and GG to form a full IPN network structure in the presence of MA, and the proposed reaction is displayed in Scheme 1. This indicates that VPL is not involved in any chemical reaction with either the polymer or the crosslinking. Characteristic peaks of VPL are also present in the FTIR spectrum of drug-loaded IPN microparticles with some broadening and reduction in intensity, indicating the absence of chemical interactions between VPL, the polymer matrix, and counter ions after the formation of the IPN structure.

The FTIR analysis confirmed the compatibility of the VPLHCl with the polymer used to prepare microspheres of GL/GG unsaturated esters containing VPLHCl by maleic and hydride-induced esterification.

3.2. DSC Study

The state of the drug whether crystalline or amorphous inside the polymer matrix is important in relation to their release characterization for its release studies is the nature of the drug present in the polymer matrix, the polymer drug and the drug-loaded microparticles have been analyzed by DSC. The DSC thermograms of pristine VPLHCl (a), placebo IPN microparticles (b),

and VPLHCl-loaded microparticles are displayed in Figure 2. The DSC thermogram of pure VPL exhibits a sharp endothermic peak at 145°C indicating its melting temperature.

Placebo IPN microparticles have shown an endothermic peak (b) at 255°C indicating the melting temperature of the polymer, whereas VPLHCl-loaded blend microparticles (c) showed an endothermic peak at 250°C. The melting point peak of the drug has not appeared in either placebo blend microparticles or drug-loaded blend microparticles, indicating an amorphous nature and molecular dispersion of the drug into the polymer matrix.

3.3. X-RD

X-RDs of pristine VPLHCl drug (a), placebo microparticles (b), and VPLHCl-loaded microparticles (c) are presented in Figure 3. Which have been used for investigating drug's polymorphism after its encapsulation into the polymer matrix. The X-RD curves of VPLHCl (a) showed intense peaks at 2θ in the range of 10-25 which are the characteristics of its crystalline nature, but these peaks have disappeared in the VPLHCl-loaded microspheres and in placebo matrix. Typically, the peak depends on crystal size, but in the present study, for the VPL-loaded formulations, the characteristic peaks of VPLHCl have merged with the polymer blend matrix, thereby suggesting that VPLHCl is in the amorphous state in VPLHCl matrix, though it is difficult to investigate drug's crystallinity at the detection limit of the crystal size in case of drug-loaded microparticles. X-RD results further suggest that drug is molecularly dispersed in the IPN blend matrix [35,36].

3.4. SEM

The morphological analysis of VPL-loaded blend microparticles made of GL/GG was visualized by SEM and is presented in Figure 4. Figure 4a shows the SEM of (80% GL +M20% GG) blend microparticles at 100 resolution, whereas Figure 4b shows SEM of (80% GL +M20% GG) blend microparticles at 500 resolution. Figure 4c shows the SEM of (80% GL +M20% GG) drug-loaded blend microparticles at 500 resolution, whereas Figure 4d shows the SEM of (80% GL +M20% GG) drug-loaded blend microparticles at 100 resolution.

By careful observation of these SEM photographs, it is noticed that these particles were almost spherical shape and dense with thick polymer coat. Elongated tails in the microparticles were also observed because of the presence of higher GL in the polymer concentration. The surface morphology of these microparticles revealed rough surface with wrinkles, which might be caused by partly collapsing the polymeric microparticles network during drying.

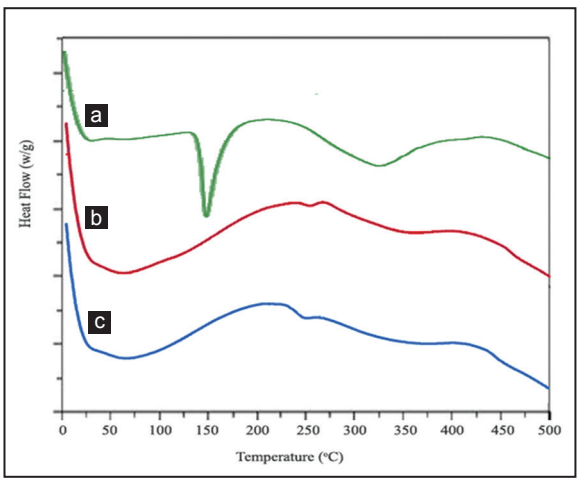


Figure 2: Differential scanning calorimetry thermograms of pure verapamil hydrochloride (a), pure blend microparticles (b), and drug-loaded blend microparticles (c).

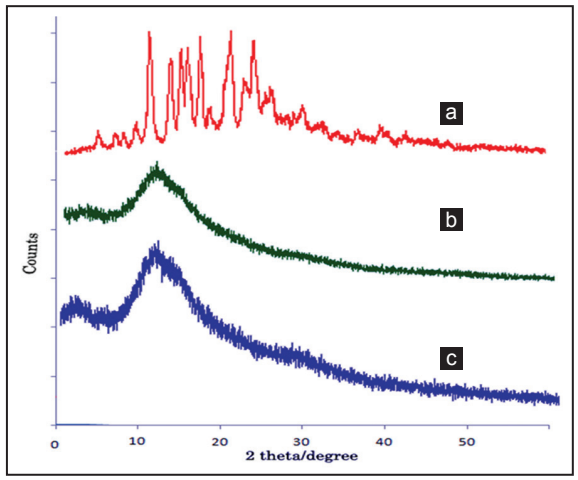


Figure 3: X-ray diffraction patterns of pure verapamil hydrochloride (a), gelatin (GL)/gellan gum(GG) blend microparticles (b), and drug-loaded GL/ GG blend microparticles (c).

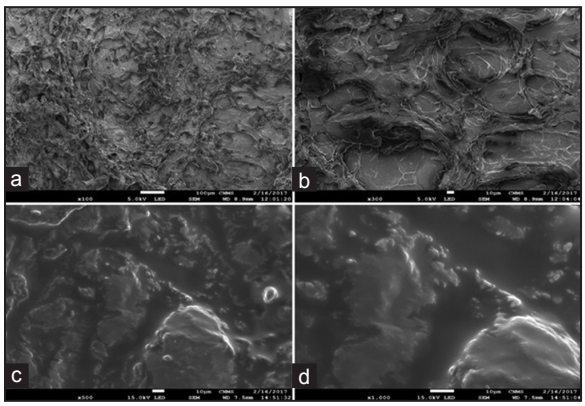


Figure 4: Scanning electron microscopy photographs without drug-loaded blend microparticles (a and b) and with drug-loaded microparticles (c and d) at different resolutions.

3.5. Encapsulation Efficiency (EE)

The percent EE of VPL in the microparticles ranged between 62% and 78%, depending on the percentage of components of the blend in the matrix. By increasing the GG concentration, EE values slightly decreased. For instance, the S3 (30% w/w GG) formulation has a smaller EE value of 49 than S2 (20% w/w GG) and S1 (10% w/w GG) formulations, which exhibited %EE of 51 and 54, respectively; this could be due to the rigid nature of the IPN matrix at a higher concentration of GG. This in turn reveals that the EE values increased with increase in GL composition in the blend microparticles. This may be due to the hydrophobic nature of GG and hydrophilic nature of GL. A similar observation was made by Ramesh Babu *et al.* [37] from their nifedipine drug release studies from GE/MC microparticles.

3.6. Equilibrium Swelling

The percentage of equilibrium water uptake of the cross-linked microspheres is presented in Table 1. It indicates that, as the amount of drug in the matrices increased from 5 to 10ml, the equilibrium water uptake increased significantly from 54.03% to 69.67%. It is noticed that formulations containing higher amounts of GG showed lower swelling rates than formulations containing a lesser amount of GG. Thus, formulation S3 (30%, w/w, GG) exhibited lower swelling than formulation S2 (20%, w/w, GG); similarly, formulation S2 exhibited a lower swelling than formulations S1 (10%, w/w, GG), due to hydrophobic nature of GG, thereby leading to lower water uptake capacity. This further indicates comparatively that the formulation containing a higher amount of GL will exhibited higher swelling comparative to GG.

3.7. In Vitro Release Study

Effect of blend composition in formulations S1, S2, and S3 on released rates is presented in Figure 5. The percentage cumulative release is decreased in the following sequence S1 > S2 > S3. This indicates that as the GG content increases in the polymer IPN blend microparticle, the release rate is decreased since the swelling matrix decreases with increase in GG content, which is due to the hydrophobic nature of GG compared to GL as explained above in swelling studies or in other works as the GL content in the polymer matrix increases, swelling of the matrix increases due to the extremely hydrophilic nature of GL.

The effect of drug loading *in vitro* release of profiles for formulations S2, S4, and S5 is displayed in Figure 6. The S5 exhibits higher releases rates than S4, and S4 exhibits higher releases rates than S2. This indicates that release rates vary depending on the amount of drug in the matrices, i.e., release is faster for those formulations having a higher amount of drug and slower rate for the formulation having lower amount of drug. The drug in the present IPN microparticles might act as inert filler by occupying the free volume of the swollen polymer matrix. This could have created a tortuous path for water molecules to permeate, but the degree of tortuosity depends on the volume fraction of the filler [38].

3.8. Drug Release Kinetics

To determine the mechanism of drug release, the initial percentage of drug release versus time profiles has been fitted to the empirical equation [39].

$$\frac{M_t}{M_\infty} = kt^n \quad (4)$$

Where $\frac{M_t}{M_\infty}$ represents the fractional drug release at time t, k is a constant characteristic of the drug-polymer system, and n is diffusion exponent, characteristics of the release mechanism. Using the least squares procedure, we have estimated the values of n, k, and r

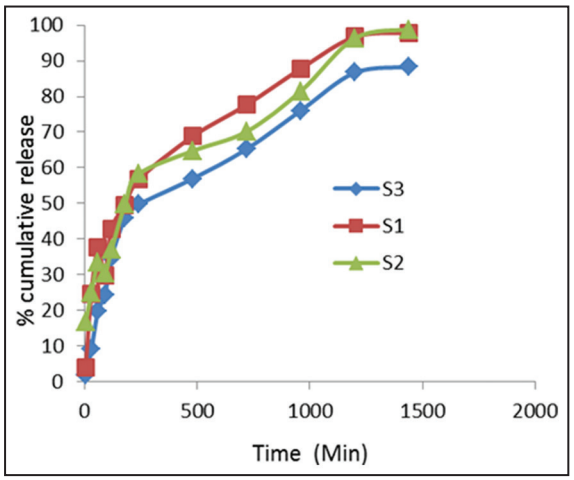


Figure 5: Effect of blend composition on *in vitro* release profile.

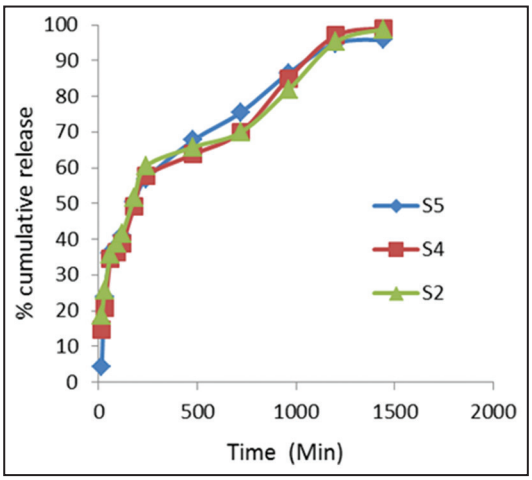


Figure 6: Effect of % drug loading on *in vitro* release profile.

Table 2: Results of % of release kinetics parameters (k, n, and r) of drug in different blend microparticles formulations.

Sample code	K	N
S2	0.157	0.86
S4	0.171	0.84
S5	0.182	0.74

for all formulations using equation 4, and these results are represented in Table 2. If n=0.5, drug diffuses and releases out of the polymer matrix following Fickian diffusion for n>0.5, anomalous or non-Fickian type drug diffusion occurs. If n=1, a completely non-Fickian or case II release kinetics is operative.

The values of k increase with increasing % loading of VPLHCl in the beads, but the values of n decrease with increasing % loading of VPLHCl. This indicates the smaller level interactions between the microparticles and the swelling media with increasing size of the microparticles. The values of n are in the range of 0.67-0.86, which indicated that the drug release was deviated only slightly from the Fickian trend and followed the anomalous trend.

4. CONCLUSION

Two naturally available carbohydrate polymers, namely, GL and gellan gum have been chosen to develop IPN blend microparticles for the effective encapsulation (by emulsification method) of VPL and studied the controlled release. FTIR was used to confirm the formation of interpenetrating network between the polymer, drug, and crosslinker. Microparticles with spherical shapes having somewhat rough surfaces were produced. Swelling kinetics were investigated in terms of the composition of IPN blend microparticles and concluded that the amount of swelling increases with increase in the content of GL which is more hydrophilic. The amount of drug loading and GL content of the matrix influenced the release of VPL. Release mechanism followed non-Fickian type behavior. It is demonstrated that microparticles of this study are useful as CR devices to control the release rates of VPL though the polymeric matrices developed.

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