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Development of Novel Interpenetrating Network Gellan Gum-Poly(vinyl alcohol) Hydrogel Microspheres for the Controlled Release of Carvedilol†

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ABSTRACT Novel interpenetrating polymeric network microspheres of gellan gum and poly(vinyl alcohol) were prepared by the emulsion cross-linking method. Carvedilol, an antihypertensive drug, was successfully loaded into these microspheres prepared by changing the experimental variables such as ratio of gellan gum:poly(vinyl alcohol) and extent of cross-linking in order to optimize the process variables on drug encapsulation efficiency, release rates, size, and morphology of the microspheres. Formation of interpenetrating network and the chemical stability of carvedilol after preparing the microspheres was confirmed by Fourier transform infrared spectroscopy. Differential scanning calorimetry and x-ray diffraction studies were made on the drug-loaded microspheres to investigate the crystalline nature of the drug after encapsulation. Results indicated a crystalline dispersion of carvedilol in the polymer matrix. Scanning electron microscopy confirmed the spherical nature and smooth surface morphology of the microspheres produced. Mean particle size of the microspheres as measured by laser light scattering technique ranged between 230 and 346 μm. Carvedilol was successfully encapsulated up to 87% in the polymeric matrices. In vitro release studies were performed in the simulated gastric fluid or simulated intestinal fluid. The release of carvedilol was continued up to 12 h. Dynamic swelling studies were performed in the simulated gastric fluid or simulated intestinal fluid, and diffusion coefficients were calculated by considering the spherical geometry of the matrices. The release data were fitted to an empirical relation to estimate the transport parameters. The mechanical properties of interpenetrating polymeric networks prepared were investigated. Network parameters such as molar mass between cross-links and cross-linking density for interpenetrating polymeric networks were calculated.

KEYWORDS Gellan gum, Poly(vinyl alcohol), Microspheres, Carvedilol, Controlled release

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INTRODUCTION

Carvedilol is a noncardioselective β-blocker used in the management of hypertension and angina pectoris. It is well absorbed from the gastrointestinal tract but is subject to considerable first-pass metabolism in the liver. Its absolute bioavailability is considerably low, i.e., about 25%, and its plasma half-life is about 6 h (McTavish et al., 1993). Since the use of controlled release (CR) formulations offer many potential advantages, like sustained blood levels, attenuation of adverse effects, and improved patient compliance, it is realized that in the case of antihypertensive agents, it is necessary to maintain constant blood levels, as otherwise, dose dumping may cause hypotension. Therefore, formulating carvedilol in CR dosage forms will increase therapeutic efficiency and patient compliance.

The chemical and physical combination methods and properties of multipolymers have been of great practical and academic interest for the CR of drugs and proteins (Chandy et al., 2002; Ebube & Jones, 2004; Mi et al., 2003; Santos et al., 2003), because they provide a convenient route for the modification of properties to meet specific needs. Among these methods, considerable interest has been given to the development of interpenetrating polymer network (IPN) hydrogels (Kurkuri & Aminabhavi, 2004; Kulkarni et al., 2001; Kumbar et al., 2003; Soppimath et al., 2000). An IPN is a composite of two polymers, which is obtained when at least one polymer network is synthesized or cross-linked independently in the immediate presence of the other. Such systems are known to increase the phase stability and, thus, enhance the mechanical properties of the final product. There are two reactive phases in an IPN. For instance, if phase A reacts earlier than phase B, then the product is called sequential IPN. If both reactions take place at the same time, it is called a simultaneous IPN. If both phases are cross-linked, it is called a full IPN, while if one is cross-linked and the other is linear, it is called a semi-IPN.

Gellan gum (GG) is an extracellular polysaccharide produced by the bacterium *Pseudomonas elodea* (Kang et al., 1982). The natural form of GG is a linear anionic heteropolysaccharide based on a tetrasaccharide repeat unit of glucose, glucuronic acid, and rhamnose in a molar ratio of 2:1:1 (Jansson et al., 1983). The natural form of GG is partially acetylated with acetyl and L-glyceryl groups located on the glucose residues (Kuo et al., 1986). The presence of acetyl groups interferes in ion-bonding ability. On the other hand, commercially available GG is a deacetylated product obtained by treatment with alkali (Kang et al., 1982). GG has a wide variety of applications, mainly in the food and pharmaceutical industries. Its pharmaceutical uses are mainly concentrated in ophthalmic drug delivery and oral sustained release preparations (Agnihotri et al., 2005; Miyazaki et al., 1999, 2001; Rozier et al., 1997; Sanzgiri et al., 1993). Due to the characteristic property of cation-induced gelation, it has been widely used in the formulation of in situ gelling ophthalmic preparations (Rozier et al., 1997; Sanzgiri et al., 1993) as well as in situ gelling oral CR formulations (Miyazaki et al., 1999, 2001). Recently, we have developed GG beads by ionotropic gelation with a mixture of calcium and zinc ions for the CR of cephalexin (Agnihotri et al., 2005).

Poly(vinyl alcohol) (PVA) is a widely used hydrophilic polymer because of its processability, strength, and pH, as well as its temperature stability. Because it is biocompatible and nontoxic, it has a wide variety of pharmaceutical applications (Kurkuri & Aminabhavi, 2004; Peppas & Wright, 1998; Soppimath et al., 2000). In the literature of pharmaceutics, we are not aware of using the microspheres prepared from IPN of GG and PVA for the CR of carvedilol; hence, the present study is aimed at developing a novel type of IPN microspheres of GG with PVA. The formulation and process variables affecting the preparation of microspheres and in vitro drug release characteristics were investigated. The swelling and deswelling kinetics of IPN microspheres were studied in an effort to investigate their applications as oral dosage formulations. The specific advantages of multiparticulate systems such as microspheres, beads, etc., over other conventional dosages forms like tablets and capsules were discussed in our earlier paper (Agnihotri & Aminabhavi, 2004a). The formation of IPN along with the chemical

TABLE 1 Formulation Codes

	GA ($\times 10^{-3}$ mL)/mg of polymer		
Ratio of GG:PVA	1.0	2.0	3.0
20:80	F1	F2	F3
40:60	F4	F5	F6
60:40	F7	F8	F9

stability of carvedilol-loaded microspheres was confirmed by Fourier transform infrared (FTIR) spectroscopy. Differential scanning calorimetry (DSC) and x-ray diffraction (x-RD) studies were performed on the drug-loaded microspheres to investigate the crystalline nature of the drug after encapsulation. Scanning electron microscopy (SEM) was employed to investigate the morphology of the microspheres. Mechanical properties, such as tensile strength of IPNs prepared, were compared with pure GG. Network parameters such as molar mass between cross-links and cross-link density were determined. Drug release data were analyzed using an empirical equation proposed by Ritger and Peppas (1987). Diffusion coefficients were computed from the modified Fick's equation.

MATERIALS

The gift samples of carvedilol and deacetylated gellan gum (Gelrite®) (M_w=880,000–920,000) (Merck and Co., Inc., New Jersey, USA) were kindly supplied by Eros Pharma Ltd. (Bangalore, India). Analytical reagent grade absolute alcohol was purchased from Hayman Limited, England. Analytical reagent grade samples of poly(vinyl alcohol) (approximately M_w=125,000, 98% hydrolyzed), glutaraldehyde (25% v/v), light liquid paraffin, Span® 80, *n*-hexane, and acetone were purchased from s.d. fine-chemicals (Mumbai, India). Double-distilled water was used throughout the work. All the chemicals were used without further purification.

METHODS

Preparation of Microspheres

Gellan gum and poly(vinyl alcohol) (GG-PVA) IPN microspheres containing carvedilol were prepared by the emulsion cross-linking method. In this method, PVA was dissolved in hot water at 80°C, and after cooling, GG was added (total polymer concentration was 4%, w/v) and stirred overnight to get uniform bubble-free solution. Carvedilol equivalent to 50% (w/w) dry weight of the polymer was dissolved in 2 mL absolute alcohol and slowly added to the above polymer solution under stirring. This produced a fine precipitation of carvedilol, which was stirred further to get a uniform suspension. This solid-in-water (S/W) suspension was emulsified into light liquid paraffin in the presence of 0.5% Span® 80 using Eurostar stirrer (IKA Labortechnik, Germany) at 300 rpm for 5 min. Then, a mixture of different quantities of glutaraldehyde (GA) and 1 mL of 5 N HCl was added slowly, and stirring was continued for 2 h. Hardened microspheres were separated by filtration and washed with *n*-hexane followed by acetone. The microspheres were dried at 50°C for 24 h and stored in desiccator until further use. All the formulations were prepared in triplicate and used for further studies. In total, nine formulations were prepared. The assigned formulation codes are given in Table 1.

Carvedilol Content

Estimation of drug content was done according to the method adopted earlier (Agnihotri & Aminabhavi, 2004b). Microspheres of known weights were soaked in 50 mL of 0.1 N HCl for 30 min and sonicated using a probe sonicator (UP 400s, dr. hielscher, GmbH, Germany) for 10 min to break the microspheres and facilitate extraction of drug. The solution was centrifuged using a tabletop centrifuge (Jouan, MR 23i, France) to remove the polymeric debris. The clear supernatant solution was analyzed for carvedilol content by ultraviolet (UV) spectrophotometer (Secomam, Anthelie, France) at the λ_{max} value of 240 nm. This method of analysis was also validated by potentiometry (British Pharmacopoeia, 2004) to confirm the results of spectrophotometry; these data were found to be in agreement. The percent encapsulation efficiency of the IPN matrix was calculated as follows:

$$\%\ \textit{Encapsulation efficiency} = \frac{\textit{Drug loading}}{\textit{Theoritical drug loading}} \times 100 \qquad (1)$$

These data for various formulations are presented in Table 2.

Particle Size Measurements

Particle size was measured by using a laser light scattering technique (Mastersizer 2000, Malvern, UK). The sizes of the completely dried microspheres of different formulations were measured by using a dry sample adapter. The volume–mean diameter (V_d) was recorded.

TABLE 2 Results of % Encapsulation Efficiency, Particle Size, % Equilibrium Liquid Uptake, Molar Mass Between Cross-links (M_c) Calculated from Eq. 5 and Cross-link Density (d_x) Calculated from Eq. 8

Formulation code	Encapsulation efficiency (%)	Volume mean particle size (μm)	% Equilibrium liquid uptake in media		M_c	$d_x \times 10^4$
			SGF	SIF		
F1	84.00	276	195.51	194.22	4779	2.80
F2	86.52	259	181.33	181.10	4216	3.24
F3	83.89	230	169.28	170.35	3784	3.68
F4	84.70	301	163.43	164.22	3405	4.07
F5	82.81	282	149.25	148.36	2936	4.81
F6	85.33	256	137.98	136.59	2441	5.90
F7	86.53	346	130.65	131.24	2201	6.50
F8	87.20	300	116.44	115.00	1852	7.88
F9	86.59	278	107.23	106.99	1591	9.35

Liquid Transport Studies

To understand the molecular transport of liquids into IPN microspheres, dynamic swelling studies were performed in the simulated gastric fluid (SGF) or the simulated intestinal fluid (SIF) (without enzymes) by mass measurements. The microspheres were soaked in either SGF or SIF media maintained at 37°C. At different time intervals, few microspheres representative of the batch were taken out and blotted off carefully in between tissue papers (without pressing hard) to remove the surface-adhered liquid droplets. The swollen microspheres were then weighed (w_1) on an electronic microbalance (Mettler, AE 240, Switzerland) with an accuracy of ±0.01 mg. Microspheres were then dried until attainment of constant mass (w_2) in an oven maintained at 60°C. These studies were performed in triplicate for each sample, but average values were considered for data analysis. The percent equilibrium water uptake was calculated as follows (see Eq. 2 below).

Drying Rates of the Microspheres

A few samples of the microspheres representative of the batch prepared were allowed to dry in an incubator (Stuart Orbital Incubator, Model S150, Staffordshire, UK) maintained at 37°C (the initial mass of microspheres should be nearly equal). Initially, microspheres were removed at short intervals of time, and later, at longer time intervals, and then, they were weighed on an electronic microbalance (Mettler, AE 240, Switzerland) with an accuracy of ±0.01 mg. These measurements were continued until attainment of constant mass, indicating complete dried equilibrium. To obtain reproducible results, experiments were carried out in triplicate, and average values were used for calculation and display.

In Vitro Release Studies

Before performing in vitro release studies, the saturation solubility of carvedilol in both SGF and SIF was determined to assure the sink conditions during in vitro release studies. An excess of drug was added to the medium, and duplicate samples were stirred for 2 days at room temperature. The samples were then filtered through a 0.45 μm filter, and the concentration of the drug was measured spectrophotometrically after appropriate dilution. In vitro drug release from different formulations of IPN matrices was investigated in SGF or SIF (without enzymes). These experiments were performed using a fully automated dissolution tester coupled with UV system (Logan Instruments corp., Model D 800, NJ, USA) equipped with six baskets at the stirring speed of 100 rpm. A weighed quantity of each sample was placed in 500 mL of SGF or SIF maintained at 37°C. The instrument automatically measures the concentration of drug released at particular time intervals by UV spectrophotometer coupled with flow-through

$$\left(\frac{Mass\ of\ swollen\ microspheres\ (w_1) - Mass\ of\ dry\ microspheres\ (w_2)}{Mass\ of\ dry\ microspheres\ (w_2)}\right) \times 100 \qquad (2)$$

cells attached to the instrument, and it then puts the solution back into the dissolution bowl. The carvedilol concentration was determined spectrophotometrically at the λ_{max} value of 240 nm. These studies were performed in triplicate for each sample, and average values were used for data analysis.

Fourier Transform Infrared (FTIR) Spectral Studies

FTIR spectra were taken on a Nicolet (Model Impact 410, Milwaukee, USA) instrument to confirm the formation of IPN and also to investigate the possible chemical interactions between the drug and the polymer matrix. FTIR spectra of GG, PVA, placebo microspheres, pristine carvedilol, and carvedilol-loaded microspheres were obtained. Also, to investigate the possible reaction between GA and carvedilol, pristine carvedilol was treated with GA. The ratio (mL/mg) and concentration of GA as well as carvedilol was kept identical to that used in the formulations. The time of exposure was also kept similar to that of microsphere preparation, i.e., 2 h. Then, carvedilol was washed with double-distilled water. After drying, FTIR spectra were recorded. The samples were crushed with KBr to get the pellets by applying a pressure of 600 kg/cm^2. Spectral scans were taken in the range between 4000 and 500 cm^{-1}.

Scanning Electron Microscopic (SEM) Studies

SEM images were taken on GG-PVA IPN microspheres prepared by cross-linking with 2×10^{-3} mL of glutaraldehyde per milligram of the polymer and loaded with 50% of carvedilol. Microspheres were sputtered with gold to make them conducting and placed on a copper stub. Scanning was done using Leica 400, Cambridge, UK, instrument at the National Chemical Laboratory, Pune, India. The thickness of the gold layer accomplished by gold sputtering was about 15 nm.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed on pristine carvedilol, pristine GG, pristine PVA, placebo microspheres, and carvedilol-loaded microspheres. DSC measurements were done on a Rheometric Scientific (DSC-SP, Surrey, UK) by heating the samples at a heating rate of 10°C/min in nitrogen atmosphere (flow rate 20 mL/min).

X-Ray Diffraction (x-RD) Studies

The crystallinities of pristine carvedilol and carvedilol-loaded microspheres were evaluated by x-RD measurements recorded for pristine carvedilol, placebo microspheres, and the drug-loaded microspheres using an *x*-ray diffractometer (x-Pert, Philips, UK). Scanning was done up to 2θ of 50°.

Tensile Strength Measurements

To measure the tensile strength, membranes were prepared from aqueous solutions of gellan gum and other formulations (without the drug); the formulation compositions are given in Table 1. The solution casting method was used to prepare the membranes on a clean glass plate. Membranes formed were peeled off from the glass plate and cross-linked by immersing in a methanol:water (80:20, v/v) mixture containing the same quantities of GA and 5 N HCl as used in the preparation of microspheres. The cross-linking period was identical to that for microsphere preparation, i.e., 2 h. The films were dried at room temperature and used for the measurements. Test specimens were prepared by cutting the membranes to 10 mm wide and 100 mm long strips using a precise cutter. The tensile testing was performed using a Hounsfield universal testing machine (Model H25KS, Surrey, UK). Two ends of the specimen were fastened to the upper and lower jaws of the instrument leaving a length of 50 mm of the film in between the two jaws. The extension speed of the instrument was 20 mm/min.

RESULTS AND DISCUSSION

Preparation and Characterization of Microspheres

In this research, initially, the preparation of GG microspheres was attempted by the emulsion cross-linking method, but such microspheres exhibited very poor mechanical strength. In order to improve the

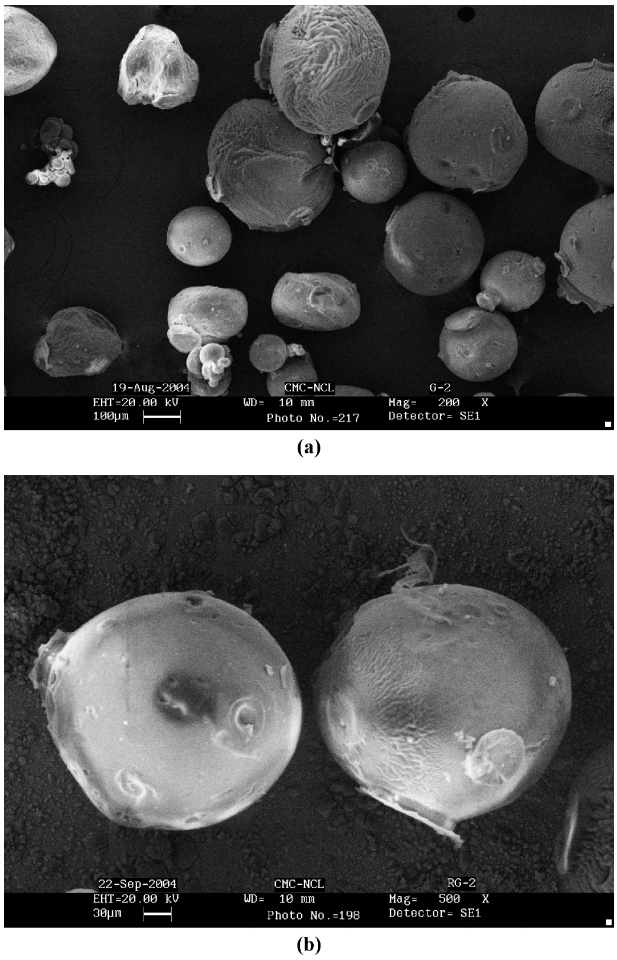

FIGURE 1 **SEM Images of the Microspheres: (a) Group of Particles and (b) Single Particle.**

mechanical strength, we prepared the GG-PVA IPN microspheres from the S/W suspension of carvedilol. Because carvedilol is slightly soluble in water, a S/W suspension of drug was prepared by controlled precipitation in polymer solution. By this method, percent encapsulation efficiency was in the range from 83 to 87. The microspheres produced were all spherical in nature, with the smooth surfaces as revealed by SEM images shown in Fig. 1. Sizes and size distributions of the microspheres were recorded by using a laser light diffraction technique (Mastersizer-2000, Malvern, UK). On a population basis, the particle size distribution was found to be unimodal with the narrow size distributions. Calculated values of volume–mean diameter of the microspheres are included in Table 2. These data showed a systematic dependence on cross-link density of the microspheres. With an increase in cross-link density, the microspheres with smaller size were produced, probably due to the formation of a more rigid network as a result of increased cross-link density. Also, by increasing the ratio of GG in the microspheres, an increase in the size of microspheres was observed, which could be attributed to the formation of bigger droplets with increasing concentration of GG during emulsification.

FTIR

FTIR was used to confirm the cross-linking of the IPN matrix. Figure 2 compares the FTIR spectra of (a) GG, (b) PVA, and (c) placebo microspheres. In the case of GG, a broad band, which appeared at 3444 cm^{-1} is attributed to the presence of hydroxyl groups of glucopyranose ring that are hydrogen bonded to various degrees. The bands appearing at 1610 and 1418 cm^{-1} indicate the presence of carboxylate group. The band at 2925 cm^{-1} is due to a stretching vibration of $-CH_2$ group, while those appearing at 1159 and 1023 cm^{-1} are due to the ethereal and hydroxylic C–O stretchings. A bending vibration of C–H appeared at 894 cm^{-1}. In case of PVA, a broad band observed at 3425 cm^{-1} is due to the O–H stretching vibrations, whereas the band at 1263 cm^{-1} is due to the O–H bending vibration. The band at 2850 cm^{-1} is attributed to the stretching vibration of $-CH_2$, while the one observed at 2912 cm^{-1} is due to the C–H stretching

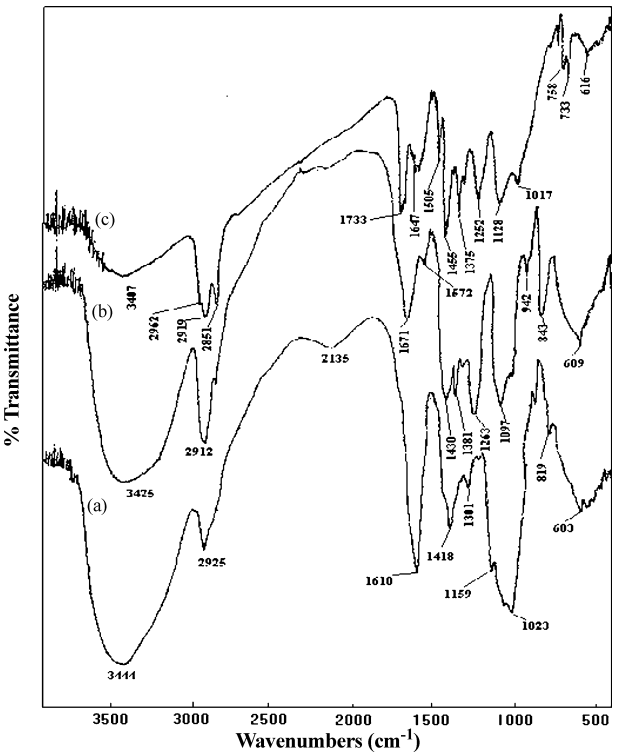

FIGURE 2 **FTIR Spectra of (a) GG, (b) PVA, and (c) Placebo Microspheres.**

vibration. The band at 1430 cm^{-1} is due to C–H bending vibration, while that at 1097 cm^{-1} indicates the C–O stretching vibration. In case of placebo microspheres, a broad band with less intensity compared to both GG and PVA matrices is due to the presence of very few uncross-linked hydroxyl groups of the glucopyranose ring that are hydrogen bonded to various degrees. The intense bands, which appeared at 2962, 2919, and 2851 cm^{-1} are due to the aliphatic C–H stretching vibrations. The bands at 1647 and 1455 cm^{-1} are due to the presence of carboxyl groups in GG. The bands appearing at 1128 and 1017 cm^{-1} are due to the presence of an acetal group, which formed due to the reaction of glutaraldehyde with the hydroxyl groups of both PVA and GG. Thus, FTIR confirms the cross-linking reaction in addition to the formation of IPN matrix. In addition, in the case of placebo microspheres, the shifting of peaks from 1610 and 1418 cm^{-1} to higher absorption frequencies indicates the interactions between IPN chains and further supports the formation of IPN structure.

FTIR spectral data were also used to confirm the chemical stability of carvedilol in the IPN microspheres. For instance, FTIR spectra of (a) placebo microspheres (b) carvedilol-loaded microspheres, and (c) pure carvedilol are displayed in Fig. 3. Pure carvedilol showed characteristic bands due to different functional groups. However, the band appearing at 3351 cm^{-1} is due to O–H/N–H stretching vibrations, while those at 2919 and 2850 cm^{-1} are due to aliphatic C–H stretching vibrations. The bands at 3061 and 2999 cm^{-1} are due to the aromatic C–H stretching vibrations, whereas those appearing at 1606, 1591, and 1503 cm^{-1} are due to aromatic C=C stretching vibrations. The N–H bending vibrations are seen at 1503 cm^{-1}. Bands at 1210 and 1190 cm^{-1} are due to C–N stretching vibrations, while the one that appeared at 1253 cm^{-1} is due to the aromatic C–O stretching vibrations. Spectra of carvedilol-loaded microspheres are not characteristically different from the spectra of the placebo microspheres. The peaks appearing at 3336, 3067, 3000, 2919, 2851, 1606, 1600, 1505, 1252, 1216, and 1174 cm^{-1} for carvedilol are also appearing in the carvedilol-loaded microspheres, indicating the chemical stability of carvedilol in IPN matrix.

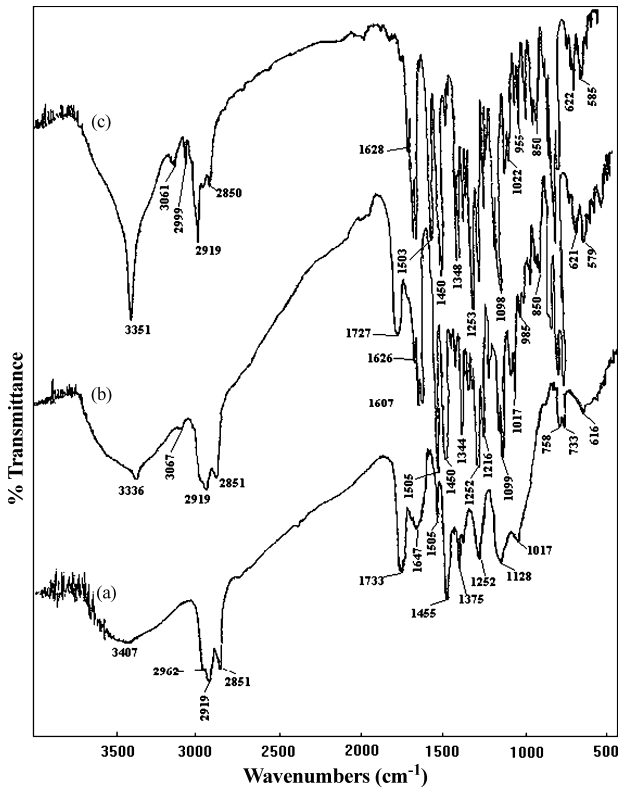

FIGURE 3 FTIR Spectra of (a) Placebo Microspheres, (b) Carvedilol-loaded Microspheres, and (c) Pure Carvedilol.

FTIR spectra of the pristine carvedilol and GA-treated carvedilol were compared (not presented in Figs. 2 and 3) to investigate the possible reactions between them. It was observed that FTIR spectrum of GA-treated carvedilol was identical to that of pristine carvedilol. In addition, a new peak observed at 1733 cm^{-1} is due to the presence of unreacted GA, which further confirms the chemical stability of carvedilol in presence of GA.

DSC

The DSC thermograms of (a) pure carvedilol, (b) plain GG, (c) plain PVA, (d) placebo microspheres, and (e) carvedilol-loaded microspheres are presented in Fig. 4. The crystallinity of carvedilol and melting temperature (T_m) of the polymer were determined. The plain GG has shown an endothermic peak at 248°C, indicating the melting temperature of the polymer. For plain PVA, two endothermic peaks were observed, one with a minimum at 210°C, which corresponds to the melting process, and the other at 290°C due to thermal decomposition. Similar peaks were observed for PVA in a study by Corradini et al. (1999). Placebo microspheres have also shown two endothermic peaks, one at 224°C due to the merging of melting peaks of GG and PVA, with the other at 320°C corresponding to thermal decomposition. In case of placebo microspheres, a shift in endothermic

peaks is observed toward higher temperatures compared to the plain GG and PVA. This could be due to the formation of more crystalline polymer matrix as a result of cross-linking and formation of IPN structure. This shift in endothermic peak toward higher temperature supports the formation of IPN structure due to chain entanglements. For pure carvedilol, an endothermic peak appeared at 116°C due to the melting of the drug. Similarly, an endothermic peak was observed at 120°C in the carvedilol-loaded microspheres, indicating the crystalline dispersion of the drug into the polymer matrix.

x-RD

The x-ray diffraction spectra recorded for (a) pure carvedilol, (b) carvedilol-loaded microspheres, and (c) placebo microspheres are presented in Fig. 5. These studies are useful to investigate the crystallinity of carvedilol in the cross-linked microspheres. Carvedilol has shown characteristic intense peaks between 2θ of 13 and 26°, but in the case of placebo microspheres, no peaks were observed between 2θ of 13 and 26°. However, in carvedilol-loaded microspheres, intense peaks were observed between 2θ of 13 and 26°, indicating the crystalline nature of the drug after entrapment into the microspheres.

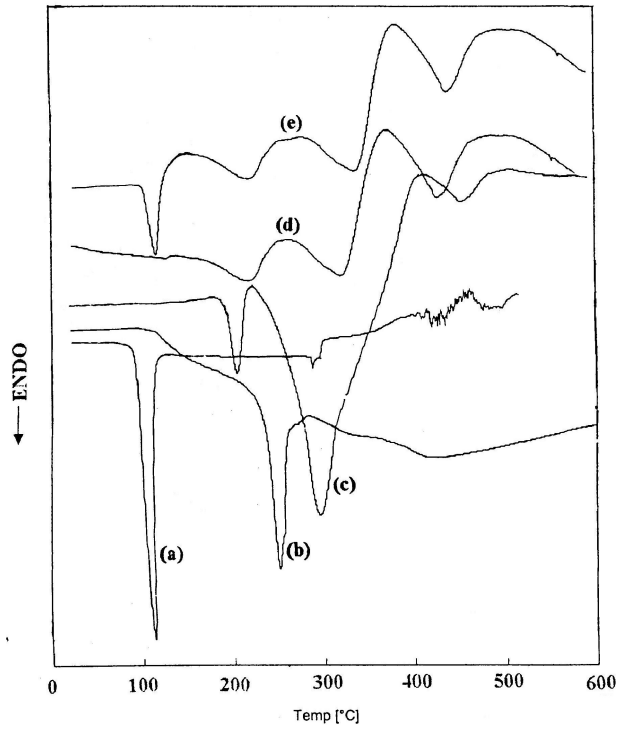

FIGURE 4 DSC Thermograms of (a) Pure Carvedilol, (b) Plain GG, (c) Plain PVA, (d) Placebo Microspheres, and (e) Carvedilol-loaded Microspheres.

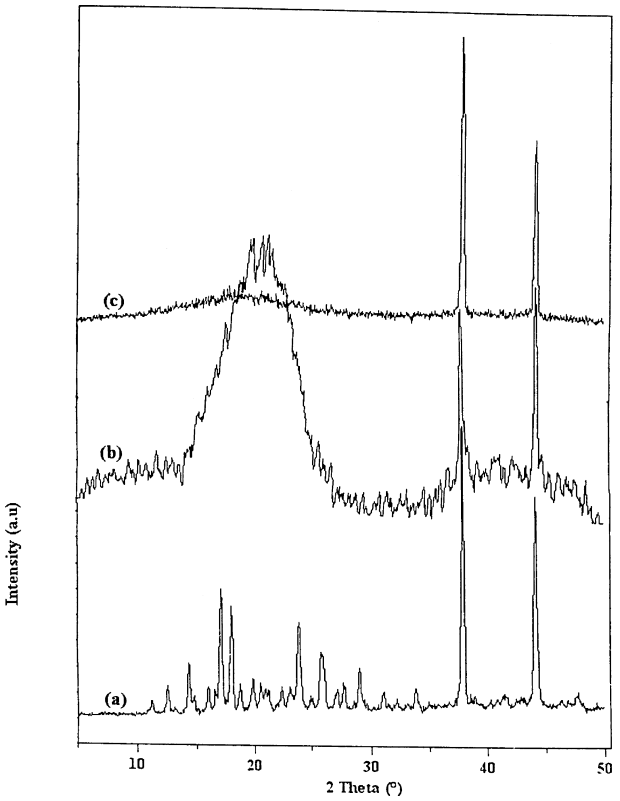

FIGURE 5 x-RD Diffractograms of (a) Pure Carvedilol, (b) Carvedilol-loaded Microspheres, and (c) Placebo Microspheres.

Tensile Strength Measurements

Tensile strengths of GG and the IPNs prepared are compared in Table 3. The tensile strength of IPNs was found to be fivefold to sixfold higher than that of pure GG. This is attributed to the formation of a large number of connections among the polymer chains after the formation of IPN, thereby increasing the strength of the material. This further supports the formation of IPN structure. Among the IPNs prepared, tensile strength increased with an increase in GA content, i.e., F1<F2<F3, indicating an

TABLE 3 Results of Tensile Strength

Formulation code	Tensile strength (MPa)
Gellan gum	13.5
F1	67.6
F2	72.2
F3	78.4

increased strength of the material with increasing cross-link density.

Liquid Transport Studies

Liquid transport studies were carried out in SGF or SIF media and percent equilibrium liquid uptake data are presented in Table 2. The percent equilibrium liquid uptake data varied from 107 to 196 for SGF and 107 to 194 for SIF media, suggesting a widely varying hydrophilic nature of the matrices. However, it can be seen that percent equilibrium uptake in SGF and SIF media showed a very small difference. It is observed that swelling capacity of the microspheres decreased with an increasing amount of glutaraldehyde. This may be due to the formation of a highly cross-linked rigid network. Swelling data obtained in SIF media are displayed in Fig. 6 typically for F1, F2, and F3 formulations. It was noticed that swelling capacity decreased with increasing amount of GG in the matrix, probably due to the formation of a rigid IPN structure due to the entanglement of both the polymer chains.

Drying Rates of the Microspheres

To optimize the drying conditions and to evaluate the effect of processing variables on the drying of microspheres, a representative collection of microspheres was selected for the drying study. The results of drying rate presented in Fig. 7 for formulations F1, F2, and F3 indicate that microspheres having a lesser extent of cross-linking dried more quickly than those with higher cross-linking. This could be attributed to

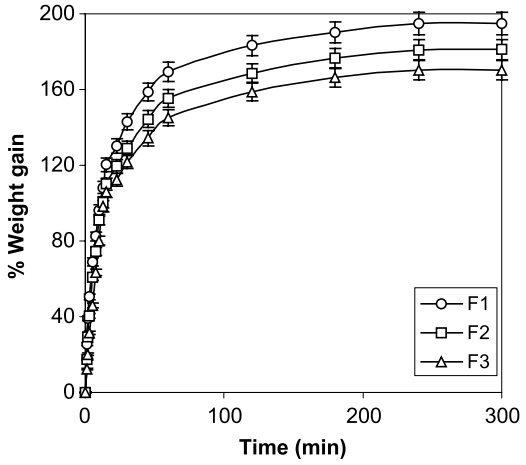

FIGURE 6 Effect of Cross-link Density on Percent Water Uptake by Microspheres.

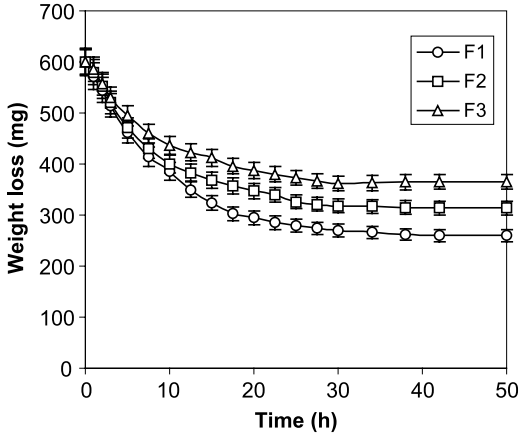

FIGURE 7 Effect of Cross-link Density on Weight Loss by Microspheres.

the formation of a more rigid IPN structure at higher cross-link densities.

Diffusion Coefficients of Liquid Transport Through Microspheres

In the present study, the drug is dispersed into polymeric microspheres. It would be interesting to model the transport process to compute the diffusion coefficient, D. Diffusion occurs due to the immersion of microspheres into the medium of interest, thereby provoking absorption of the liquid by the polymer. Mathematical models are available (Crank, 1975; Vergnaud, 1991) to describe the sorption and desorption processes under the simulated test conditions. Diffusion in spherically shaped matrices could be described by using the Laplace transformation of Fick's equation to calculate the mass uptake by the microspheres using

$$\frac{M_t}{M_\infty} = 6\sqrt{\frac{Dt}{\pi^2}}\left(\frac{1}{\sqrt{\pi}} + 2\sum_{n=1}^{\infty} \text{ierf}\,\frac{nr}{\sqrt{Dt}}\right) - 3\frac{Dt}{r^2} \quad (3)$$

where M_t is the amount of liquid released at time, t and M_∞ is the total amount of liquid in the microsphere. Equation 3 is complicated to solve. Baker and Lonsdale (1974) derived the following equation appropriate for the present case using the following initial and boundary conditions:

Initial : $t = 0 \;\; r < R \;\; C = C_{in}$

(inner part of microspheres)

Boundary : $t > 0 \;\; r = R \;\; C = C_{eq}$

(surface of microspheres)

In the above equations, r is initial radius, R is radius of the swollen microsphere, and C_{in} and C_{eq} are the concentrations at the beginning and at the end of the diffusion process, respectively. The diffusion coefficient can then be calculated for water absorption or drug release by the microspheres using

$$D = \left(\frac{r\theta}{6M_\infty}\right)^2 \pi \qquad (4)$$

where θ is slope of the linear portion of the plot of M_t/M_∞ vs. $t^{1/2}$, r is initial radius of the microspheres, and M_∞ is maximum (equilibrium) value of sorption. To calculate D for liquid desorption from the microspheres during drying, θ was calculated by plotting ln $(1- M_t/M_\infty)$ vs. time, t. Values of D calculated for sorption and desorption processes in both SGF and SIF media are included in Table 4. For the values of D calculated in SGF and SIF media, no significant difference ($P<0.01$) was observed. In general, D values for sorption were higher than those observed for desorption by nearly two orders of magnitude. This could be attributed to the slow drying of the microspheres compared to those computed during the sorption. It is observed that D for sorption and desorption processes decrease systematically with increasing cross-linking as well as with increasing amount of GG in the microspheres. This indicates that with increasing cross-linking density, a stiffer IPN matrix is likely to be formed. Also, with increasing amount of GG in the matrix, the rate of swelling and deswelling of microspheres has decreased due to the increased entanglement of GG and PVA chains.

Network Parameters

Release of the active agents from the polymer matrix is a function of the extent of cross-linking. In order to understand the cross-linking of the polymer network, we have calculated two important structural parameters, i.e., molar mass between cross-links (M_c) and cross-link density (d_x), as these parameters were widely studied (Flory, 1953). However, one of the most popular techniques to evaluate the network property of a polymer is based on the equilibrium swelling study. When a polymer is placed in a solvent, it swells until elastic forces due to stretching of the polymer chain segments balance osmotic forces that could dissolve the polymer. These elastic retractive forces are inversely proportional to the molar mass of the polymer between the points of cross-linking. Thus, molar mass between two junction points in a network would be rigid and exhibit limited swelling. When M_c is large, the network is more elastic and swells rapidly if brought in contact with a compatible liquid.

In order to assess the M_c values, the Flory–Rehner equation (Flory, 1953) in the following form was used:

$$M_c = -\rho_p V_s \phi^{1/3} [\ln(1-\phi) + \phi + \chi\phi^2]^{-1} \qquad (5)$$

TABLE 4 Results of Parameters *k* and *n*, Correlation Coefficient (*r*) Calculated from Eq. 9 and Diffusion Coefficients (*D*) Calculated from Eq. 4 for Sorption and Desorption Processes in SGF and SIF Media at 37°C

	SGF					SIF				
Formulation code	k	n	r	$D_{(sorption)} \times 10^6$ (cm²/s)	$D_{(desorption)} \times 10^8$ (cm²/s)	k	n	r	$D_{(sorption)} \times 10^6$ (cm²/s)	$D_{(desorption)} \times 10^8$ (cm²/s)
F1	0.126	0.79	0.977	9.22	7.81	0.122	0.80	0.971	9.10	7.64
F2	0.123	0.79	0.973	8.71	7.12	0.119	0.82	0.968	8.62	7.01
F3	0.121	0.83	0.984	8.15	6.61	0.115	0.85	0.974	8.03	6.62
F4	0.110	0.80	0.964	7.64	6.74	0.108	0.83	0.982	7.61	6.70
F5	0.110	0.84	0.987	7.16	5.90	0.106	0.84	0.968	7.18	5.95
F6	0.091	0.89	0.968	6.45	5.28	0.090	0.91	0.987	6.38	5.24
F7	0.105	0.84	0.988	7.60	5.60	0.099	0.87	0.975	7.49	5.58
F8	0.098	0.89	0.975	7.09	5.20	0.093	0.90	0.988	7.02	5.23
F9	0.080	0.93	0.970	6.32	4.47	0.076	0.96	0.979	6.29	4.43

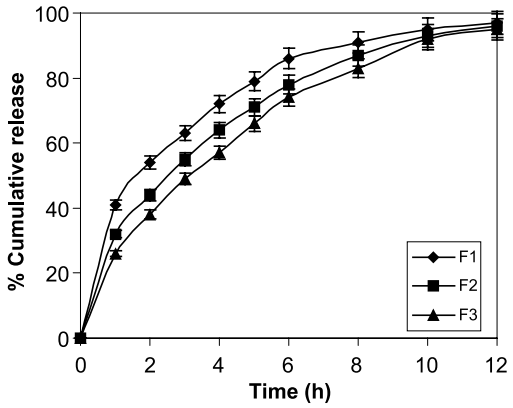

FIGURE 8 **Effect of Cross-link Density on In Vitro Release Profiles.**

The volume fraction, ϕ of the polymer in the swollen state has been calculated as follows:

$$\phi = \left[1 + \frac{\rho_p}{\rho_s}\left(\frac{M_a}{M_b}\right) - \frac{\rho_p}{\rho_s}\right]^{-1} \quad (6)$$

In the above equations, ρ_p and ρ_s are the densities of polymer and solvent, respectively; M_b and M_a are, respectively, the mass of polymer before and after swelling, and V_s is molar volume of the solvent. The interaction parameter, χ can be calculated using the equation proposed by Bristow and Watson (1958):

$$\chi = \beta + \left(\frac{V_s}{RT}\right)(\delta_s - \delta_p)^2 \quad (7)$$

Here, β is a lattice constant, with a value that is taken to be 0.34, V_s is molar volume of the solvent, R is molar gas constant, and T is temperature in Kelvin. The symbols δ_s and δ_p are solubility parameters of solvent and polymer, respectively.

For the analysis of cross-linked structure of the hydrogels, the cross-link density, (d_x) was calculated using the following equation (Savas and Guven, 2001);

$$d_x = \frac{1}{vM_c} \quad (8)$$

Here, v is the specific volume of the polymer. The results of M_c and d_x are presented in Table 2. As indicated by the data in Table 2, the M_c values decreased with an increase in GA content of the formulation and the network becomes denser. Also, the M_c values decreased with an increase in GG content of the formulation, indicating a dense structure. Similarly, the cross-link density of IPNs was significantly affected by GA and GG contents in the formulations.

In Vitro Drug Release

In vitro drug release studies were performed in SGF or SIF media (without enzymes) for 12 h and percent cumulative release vs. time data are presented in Fig. 8 for different cross-link densities of the microspheres studied in SIF media. In an effort to study the extent of cross-linking on the release rates, we have taken the formulations F1, F2, and F3 (with the respective 1, 2, and 3×10^{-3} mL of GA/mg of polymer). It is found that release rates varied depending upon the amount of GA used for cross-linking, i.e., release was slower for those formulations in which a lower amount of GA was used compared to those in which a higher amount was used. This is due to the fact that at higher cross-linking, free volume of the matrix will be less, thereby hindering the easy transport of drug molecules through the matrix. This also reduces the rate of swelling as well as the rate of drug release from the matrix.

The percent cumulative release vs. time for the microspheres prepared with different ratios of GG:PVA loaded with 50% of carvedilol are presented in Fig. 9. Drug release rates are higher for microspheres having a lower amount of GG compared to those prepared with higher amount of GG. This further explains the formation of stiffer polymer chain entanglements at higher proportion of GG, thus reducing the rate of swelling and release of drug. The release data are in agreement with the equilibrium swelling data, wherein swelling capacity of the microspheres decreased with increasing amount of GG in the microsphere. Drug release was also affected by the composition of the release media. The release rates of

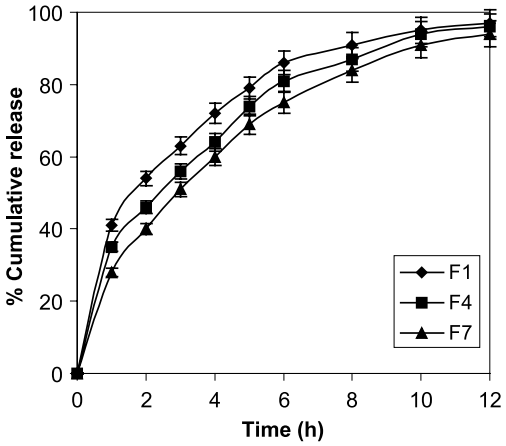

FIGURE 9 **Effect of GG:PVA Ratios on In Vitro Release Profiles.**

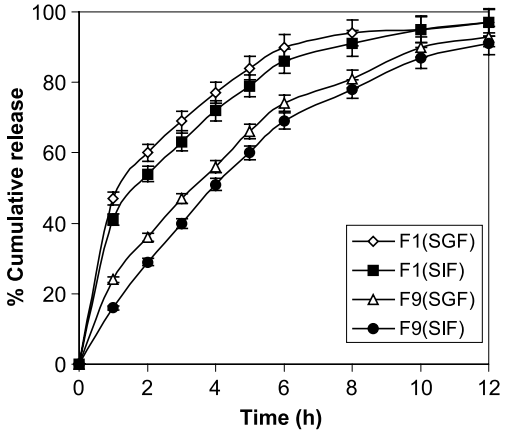

FIGURE 10 **Effect of Dissolution Media on In Vitro Release Profiles.**

carvedilol in SGF and SIF media are compared in Fig. 10, wherein it was seen that carvedilol release from the microspheres was faster in the case of SGF compared to SIF media. This could be explained from the saturation solubility results obtained before the in vitro release studies, wherein it was noticed that the saturation solubility of carvedilol was almost double in SGF compared to SIF media. Because there was no significant difference in the swelling of the microspheres in SGF and SIF media, the possible reason may be due to the difference in the solubility of drug in both the media. Thus, the release of carvedilol in SGF was faster than in SIF media.

The release data were further analyzed using an empirical equation (Ritger & Peppas, 1987). The initial 60% drug release data were fitted to Eq. 9 to estimate the release kinetic parameter, k and the diffusional exponent, n:

$$\frac{M_t}{M_\infty} = kt^n \qquad (9)$$

By applying the least-squares method to the release data at 95% confidence level, we estimated the values of k and n. These data along with the correlation coefficients, r are presented in Table 4. The values of k decrease with increasing cross-lnking density as well as with increasing ratio of GG in the IPN matrix. The k values ranged between 0.080 and 0.126 in SGF, whereas in SIF, these varied between 0.076 and 0.122. Such small values of k indicate mild interactions between the drug and the polymer matrix. On the other hand, the values of n increased with increasing cross-link density as well as with increasing ratio of GG in the IPN matrix. These values ranged between 0.79 to 0.93 in SGF and 0.80 to 0.96 in SIF, indicating that the drug release followed Case II transport (Ritger & Peppas, 1987).

CONCLUSIONS

Gellan gum-poly(vinyl alcohol) interpenetrating microspheres were prepared by the emulsion cross-linking. The IPNs prepared demonstrated a better mechanical property as compared to pure GG, indicating the suitability of the IPNs for microsphere preparation. The cross-link density was significantly affected by the content of GA and GG in the formulations. Carvedilol was successfully entrapped into the IPN matrix. Microspheres were spherical with smooth surfaces. The release of carvedilol was found to depend upon the extent of cross-linking of the matrix as well as the amount of gellan gum present in the matrix. Higher release rates were observed for microspheres with lower cross-link density and lower amounts of gellan gum in the matrix. No significant difference was observed in the diffusion coefficients in the simulated gastric and intestinal fluids. The n values varied in the range from 0.79 to 0.93 in SGF and 0.80 to 0.96 in SIF media, indicating the release mechanism to follow Case II transport.

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