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Interpenetrating polymer network of locust bean gum-poly (vinyl alcohol) for controlled release drug delivery

Santanu Kaity, Jinu Isaac, Animesh Ghosh*

Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi 835 215, Jharkhand, India

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ABSTRACT

A novel interpenetrating polymer network (IPN) microspheres of locust bean gum (LBG) and poly (vinyl alcohol) (PVA) was developed for oral controlled release of buflomedil hydrochloride (BH) by emulsion crosslinking method using glutaraldehyde as crosslinker. The effects of gum–polymer ratio, concentration of crosslinker and internal phase viscosity were evaluated thoroughly. Drug entrapment efficiency, particle size distribution, swelling property and in vitro release characteristics with kinetic modelling of microspheres were evaluated. The microspheres were characterised by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), solid state C^{13} NMR, X-ray diffraction study (XRD) and differential scanning colorimetry (DSC). The microspheres showed control release property without showing any incompatibility in IPN device. Hence, IPN microspheres of LBG and PVA can be used as a potential carrier for controlled oral delivery of highly water soluble drugs like BH.

1. Introduction

Drug delivery research is primarily focussed on targeted delivery of the drug to the desired organ system to minimise toxicity and maximise therapeutic efficacy. As oral route is the most popular route of administration, a large emphasis is given on the development of controlled oral drug delivery systems. Drug substances with high water solubility and short half life (elimination half-life 2–3 h) get readily absorbed and eliminated, thus requiring frequent dosing (Alavijeh, Chishty, Qaiser, & Palmer, 2005). This may lead to decrease in patient compliance and increase chances of side effects due to dose dumping (Phutane, Shidhaye, Lotlikar, Ghule, Sutar, & Kadam, 2010; Ray et al., 2010). Thus, the drugs having high water solubility and short half-life warrants extensive research to reduce frequent dosing and dose related side effects by controlling their release rate. Fabrications of drug delivery devices like micropaticles or nanospheres are some of the approaches to control the release of highly water soluble drugs. Recently researchers has given a strong emphasis on natural polymer based approaches to develop controlled drug delivery systems. The use of synthetic polymers for drug delivery purposes is of limited application due to problems in biodegradability, use of organic solvents resulting in environmental pollution etc. (Halder, Mukherjee, & Sa, 2005). However natural polymers are considered to be much safer since organic solvents are not required for their processing and they are biodegradable in nature (Vijan et al., 2012; Vyas and Khar, 2002). A lot of natural gums like alginate (Das and Senapati, 2008; Yegin et al., 2007), chitosan (Win, Shin-ya, Hong, & Kajiuchi, 2003), guar gum (Thimma and Tammishetti, 2001), xanthan gum (Ray, Maiti, & Sa, 2008) have been extensively studied for the fabrication of drug delivery systems. However, the use of these gums has their own problems like uncontrolled swelling and premature release of loaded drug. The concept of polymeric blend microsphere is quite effective in overcoming the above problems. Interpenetrating polymer network (IPN) is one such formulation which is considered to be promising in delivery of bioactive molecules, particularly in controlled release applications (Changez, Burugapalli, Koul, & Chowdary, 2003). An IPN is a composite of two polymers, which is obtained when at least one polymer network is synthesised or cross-linked independently in the immediate presence of the other. Recently a large number of IPN microspheres have been developed (Agnihotri and Aminabhavi, 2005; Ray et al., 2010; Soppimath, Kulkarni, & Aminabhavi, 2000). Poly (vinyl alcohol) (PVA) based IPN systems are extensively studied due to its inherent non-toxicity, non-carcinogenicity, biocompatibility and desirable physical properties such as rubbery or elastic nature and high degree of swelling in aqueous solution leading to its extensive industrial use. Chemical

Abbreviations: LBG, locust bean gum; PVA, poly (vinyl alcohol); BH, buflomedil hydrochloride; GA, glutaraldehyde; IPN, interpenetrating polymer network; FTIR, Fourier transform infra-red; NMR, nuclear magnetic resonance; XRD, X-ray diffraction; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; KBr, potassium bromide; UV–vis, ultraviolet–visible; C, carbon; N, nitrogen; O, oxygen; DEE, drug encapsulation efficiency.

* Corresponding author. Tel.: +91 9470339587; fax: +91 6512275290.
E-mail addresses: aghosh@bitmesra.ac.in, anim_1607@yahoo.co.in (A. Ghosh).

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crosslinking between these polymers provides improved mechanical properties and thermal stability (Mishra, Bajpai, Katare, & Bajpai, 2006).

In this present study, screening of a new PVA based natural polysaccharide IPN system has been focussed. Locust bean gum (LBG), a natural polysaccharide, was chosen for the study. Locust bean gum is a high molecular weight branch polysaccharide and is extracted from the seeds of carob tree *Ceratonia siliqua*. It consists of a (1,4)-linked β-D-mannopyranose backbone with branch points from their 6-positions linked to α-D-galactose (1,6-linked α-D-galactopyranose. The molecular weight of LBG is 300,000–360,000. It is less soluble in water and needs heating to dissolve. Being non-ionic, it is not affected by pH or ionic strength. It is dispersible in either hot or cold water, forming a sol having a pH of 5.4–7.0, which may be converted to a gel by the addition of small amounts of sodium borate. Previously, we have reported ion crosslinked hydrogel beads of carboxymethyl LBG for controlled oral delivery of glipizide (Maiti et al., 2010). Recently we have developed IPN hydrogel microspheres composed of sodium carboxymethyl cellulose and PVA to encapsulate diclofenac sodium (Banerjee et al., 2012). In continuation to the above works, IPN hydogel microspheres of locust bean gum and poly (vinyl alcohol) has been prepared and further characterised by FT-IR, SEM, XRD and DSC. An attempt was made to prepare an IPN hydrogel microsphere by using locust bean gum and PVA for oral controlled delivery of buflomedil hydrochloride (BH). Buflomedil is readily absorbed in the gastrointestinal tract and has a plasma half-life of approximately 2–3 h. Pharmacologically, buflomedil increases perfusion to impaired vascular beds of the microcirculation, increases arterial perfusion with minimal effects on central haemodynamics, exhibits apparent oxygen "sparing" effects in animal experiments, demonstrates inhibitory effects on platelet aggregation, and, in preliminary experiments, appears to improve deformability of erythrocytes with abnormal fluidity. A non-specific α receptor blocking activity appears to be involved, at least in part, in these pharmacologic effects (Dubourg & Scamuffa, 1981).

2. Materials and methods

2.1. Materials

Buflomedil hydrochloride was obtained as gift sample from Fresenius Kabi Oncology Limited (Kalyani, West Bengal, India). Locust bean gum (Molecular weight 310,000) was supplied by Hindustan Gums (Hariyana, India). Poly(vinyl alcohol) (PVA: 98% hydrolysed, average molecular weight 125,000), light liquid paraffin (LLP, viscosity 25–80 mPa at 20 °C) was procured from HiMedia Laboratories Private Limited (Mumbai, India). Span 80 was procured from Pioneer in-organics (Delhi, India). Hydrochloric acid (HCl, 30% ultra pure) was obtained from HiMedia Laboratories Private Limited (Mumbai, India). Glycine, glutaraldehyde (GA: 25%, v/v) was supplied by Merck Limited (Mumbai, India). Acetone was procured from Qualigens fine chemicals (Mumbai, India). Water used was of Milli-Q grade. All other reagents were of analytical grade and used without further purification.

2.2. Preparation of IPN hydrogel microspheres

Microspheres composed of LBG and PVA containing buflomedil hydrochloride (BH) were prepared by water-in-oil (w/o) emulsion-crosslinking method (Banerjee et al., 2010). Briefly, 20 mL of 5% (w/v) aqueous polymeric solution (total polymer amount was kept constant) was prepared by dissolving varying amount of LBG and PVA. First, PVA was dissolved in water (80 °C) by continuous stirring until a transparent solution was obtained. LBG was then dispersed in hot PVA solution with continuous stirring and kept for cooling at room temperature. Required amount of drug was added in the polymeric dispersion and stirred overnight with the help of magnetic stirrer to obtain a homogeneous, bubble free drug polymer mixture. The drug polymer mixture was slowly added to light liquid paraffin (100 g, w/w) containing 1% (w/w) Span-80 under constant mechanical stirring at 800 rpm for 10 min. A milk white (w/o) emulsion was formed. To this (w/o) emulsion, GA containing 0.5 mL of 1 N HCl was added slowly and stirring was continued for 3 h in order to produce hardened microspheres. The microspheres were then filtered and washed with acetone, 0.1 M glycine solution and water to remove excess amount of liquid paraffin, unreacted GA and surfactant, respectively. Complete removal of unreacted GA was tested by treating the filtrate with Fehling's reagent. A negative result was obtained which confirmed the absence of unreacted GA. Hardened microspheres were vacuum dried at 40 °C for 24 h and stored in desiccator until further use.

For the preparation of blank microspheres similar method was adopted except the addition of drug into the system.

2.3. Formulation variables

Total seventeen formulations were prepared by varying the three parameters, i.e., extent of crosslinking, percentage of drug loading and ratio of LBG and PVA in order to study the effects of formulation variables. The total polymer concentration was kept constant (5% w/v). The assigned formulation codes are given in Table 1. Proposed reaction scheme for the IPN hydrogel microsphere preparation is given in Fig. 1

2.4. Measurement of viscosity of internal phase

The viscosity of LBG and PVA blends (internal phase) were determined by a programmable Brookfield viscometer (Model DV-II+ Pro, Brookfield Engineering Labs., Inc., Middleboro, MA) at 32.7 °C. The spindle (Spindle No. CPE41) was rotated at 2 rpm (Maiti et al., 2010).

2.5. Estimation of percentage yield

The percent yield of microspheres was calculated by considering the total amount of microspheres obtained. The total weight of drug and polymers used during the preparation was taken to be the theoretical weight. The percent yield was calculated as follows (Maiti et al., 2009).

$$\text{Yield}\,(\%) = \left[\frac{\text{amount of microsphere}}{\text{amount of drug} + \text{total amount of polymer}}\right] \times 100 \quad (1)$$

2.6. Drug entrapment efficiency (DEE) study

Accurately weighed, 10 mg of dried microspheres were crushed with the help of a mortar and pestle and were transferred into 50 mL of pH 6.8 phosphate buffer solution and heated at 50 °C to extract the drug. After 24 h, the suspension was filtered and centrifuged to remove the polymeric debris. Then, the supernatant was taken and analysed with a spectrophotometer (UV-1800, Shimadzu, Japan) at λ_{max} = 282 nm. All samples were analysed in triplicate. The drug entrapment efficiency (%) was calculated by using the following equation (Maiti et al., 2010):

$$\text{Entrapment efficiency}\,(\%) = \left(\frac{\text{actual drug content}}{\text{theoretical drug content}}\right) \times 100 \quad (2)$$

Table 1
Formulations with their respective codes, internal phase viscosity and glutaraldehyde used.

Gum:polymer ratio (LBG:PVA)	Viscosity (cP)	Amount of glutaraldehyde used (mL)		
		2.5	5	7.5
3:1	368.0	F1	F2	F3
2:1	276.5	F4	F5	F6
1:1	193.9	F7	F8	F9
1:2	122.6	F10	F11	F12, F16, F17
1:3	103.6	F13	F14	F15

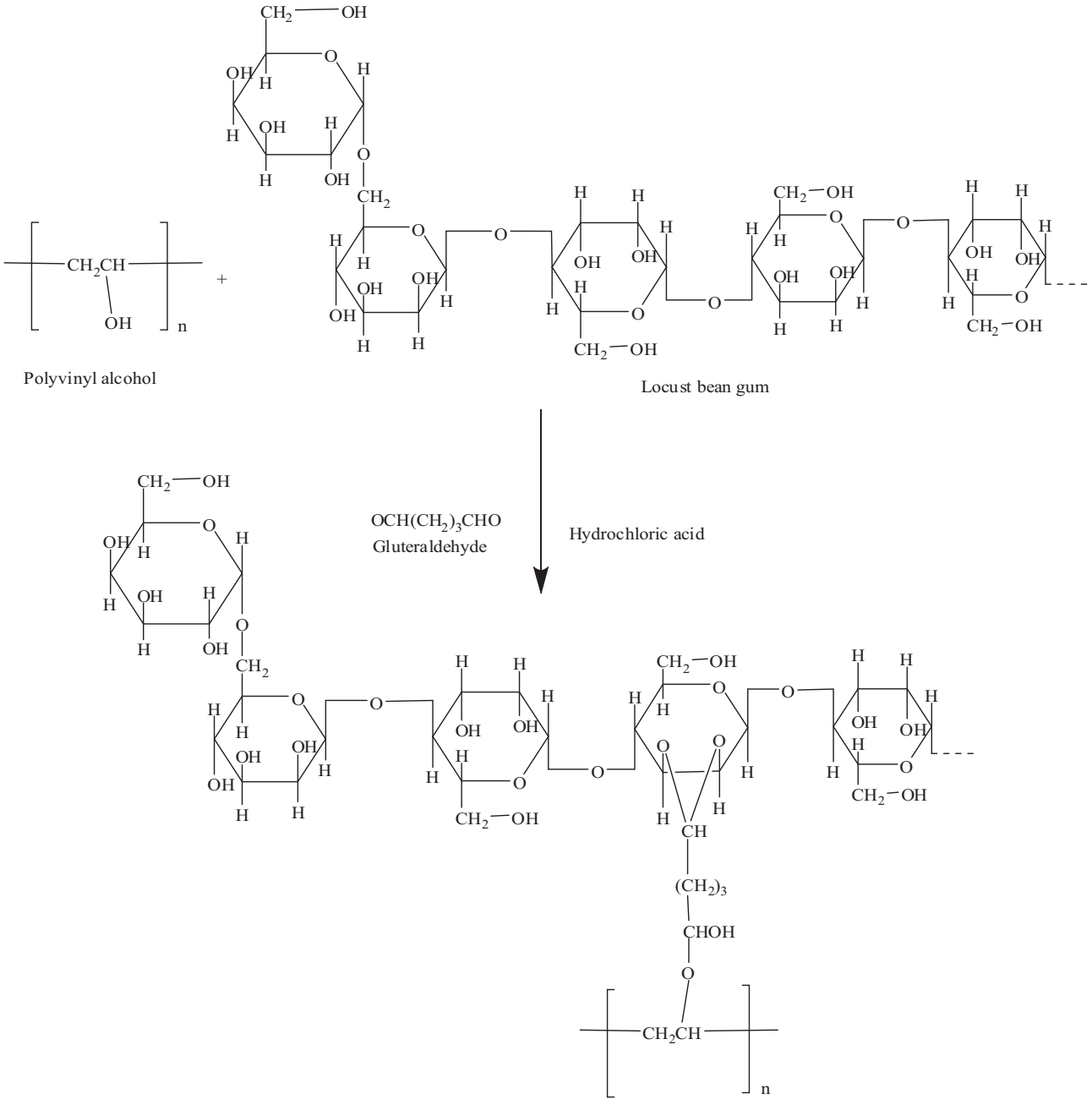

Fig. 1. Proposed scheme of formation of PVA–LBG interpenetrating polymer network crosslinked by GA.

2.7. Swelling study

The pH-dependent equilibrium water uptake of the blank microspheres was measured by immersing the microspheres (10 mg) in 50 mL buffer media having pH 1.2 and pH 6.8. In order to ensure complete equilibration, microspheres were allowed to swell completely for about 24 h at 37 °C temperature. The surface adhered liquid droplets of particles were removed by blotting with soft

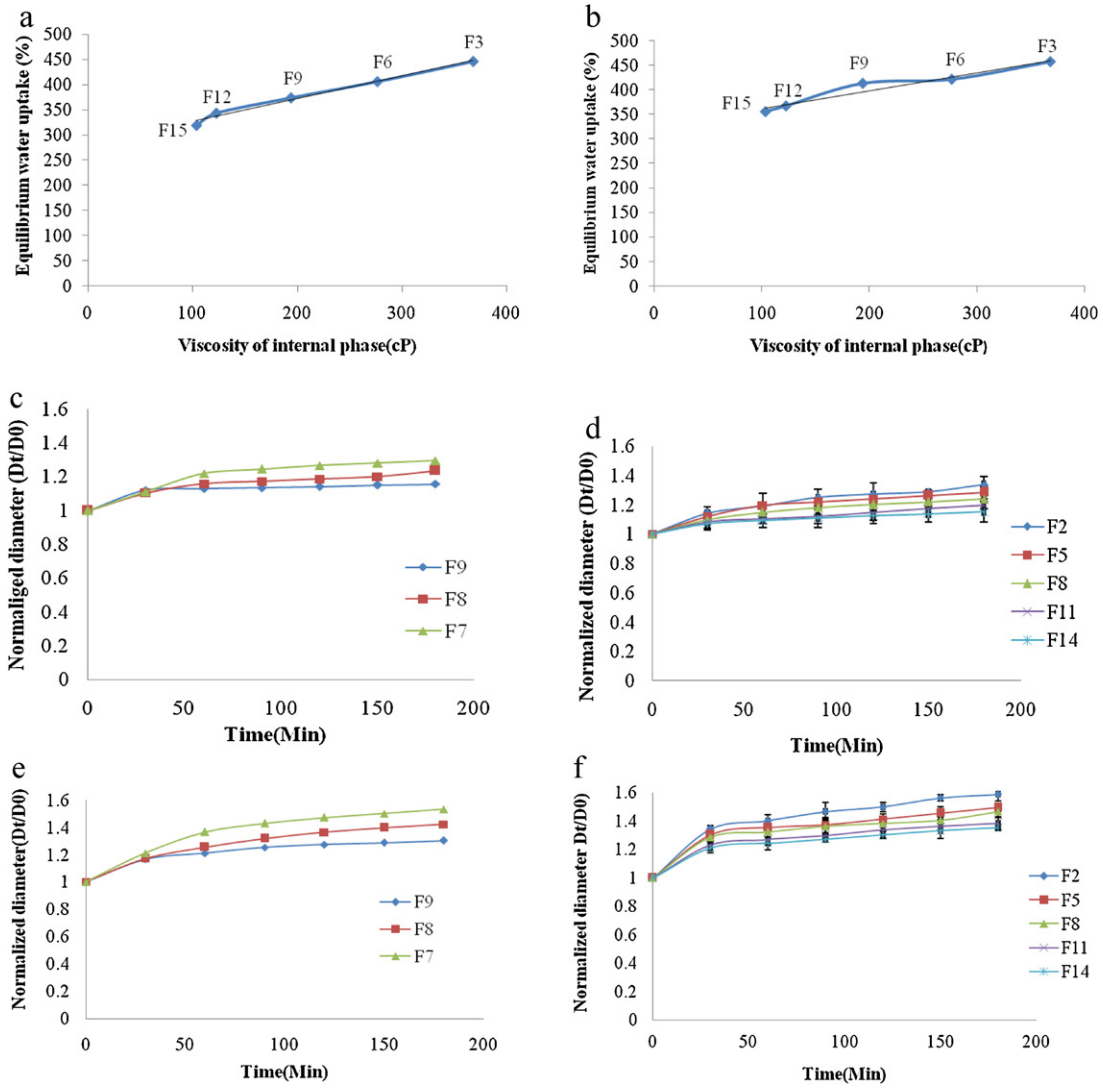

Fig. 2. Swelling study (a) effect of internal phase viscosity on equilibrium swelling (in pH 1.2), (b) effect of internal phase viscosity on equilibrium swelling (in pH 6.8), (c) effect of extent of crosslinking on dynamic swelling (in pH 1.2), (d) effect of amount of locust bean gum on dynamic swelling (in pH 1.2), (e) effect of extent of crosslinking on dynamic swelling (in pH 6.8), (f) effect of amount of locust bean gum on dynamic swelling (in pH 6.8).

tissue papers without pressing hard and then swollen microspheres were weighed to an accuracy of ±0.01 mg on an electronic microbalance (Mettler, model AT120, Greifensee, Switzerland). The percentage equilibrium water uptake was calculated as follows (Bhattacharya et al., 2011):

$$\text{Equilibrium water uptake (\% Qeq)} = \left[\frac{\text{mass of swollen microsphere} - \text{mass of dry microsphere}}{\text{mass of dry microsphere}}\right] \times 100 \quad (3)$$

Dynamic swelling study was conducted by microscopic technique (Ray et al., 2010). Microspheres were allowed to swell in pH 1.2 and pH 6.8 and at different time intervals microspheres were withdrawn from the medium. After removing the adhered liquid droplets from microsphere surface, the changes in diameter were measured by an optical microscope (Olympus Model HB, India). In this study changes in diameter of microspheres were monitored as a function of time. All the experiments were carried out in triplicate, but average values were considered for calculation (Fig. 2).

Formulation variables, yield (%), mean particle size, drug (%) entrapment efficiency (DEE) and equilibrium water uptake in pH 1.2 and pH 6.8 are shown in Table 2.

2.8. In vitro drug release studies

The in vitro drug release from the IPN hydrogel microspheres were studied in triplicate in both acidic (pH 1.2, up to 3 h.) and alkaline (pH 6.8, up to 12 h.) media. USP-I rotating basket type dissolution test apparatus (Electro Lab TDT-08L, India) equipped with eight baskets (glass jars) at the stirring speed of 50 rpm at 37 °C temperature under sink condition was used in this experiment. Drug release from the IPN microspheres having different drug loading, IPN composition, and extent of cross-linking were studied. At regular time intervals, 10 mL aliquot samples were withdrawn and replaced by an equal volume of buffer solution. The amount of drug released was analysed using a double beam UV–vis spectrophotometer (UV-1800, Shimadzu) at the wavelength of 282 nm. From this, cumulative percentage drug release was calculated and plotted as function of time to study the pattern of drug release (Angadi, Manjeshwar, & Aminabhai, 2011).

Table 2
Formulation variables, yield (%), particle size, drug (%) entrapment efficiency (DEE) and equilibrium water uptake in pH 1.2 and pH 6.8.

Formulation Code	LBG:PVA	BH loading (%)	GA (mL)	Yield (%)	Particle size [d (0.5)] (μm)	PDI	DEE (%) (±SD, $n=3$)	Equilibrium water uptake (%)	
								pH 1.2	pH 6.8
F1	3:1	50	2.5	62.27	734.18	1.07	29.73 ± 2.14	445.72	457.21
F2	3:1	50	5.0	69.51	698.90	1.40	33.52 ± 1.73	437.33	449.69
F3	3:1	50	7.5	65.33	673.22	1.53	35.33 ± 1.91	421.51	428.56
F4	2:1	50	2.5	71.59	642.19	1.27	32.61 ± 1.52	405.83	421.47
F5	2:1	50	5.0	72.82	608.64	1.60	37.11 ± 1.12	397.31	416.98
F6	2:1	50	7.5	78.80	571.43	1.42	41.83 ± 2.30	389.65	411.37
F7	1:1	50	2.5	78.35	533.18	1.30	43.26 ± 1.33	373.75	413.19
F8	1:1	50	5.0	81.22	420.83	1.14	49.37 ± 1.87	369.54	395.77
F9	1:1	50	7.5	80.78	337.25	1.03	51.72 ± 1.62	353.11	387.65
F10	1:2	50	2.5	82.14	441.64	1.31	55.42 ± 2.31	343.44	367.22
F11	1:2	50	5.0	83.55	395.26	1.54	59.41 ± 1.44	335.81	361.23
F12	1:2	50	7.5	83.17	337.24	0.95	68.59 ± 1.21	327.13	352.52
F13	1:3	50	2.5	85.62	429.13	1.22	57.11 ± 2.63	319.20	355.63
F14	1:3	50	5.0	85.35	322.71	1.13	61.63 ± 1.85	307.33	351.49
F15	1:3	50	7.5	84.71	293.17	1.18	65.17 ± 2.21	298.35	337.85
F16	1: 2	25	7.5	73.79	347.31	1.29	42.58 ± 2.32	345.23	352.14
F17	1:2	75	7.5	81.34	359.87	1.47	65.39 ± 1.72	348.42	356.25

The effect of gum:polymer ratio and amount of GA on drug release was analysed by Design Expert software (Design Expert trial version 8.0.7.1, Stat-Ease, Inc., Minneapolis, MN). Polynomial models including interactions and quadratic terms were generated for all the response variables using multiple linier regression analysis (MLRA) approach. Statistical validity of the polynomials was established on the basis of ANOVA (p value < 0.05). Two sets of gum:polymer ratio (1:1, 1:2, 1:3 and 1:1, 2:1, 3:1) with the different amount of GA (2.5, 5.0 and 7.5 mL) were used to generate the mathematical relationship with the response variables (CPR at 3 h in 0.1 N HCl and CPR at 12 h in pH 6.8 phosphate buffer).

2.9. Kinetics modelling of drug release

The kinetics of drug release from the IPN microspheres were tested by using various empirical equations such as zero-order (Cumulative percentage drug release vs. time), first-order (Log cumulative percentage drug remaining to be released vs. time), Higuchi square root equation (Cumulative percentage release vs. square root of time), Hixson–Crowell cube root equation (cube root of drug (%) remaining to be released vs. cube root of time), and the power law equation which is also known as Korsmeyer–Peppas equation and can be expressed as:

$$\frac{M_t}{M_\infty} = Kt^n \tag{4}$$

where M_t and M_∞ are the amount of drug released at time t and at infinite time, respectively. K represents a constant, incorporating structural and geometrical character of the dosage form, and n values denote the diffusion exponent indicative of the mechanism of drug release. The mechanism of drug release from spherical polymeric devices may be Fickian diffusion when the value of $n = 0.43$ or less, anomalous (non-Fickian) transport when the value of n lies between 0.43 and 0.85, and case II transport when $n = 0.85$. Exponent values of n greater than 0.85 signifies the super case II transport mechanism (Higuchi, 1961; Korsemeyer, Gunny, Peppas et al., 1983; Peppas, 1985; Ritger and Peppas, 1987).

2.10. Particle size analysis

Particle size analysis was done by Mastersizer 2000 Ver.5.40 (Malvern Instruments Ltd., UK) which allows sample measurement in the range of 0.020–2000 mm. The particles were dispersed in water and size was measured by the laser light scattering technique. Polydispersity index was determined according to the following equation:

$$\text{Polydispersity index} = \frac{d(0.9) - d(0.1)}{d(0.5)} \tag{5}$$

where d (0.9) corresponds to the particle size by volume immediately above 90% of the sample, d (0.5) corresponds to the particle size immediately above 50% of the sample; d (0.1) corresponds to the particle size immediately above 10% of the sample. (Maiti, Kaity, Ray, & Sa, 2011).

2.11. Scanning electron microscopy

The surface morphology and shape of the LBG–PVA network microspheres were investigated by scanning electron microscope (JSM-6390LV, Jeol, Japan). Prior to examination, the samples were mounted onto stubs using double-sided dried carbon tape and vacuum coated with gold palladium film (thickness 2 nm) using sputter coater (Edward S-150, UK) to render them electrically conductive.

2.12. Fourier transform infrared spectroscopy

FTIR spectral analysis of LBG, PVA, pure buflomedil hydrochloride (BH), placebo microsphere and drug loaded microspheres was done using FTIR-8400S (Shimadzu, Japan) to confirm the compatibility of different ingredients of the formulation. A small amount of each material was mixed with KBr (1% w/w sample content), taken into sample holder and scanned in the range of 600–4000 cm^{-1}.

2.13. Solid state C^{13} NMR

A Bruker AMX 300 spectrophotometer operating at 75 MHz for 13C solid state NMR was used for analysis of LBG and LBG–PVA IPN. Placebo IPN microspheres and native LBG were used for NMR study to confirm the formation of GA crosslinked IPN matrix of LBG and PVA.

2.14. Differential scanning calorimetry

DSC thermogram of native gum, PVA, pure drug (BH), placebo microsphere and drug loaded microspheres were studied by using DSC-60 (Shimadzu, Japan). Each sample (3–7 mg) was accurately weighed into a 40 μL aluminium pan in a hermetically sealed condition. The measurements were performed in an atmosphere of

nitrogen between 20 °C and 250 °C at a heating rate of 10 °C/min (Bruylants, Wouters, & Michaux, 2005).

2.15. Qualitative X-ray diffractometry

Grinded samples of pure drug (BH), placebo microsphere and drug (BH) loaded microspheres were scanned from 10° to 60° 2θ, using an X-ray diffractometer (Bruker AXS D8 Advance, Germany, Configuration: Vertical, Theta/2 Theta geometry) X-ray Cu, Wavelength 1.5406 A°, detector: Si (Li) PSD. The diffractometer was run at a scanning speed of 2°/min and a chart speed of 2°/2 cm per 2θ and the angular range was fixed from 10° to 60°. The peaks at 2θ value 10.577° and 11.904° were considered as the representative peaks for the calculation of relative degree of crystallinity (RDC) as the other peaks of pure drug obtained merged in the case of drug loaded formulation. The RDC was determined by comparing the representative peak intensity at different 2θ values in the diffraction pattern of BH with drug loaded formulation by using the following equation.

$$\mathrm{RDC} = \frac{I_F}{I_{\mathrm{Drug}}} \times 100 \quad (6)$$

where, I_F is the peak intensity of drug loaded formulations and I_{Drug} is the peak intensity of the pure drug (Vippagunta, Brittain, & Grant, 2001).

3. Results and discussion

3.1. Formation of microsphere

In the present study, we have prepared the interpenetrating polymer network hydrogel microsphere by emulsion crosslinking method using gluteraldehyde as a crosslinker. Being a natural polysaccharide, LBG has an inherent property to swell in presence of water. But this swelling behaviour is abrupt. To control this abrupt swelling property, we have crosslinked native gum and PVA by GA. The crosslinker, GA, is a bi-functional crosslinker and help to form the three dimensional polymer networks. This three dimensional network helped to entrap the drug properly and showed controlled drug release profile. During the formation of microsphere an acetal ring was formed between the hydroxyl groups of LBG–PVA polymer strands and aldehyde groups of GA to produce IPN. It made the matrix network rigid and insoluble.

3.2. Viscosity of internal phase

The viscosity of internal phase, as shown in Table 1, was found to decrease gradually with the reduction of LBG. This happened because of water uptake capacity of the polysaccharide units present in the polymer blend. LBG is composed of galactose and mannose units and the longer the galactose side chain greater will be the viscosity. In case of LBG, Mannose:Galactose content is about 4:1 (Englyst and Commings, 1988) and hence the mixture of LBG and PVA solution showed higher viscosity in presence of high LBG. Viscosity did not increase markedly when the amount of PVA was increased. This occurred owing to the fact that, PVA solubilised easily in water by forming hydrogen bonding and hence prevented increase in viscosity in significant manner like LBG.

3.3. Percentage yield

As the amount of LBG was gradually decreased and the amount of PVA was gradually increased, the percentage yields value of IPN microspheres increased from 62.27% to 85.62% (Table 2). This may be due to the higher availability of PVA chains, which was used as backbone to LBG chains for their attachment with the help of the crosslinker. In case of formulations F1–F6 (more LBG and less PVA), less amount of PVA backbone chain leaded to less production yield. A high impact of GA was also found in increasing the percent yield value. When the amount of GA was increased from 2.5 mL to 7.5 mL (Table 2), the yield value also increased significantly due to the frequent breaking of polymer chains and crosslinking. However, no significant effect of drug variation on the yield percentage was observed.

3.4. Drug entrapment efficiency (DEE)

Percentage entrapment efficiency of the formed microspheres was found in the range of 29.73% ± 2.14%–68.59% ± 1.21% (Table 2). The percentage DEE was highly dependent on gum polymer ratio and the amount of the crosslinker used. In case of formulations F1–F6 (more LBG and less PVA), less number of LBG chains were involved in the formation of the network structure leaving the remaining LBG chains to be crosslinked by GA. This lead to formation of a looser network of which most of the portion was made up of LBG only. Later, when PVA was increased from 1:1 to 1:2 (F7–F12), a well defined IPN network was formed due to the availability of PVA backbone and this rigid network structure prevented the leaching of drug in the external phase which was predominant in the previous case. Whereas more increment of PVA (1:3) (F13–F15) was not found beneficial as the attachment sites of LBG with PVA chains got saturated and the remaining PVA chains remained out of the network system. The amount of GA showed high impact on the DEE. As the amount of GA was increased, DEE (%) also increased for all the gum polymer ratios. This was because of the formation of highly crosslinked rigid network structure which in turn prevented the leaching of drug into the external phase. An increase in drug loading (F17) was not found beneficial due to saturation of polymer matrix or due to a high drug concentration gradient between the internal and external phase which indirectly helped to leach out higher amount of drug from IPN matrix. Drug entrapment efficiency was seen less in case of less drug loaded formulation (F16) with fixed amount of crosslinker. This may be due to less amount of drug in comparison to total amount of polymer. Though the viscosity of internal phase at 1:3 ratio of LBG and PVA was maximum, it was unable to entrap more amount of drug due to lack of formation of a well defined network structure.

3.5. Swelling study

3.5.1. Equilibrium water uptake study

The equilibrium water uptake study data (Table 2) showed that as the amount of LBG was increased, the swelling capacity of the hydrogel network was also increased. This phenomenon can be attributed due to the hydrophilic nature of native gum. The increment of PVA did not affect the water uptake because of its low water uptake capacity compared to LBG. The amount of crosslinker also had significant influence on the water uptake capacity of the microsphere. In higher amount of GA, water uptake capacity of the microsphere was decreased due to the rigid matrix structure which in turn reduced the surface pore volumes and made it difficult for the buffer to penetrate into the network. The viscosity of internal phase also played a vital role in water uptake. As the viscosity of the internal phase was increased, larger particles were formed (Table 2) which helped the microsphere to arrest a higher amount of water into its network. The relationship between the viscosity of the internal phase and equilibrium water uptake is shown in Fig. 2a and b.

3.5.2. Dynamic swelling

In case of dynamic swelling, change in microsphere diameter, as a function of time was measured with the help of an optical

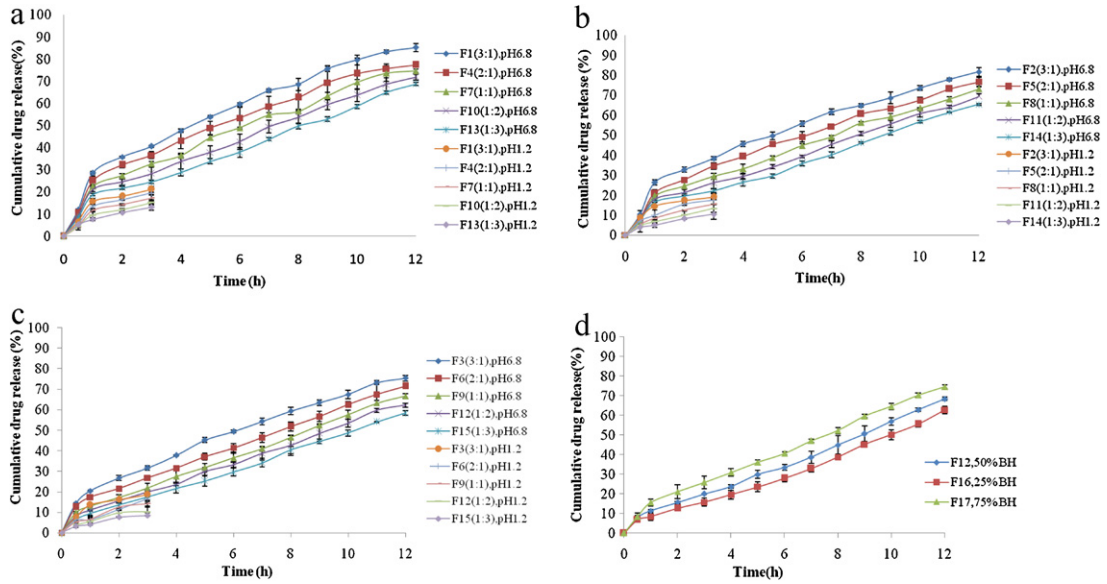

Fig. 3. Release profile of drug (a) in presence of 2.5 mL GA in microsphere, (b) in presence of 5.0 mL GA in microsphere, (c) in presence of 7.5 mL GA in microsphere, (d) effect of drug variation.

microscope. In Fig. 2c–f, normalised diameter, D_t/D_0 (D_0 is initial diameter, D_t is diameter at time t) of different formulations containing different amounts of GA are plotted as a function of time. It is evident that in pH 1.2 and 6.8, normalised diameter value decreased with the increase of GA concentration, which could be due to the rigid network structure formed at higher amounts of GA (Fig. 2c and e). Increased normalised diameter with the LBG concentration (Fig. 2d and f) may be due to the hydrophilic nature of LBG. As the microspheres formed a looser network in high LBG ratio (LBG:PVA, 3:1), the microspheres so formed had higher tendency to swell than the other formulations and hence significant increase in their diameter was observed.

3.6. *In vitro drug release*

In case of in vitro drug release study the cumulative percentage of drug release (CPR) from various formulations were correlated with the IPN blend composition, extent of crosslinking and drug loading.

3.6.1. *Effect of LBG and PVA blend ratio*

The CPR of drug from the IPN vs. time plot as shown in Fig. 3a–c, indicates that the amount released was dependent on the gum polymer ratio at a fixed crosslinker concentration. When the ratio of LBG and PVA was decreased from 3:1 (F1, F2, F3) to 1:3 (F13, F14, F15), the drug release became slower indicating the role of LBG amount in increasing the swelling of microspheres in dissolution medium.

3.6.2. *Effect of amount of glutaraldehyde*

The extent of crosslinking also showed high impact on the CPR of drug from IPN (Fig. 3a–c). As the amount of GA was increased the CPR was found to decrease. In formulations with fixed gum: polymer ratio (3:1; F1, F2 and F3), the amount of gluteraldehyde added (2.5 mL, 5 mL and 7.5 mL, respectively) resulted in reduction of CPR from 85.23% to 81.64% to 75.36%. This may be due to fact that higher crosslinker concentration increased the rigidity of the network, which in turn prevented the imbibition of buffer into the polymer matrix. So there was a reduction in network erosion and loosening leading to reduction in drug release.

3.6.3. *Effect of percentage drug loading*

The amount of drug loading also showed some impact on drug release as shown in Fig. 3d. As the amount of drug loading was increased from 50% (F12) to 75% (F17), drug release also increased from 65.24% to 74.66%, and when drug loading was decreased to 25% (F16), the release was also decreased to 63.42%. This happened due to the fact that, as the amount of drug was high in gum polymer blend with same crosslinker concentration, due to the concentration gradient, drug leached out from the matrix during the formation of microspheres. It formed a looser network having less entrapped drug molecules and helped the drug molecules to come out from the matrix more easily during dissolution. In case of F12, optimum drug entrapment and moderate cumulative release profile was seen. In F16, with less amount of drug, the high rigidity of matrix leaded to lowering of driving force for dissolution and hence reduction in percentage of drug release was observed. Moreover due to less amount of drug dispersed in the same volume of polymer matrix, higher obstacles was experienced (by drug particles) here as compared to the previous two formulations (F12, F17) in coming out of the matrix.

3.6.4. *Statistical analysis of the dependence of release rate on gum:polymer, amount of GA and pH of the dissolution media*

Mathematical relationship generated using MLRA for the studied response variables are expressed in Eq. (7)–(10).

$$y_1 = 69.27 - 3.65x_1 - 4.65x_2 - 0.59x_1x_2 + 0.042x_1^2 - 2.18x_2^2; \quad (R^2 = 0.990) \tag{7}$$

$$y_2 = 13.15 - 2.51x_1 - 2.12x_2; \quad (R^2 = 0.9789) \tag{8}$$

$$y_1 = 75.81 + 4.62x_3 - 3.93x_2; \quad (R^2 = 0.9394) \tag{9}$$

$$y_2 = 17.76 + 1.97x_3 - 1.43x_2; \quad (R^2 = 0.9751) \tag{10}$$

where, y_1 and y_2 are CPR at 12 h in pH 6.8 phosphate buffer and at 3 h in 0.1 N HCl, respectively. x_1, x_2 and x_3 are first set of gum:polymer ratio (1:1, 1:2 and 1:3), amount of GA and second set of gum:polymer ratio(1:1, 2:1 and 3:1). All the models and the interaction terms were significant as the probability values in all cases were less than 0.05.

Table 3
Kinetic modelling of cumulative drug release data in pH 6.8.

Formulation code	Zero order	First order	Higuchi kinetic	Hixson Crowell	Korsemeyer–Peppas	
					n	R^2
F1	0.958	0.775	0.99	0.963	0.558	0.963
F2	0.959	0.763	0.991	0.96	0.572	0.959
F3	0.989	0.902	0.99	0.997	0.529	0.991
F4	0.96	0.778	0.99	0.965	0.568	0.965
F5	0.97	0.774	0.993	0.969	0.629	0.967
F6	0.999	0.94	0.969	0.991	0.553	0.975
F7	0.975	0.804	0.985	0.971	0.602	0.966
F8	0.982	0.801	0.983	0.971	0.648	0.963
F9	0.998	0.943	0.961	0.989	0.623	0.973
F10	0.987	0.855	0.975	0.977	0.586	0.969
F11	0.991	0.867	0.971	0.981	0.621	0.972
F12	0.998	0.943	0.96	0.991	0.662	0.979
F13	0.989	0.876	0.96	0.974	0.603	0.963
F14	0.99	0.887	0.954	0.974	0.629	0.964
F15	0.998	0.932	0.955	0.991	0.698	0.982
F16	0.987	0.966	0.923	0.966	0.614	0.947
F17	0.996	0.897	0.969	0.992	0.66	0.988

3.7. Kinetic modelling of release data

From the kinetic modelling of release data it was observed that the formulations showed zero order release kinetic (Table 3), suggesting that the release rate was independent of the concentration of dissolved species. In case of Korsemeyer–Peppas equation it was observed that, '*n*' values were in the range of 0.529 (F3)–0.698 (F15) indicating that BH release from the microspheres followed non-Fickian release kinetic.

3.8. Particle size analysis

Results of particle size analysis of IPN microspheres presented in Table 2 showed that arithmetic mean diameter varied from 734.18 μm to 293.17 μm. It is clear that particle size was dependent on the amount crosslinker added, IPN blend ratio used and amount of drug loading. The result showed that, in a fixed IPN blend composition, when the amount of crosslinker was increased from 2.5 mL (F1) to 7.5 mL (F3) the diameter was reduced from 734.18 μm to 673.22 μm. This can be explained by the fact that as the amount of crosslinker was increased, it squizzed the polymer matrix to a higher extent as compared to less crosslinker containing formulations. It also helped in formation of smaller particles with higher rigidity of the matrix and less void spaces inside the matrix. It also helped to entrap higher amount of drug. As the amount of LBG was increased in the formulation (F7–F4 and F1) the diameter of the particle also increased. This can be explained on the basis of hydrodynamic viscosity concept, i.e., as the amount of LBG increased, interfacial viscosity of the polymer droplets in the emulsion also increased (Table 1) and the number of free sites available for crosslinking was less, so the size of the microspheres was also increased. An increase in drug loading (F16 and F17) also resulted in the formation of larger particles. This can be explained by the fact that, drug molecules might have occupied the free volume spaces within the matrix, thereby hindering the inward shrinkage of the polymer matrix. The polydispersity index of the particles was calculated and it showed a moderately narrow distribution range of IPN particles. The histogram plot of best optimised formulation (F12) is shown in Fig. 4.

3.9. Scanning electron microscopy

The scanning electron microscopy of group of particles (Fig. 5a), individual particle (Fig. 5b), and surface of the particle (Fig. 5c and d) were done. Fig. 5c shows that GA crosslinked particles have some pores on the surface. This happened in case of particles having less crosslinker. It may be due to less shrinkage of the polymer matrix. When the amount of crosslinker was increased the polymer matrix became highly dense leading to disappearance of pores as shown in Fig. 5d. This was due to the reduction of voids of polymer matrix in highly squeezed condition.

3.10. Fourier transform infrared spectroscopy

The FT-IR spectral analysis of PVA, buflomedil hydrochloride, placebo microsphere, LBG and drug loaded formulation (Fig. 6) was done to examine the stability of drug in the formaulation and to confirm the formation of IPN matrix structure. PVA (Fig. 6a) showed its characteristics peaks related to hydroxyl and acetate groups. The small peak observed near 3550 cm^{-1} were due to the stretching vibration of O—H from the intermolecular and intramolecular hydrogen bonds of PVA. The vibrational band observed between 2840 and 3000 cm^{-1} referred to the stretching vibration of C—H from alkyl groups. A sharp peak obtained near 1400 cm^{-1} indicated the bending vibration of CH_2 groups. Buflomedil hydrochloride (Fig. 6b) showed its characteristic peaks near abot 2800 cm^{-1} for methoxy C—H stretching (CH_3—O—). A small peak at about 1300 cm^{-1} was observed for aromatic C—N stretching. Absorption band at 1695 cm^{-1} was of ketonic carbonyl group. LBG showed characteristic peaks (Fig. 6d) at 3300 cm^{-1} for O—H stretching of hydroxyl group and near about 2900 cm^{-1} for —CH_2 bending/wagging. A broad peak at 1050 cm^{-1} represented C—O—H stretching. In case of placebo formulation (Fig. 6c) all the peaks of PVA and LBG were present. In drug loded microsphere (Fig. 6e), peaks of LBG, PVA and BH were present which proved the physical stability of drug in fromulation. During the formation of

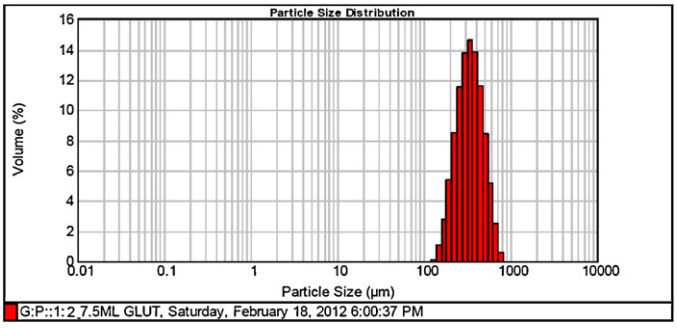

Fig. 4. Histogram of best optimised formulation (F12).

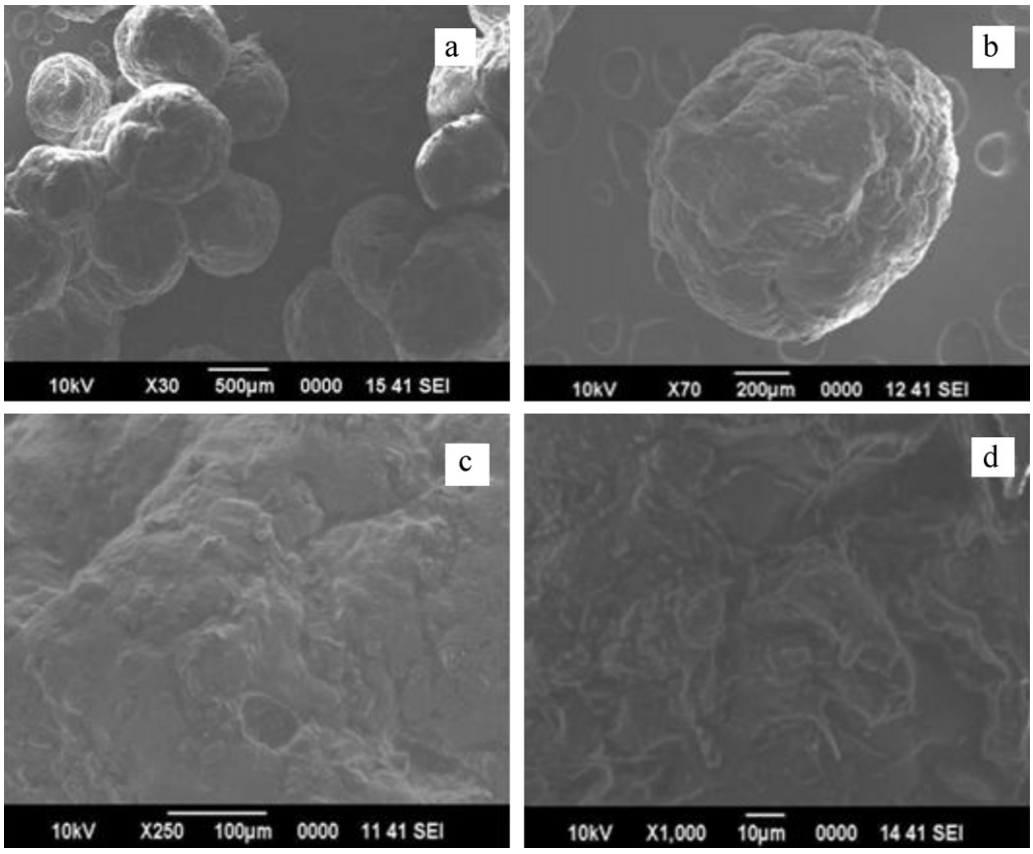

Fig. 5. Scanning electron microscopy of (a) Group of microsphere, (b) individual microsphere, (c) surface of microsphere with pores, (d) highly crosslinked polymer matrix.

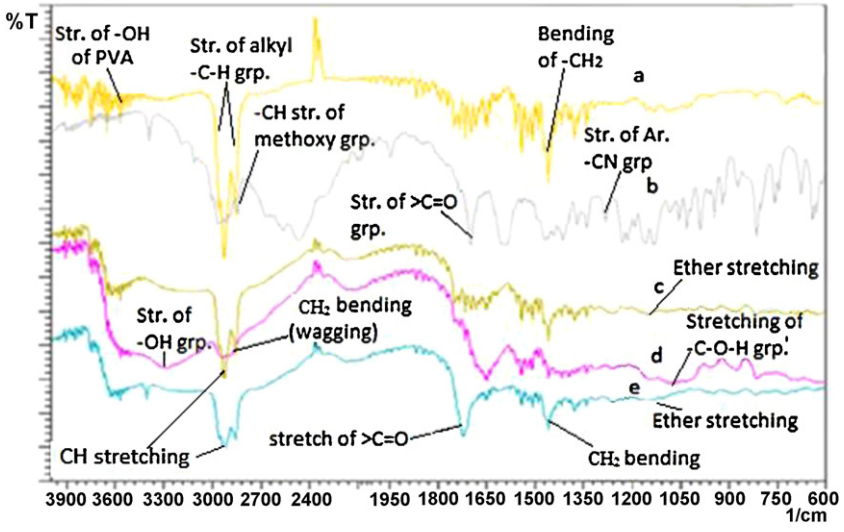

Fig. 6. FT-IR spectra of (a) PVA, (b) pure drug, (c) Placebo formulation, (d) LBG, (e) drug loaded formulation.

IPN crosslinked by GA, acetal linkage was formed with the hydroxyl groups of gum. FTIR spectrum in Fig. 6c and 6e are associated with LBG and PVA crosslinked by glutaraldehyde. It can be observed that two important peaks at $n = 2860$ and $2940\ cm^{-1}$ of C—H stretching are related to aldehydes, a duplet absorption with peaks attributed to the alkyl chain (Meyers, 2000; Mansur, Oréfice, & Mansur, 2004). By crosslinking PVA and LBG with GA, the intensity of O-H stretching vibration peak ($n = 3300\ cm^{-1}$) was decreased as compared to pure LBG. This result suggests that the hydrogen bonding became weaker in crosslinked LBG than in pure LBG because of the diminution in the number of OH groups and acetal formation (Fig. 6c and e). In addition, the C—O stretching at approximately $1100\ cm^{-1}$ in pure PVA is replaced by a broader absorption band (from $n = 1050$ to $1140\ cm^{-1}$), which can be attributed to the ether (C—O) and the acetal ring (C—O—C) bands formed by the crosslinking reaction of PVA and LBG with GA. Therefore, it can be assumed that GA has acted as chemical crosslinker among PVA and LBG polymer chains.

3.11. Solid state C^{13} NMR

The 13C nuclear magnetic resonance spectrum of the LBG is shown in Fig. 7(a). Three distinct peaks were observed from the δ

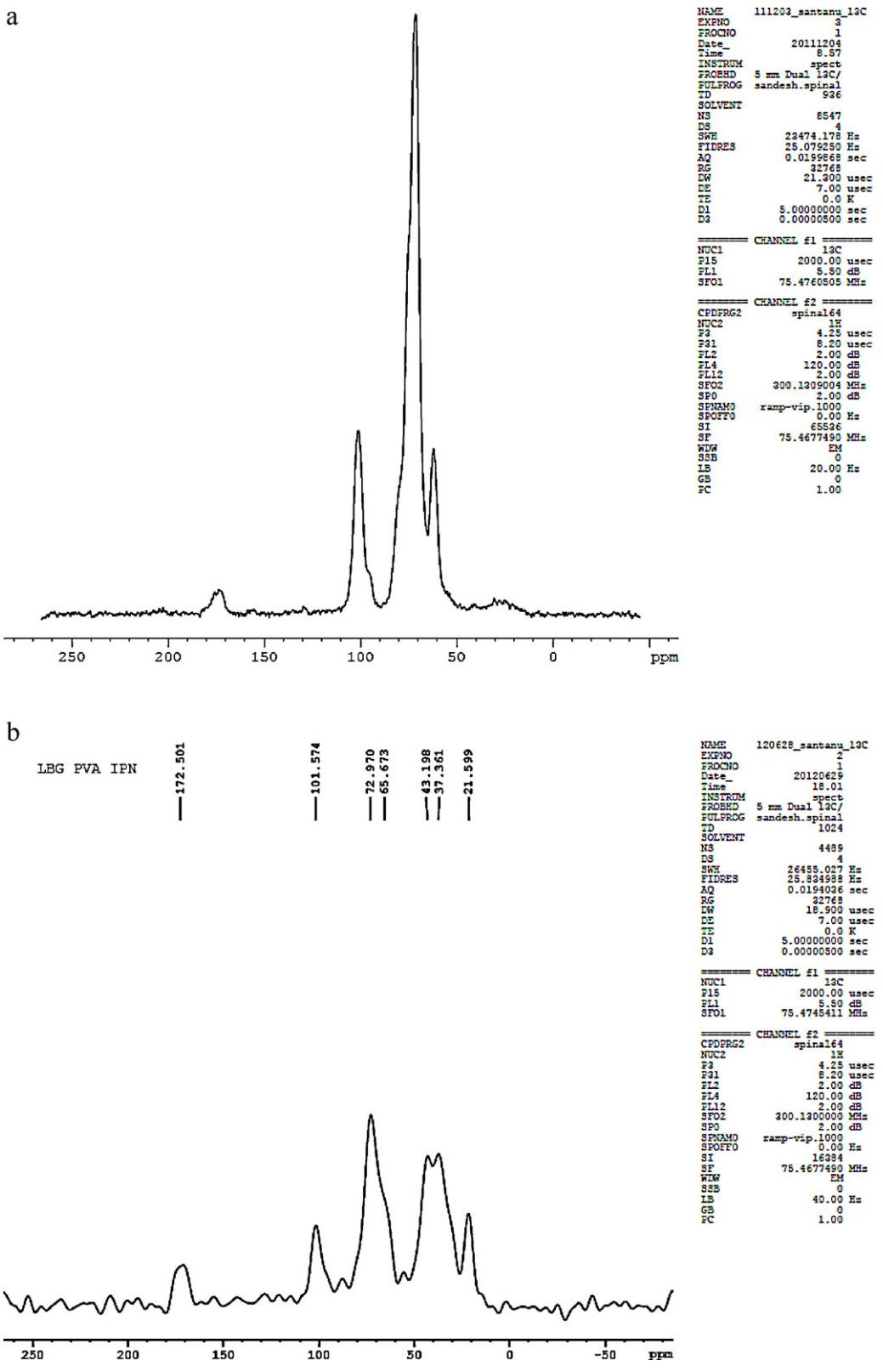

Fig. 7. NMR spectra of (a) native LBG (b) LBG–PVA IPN.

values of LBG 13C NMR spectra. The absorption peak at $\delta = 62$ ppm is for the C-6 carbon of mannan unit. The absorption peak at $\delta = 70$ ppm broadened upto 80 ppm because the signals of C-2, C-3 and C-5 have merged into it. Some notches on that peak give strong evidence of this fact. The small hump at $\delta = 81$ ppm is for C-4 mannan carbon atom. The sharp absorption peak at $\delta = 102.7$ ppm is due to C-1 mannan carbon (Newman & Hemmingson, 1998).

Yang, Hu, Horii, Endo and Hayashi (2006) reported the NMR spectra of PVA, where a sharp peak at $\delta = 43$ was for CH_2 carbon

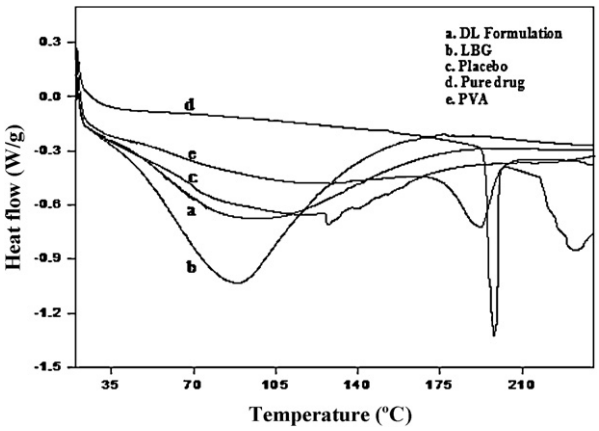

Fig. 8. DSC thermogram of (a) LBG, (b) PVA, (c) pure drug (BH), (d) drug loaded formulation, (e) placebo formulation.

of PVA and three splited peaks at about $\delta = 60–80$ ppm were for resonance of CH carbons of PVA.

^{13}C NMR spectrum of placebo IPN microspheres is shown in Fig. 7(b). Absorption peak at $\delta = 21.60$ ppm may be due to aliphatic carbon of $-CH_2$ present in PVA. Absorption peaks in the range of 37–43 ppm corresponds to aliphatic carbons of glutaraldehyde. The absorption peak at $\delta = 65.67$ ppm is for C-6 carbon atom of mannan residue of LBG. The broad absorption peak at $\delta = 72.97$ is for C-2, C-3 and C-5 carbon of LBG mannan unit. The CH resonance peaks of PVA may have merged into this broad peak and hence is not visible distinctly. Peak at $\delta = 101.57$ ppm is due to the actal carbon atom (Webster, Chudek, & Hopkins, 2000) which formed between the LBG and PVA hydroxyl group due to cross linking by glutaraldehyde proves the formation of IPN. The C-1 mannan carbon of LBG may be overlapped with the peak of acetal carbon atom. Hence the solid state NMR spectra of the IPN particles showed a strong evidence of formation of glutaraldehyde crosslinked IPN matrix.

3.12. Differential scanning calorimetry

DSC thermogram of LBG, PVA, pure drug, drug loaded formulation and placebo formulation are given in Fig. 8. In case of pure drug a sharp endothermic peak at 197.98 °C represented the melting point of the drug. LBG showed an endothermic peak at 88.63 °C which may be due to loss of moisture. PVA showed its characteristic melting at 192.19 °C. The placebo formulation showed a sharp peak at 127.03 °C which may be due to polymer-inducing amorphous phase. However, the drug loaded formulation showed a broad peak at 93.11 °C. This can be explained by the fact that the drug might have remained in the polymer matrx as a molecular dispersion. Due to that, the amorphus nature of the drug increased in the encapsulated form which in turn reduced the degradation temparature of the drug loaded formulation than the placebo formulation. No peak was observed at 197.98 °C in drug loaded formulation, indicating molecular dispersion of drug in polymer matrix.

3.13. Qualitative X-ray diffractometry

X-ray difractrogram of pure buflomedil hydrochloride, placebo formulation and drug loaded formulation are given in Fig. 9. From the difractogram it is possible to understand whether the drug is in crystaline or in amorphus state in its native form and also in encapsulated form. The XRD of pure drug showed sharp peaks at $2\theta = 10.5°$, 12°, 14°, 15.5°, 20°, and 24° which proved its crystalline nature in pure form. However, these sharp peaks broadened in case of drug loaed IPN microspheres. This can be attributed to the reduced crystal size of drug leading to molecular dispersion

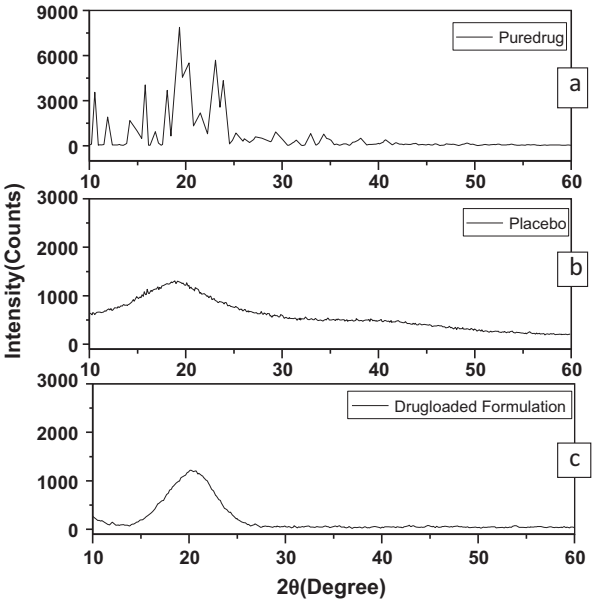

Fig. 9. X-RD spectra of (a) pure drug, (b) placebo formulation and (c) drug loaded formulation.

formation in the polymer induced amorphus phase. The peak intensity of the drug loaded formulations was found more than that of the placebo formulation due to presence of drug in polymer matrix. The RDC value of BH in drug loaded formulation at 2θ value 10.577° is 5.04 and at 2θ value 11.904° it was 6.58, thus indicating the change of drug from its crystalline to amorphous state.

4. Conclusion

The IPN microspheres of PVA and LBG was successfully prepared using GA as a crosslinker for encapsulating buflomedil hydrochloride having short elimination half life (2–3 h). The IPN microsphere showed extended release profile following zero order release kinetic. The release of drug was highly depeendent on the IPN blend ratio, crosslinker amount and percentage drug loading. The FT-IR study confirmed the stability of drug in formulation and also confirmed the formation of IPN matrix. The NMR study confirmed gluteraldehyde crosslinked LBG–PVA IPN matrix formation. The DSC and XRD study suggested the amourphus nature of drug in its molecular dispersion form in the polymer matrix. Thus, this type of formulation can be a potential candidate for controlling the release rate of drugs having short half life and high water solubility.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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