Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **28** (5): 747-55 (2009)

Original Article

Received: May 1, 2009
Accepted: June 14, 2009

Delivery of Antihypertensive Drug Through Synthesized Hydrogel Network, a Comparative Study

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***SUMMARY*. The present study aims at developing synthesized (PVA-co-PAA)/NaCl normal hydrogel(H), hydrogel microspheres(HM) and comparing the antihypertensive activity of Diltiazem hydrochloride(DL) from two formulations. The hydrogel microspheres were crosslinked by using glutaraldehyde-saturated toluene. The hydrogel showed more swelling in simulated intestinal fluid (SIF). (PVA-co-PAA)/NaCl HM formulation A1 showed comparatively higher entrapment (79%) and better control over DL release. In normotensive rats, HM formulation A1 found more effectively in reducing blood pressure to 40.1%. The experimental results demonstrated that (PVA-co-PAA)/NaCl HM had the greater potential than normal hydrogel to be used as a drug carrier.**

INTRODUCTION

Polymeric drug carriers of various size have attracted increasing attention in recent years. Now-a-days, it would be most desirable for drug release to match a patient's physiological needs at the proper time and/or the proper site. This is why there is a great interest in the development of controlled delivery systems [1]. Polymeric hydrogels are of considerable interest as biomaterials in drug delivery research [2-9]. The crosslinked hydrogels are gaining importance in a wide variety of applications as superabsorbents in wound dressings, as drug carriers, as artificial organs, etc. Such systems are highly advantageous over conventional materials because their water uptake properties can be altered by chemically modifying their structures. Hydrogels can respond to external stimuli such as pH, ionic strength, temperature, and electric current. Such polymeric systems are useful as stimulus responsive drug carriers and are often called 'intelligent' or 'smart' materials because of their quick response to the external stimuli. The pH-sensitivity of the hydrogels is due to the presence of weakly acidic and/or basic functional groups on the backbone. Their water uptake properties are attributed to the ionization of functional groups, which depend upon the pH and ionic strength of the external medium thereby making them useful as pH-sensitive drug delivery systems. Such systems are also called pH-dependent "switch-on and -off systems" [10] .From last few years, hydrogel microspheres have shown their efficacy in delivering various drugs to different areas of body [11-14]. By controlling size of the microspheres, the targeted delivery of the drugs to the required area of various organs becomes realizable. Poly vinyl alcohol (PVA) has been used in a wide variety of fields since its discovery in 1924, because of its desirable properties such as non toxicity and non carcinogenicity [15]. PVA [16-19] and Poly acrylic acid (PAA) [20-23] finds extensive biomedical applications including drug delivery to specific areas of

KEY WORDS: Drug delivery, Hydrogel microspheres, Normal hydrogel, Stability, Swelling.

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body. In our previous work [23], we have synthesized copolymeric hydrogel of polyacrylamide, PAA and studied the size dependent delivery of 5-Florouracil to the colon.

Diltiazem hydrochloride(DL) is a benzothiazepine calcium–channel blocker with peripheral and coronary vasodilating effect with limited negative inotropic activity. Its short biological half-life and thus frequent administration (usually three to four times a day) makes it a potential candidate for sustained or controlled release dosage forms [24].The present study aims at developing newly synthesized (PVA-co-PAA)/NaCl normal hydrogel (H), hydrogel microspheres (HM) and comparing the antihypertensive activity of DL from two formulations.

MATERIALS AND METHODS

Materials Used

Poly vinyl alcohol (PVA) with an average molecular weight of 65,000-86,000 and NaCl were purchased from CHD India Ltd., Acrylic acid (AA) and benzoyl peroxide (BPO) were purchased from E.Merck, India. Diltiazem hydrochloride (DL) and Methyl Prednisolone Acetate (MPA) were gift samples from Dr. Reddy's laboratories, India. Glutaraldehyde (GA) was purchased from S.D. Fine Chemicals, Mumbai. All other chemicals were of reagent grade and used without further purification.

Synthesis of (PVA-co-PAA)/NaCl Interpenetrating network hydrogel

The polymerization was carried out in a specially designed jacketed reaction vessel having an inlet and an outlet port. The inlet port was connected with the nitrogen line. (PVA-co-PAA)/NaCl interpenetrating network (IPN) hydrogel were synthesized as per our previous work [9]. Briefly, PVA and AA solutions in deionized water were charged into the reaction vessel at different (wt %) ratio as per Table 1. The solution was stirred at 400-500 rpm for 15 min. Then the initiator, BPO [2.5%(w/v)] in acetone and NaCl [1.0%(w/v)] in water were added without cross-linking agent and the temperature was maintained at 80 °C with stirring. After 3 h of reaction, a gel was formed, washed repeatedly with distilled water, and then dried in a vacuum oven at 60 °C to get a constant weight of the hydrogel (PVA-co-PAA)/NaCl.

Fabrication of (PVA-co-PAA)/NaCl hydrogel microspheres

(PVA-co-PAA)/NaCl HM were prepared by slight modification of emulsion crosslinking method [25]. 500 mg of the prepared (PVA-co-PAA)/NaCl IPN hydrogel was dispersed in 10 ml phosphate buffer (20 mM, pH 7.4) and mixed using a cyclo-mixer. This mixture was added dropwise to liquid paraffin (100 ml), while stirring the whole system at 1500 rpm.1% w/v Span 80 was added as surfactant to the oil phase. After 15 min of stirring, varying amount (2.5 ml, 5.0 ml, 7.5 ml) of glutaraldehyde-saturated toluene was added and stirring was continued for 8 h. The cross-linked microspheres were separated from the oil phase by filtration and were washed repeatedly with n-hexane (100 ml) to remove the excess oil. The HM obtained were then dried at room temperature.

Fourier transformed infrared (FTIR) spectrophotometry

FT-IR studies of PVA and (PVA-co-PAA)/NaCl hydrogel were carried out at room temperature by powder diffuse reflectance in a FT-IR Spectrophotometer (FT-IR , Shimadzu 8400S) using KBr .All the spectra were recorded in the range of 500-4000cm^{-1}.

Thermo gravimetric analysis (TGA) studies

Thermogravimetry is the study of the relationship between samples mass and its temperature to examine the thermal stability of samples. TGA of the samples were carried out using a

DL loaded (PVA-co-PAA)/NaCl H				DL loaded (PVA-co-PAA)/NaCl HM			
Batch	**Monomer feed (wt.%) PVA :AA**	**% Yield Mean±S.D.**	**% Drug Entrapped**	**Batch**	**Monomer feed (wt.%) PVA :AA**	**% Yield Mean±S.D**	**% Drug entrapped**
A	25:75	81 ± 1.47	69 (2.1*)	A1	25:75	83 ± 2.7	79 (1.8*)
B	50:50	73 ± 2.61	58 (1.8*)	B1	50:50	87 ± 2.3	72 (2.0*)
C	75:25	80 ± 1.80	48 (1.9*)	C1	75:25	78 ± 3.6	69 (1.2*)
D	90:10	80 ± 1.80	47 (1.4*)	D1	90:10	81 ± 2.9	66 (1.6*)

Table 1. Formulations of DL loaded (PVA-co-PAA)/NaCl hydrogel (H) and hydrogel microspheres (HM); NaCl 1%(w/v) in all the formulations. * is the co-efficient of variance value.

Shimadzu DTG-50 Thermal Analyzer. The samples were heated in air to a temperature of 500 °C at the rate of 10 °C/min starting from room temperature, *i.e.* 28 ± 2 °C.

Scanning electron microscopy (S.E.M.) studies

The external morphology and size of the (PVA-co-PAA)/NaCl H and (PVA-co-PAA)/NaCl HM were analyzed using Scanning Electron Microscopy (SEM). The dried formulations were fixed on support with carbon-glue, and coated with gold using a gold sputter module in a high vacuum evaporator. Sample was then observed with the Scanning electron microscope– JEOL JSM-5000, Japan at 15 kV.

Drug loading

(PVA-co-PAA)/NaCl H and (PVA-co-PAA)/NaCl HM were loaded with Diltiazem hydrochloride (as model drug) by soaking in an aqueous solution containing 10% (w/v) of the drug. Soaking was done for nearly 2 days in order to achieve complete equilibrium. The formulations were filtered and the surface-adhered drug solution was removed by washing and blotting with soft filter paper and dried in air before storing in a desiccator.

Drug entrapment efficiency (%)

DL loaded (PVA-co-PAA)/NaCl H and (PVA-co-PAA)/NaCl HM (50 mg) from each batch were dispersed separately in methanol & kept for 24 h, filtered through 0.22 µm microfilter and absorbance was measured using UV/VIS spectrophotmeter (Genesis-2,USA) at 237 nm. DL content were determined and expressed in terms of weight of DL per weight of HM, thus determining the actual entrapment ratio (AER) defined by the equation [**1**]:

$$\% \text{ of Entrapment efficiency} = AER / TER \times 100 \quad [\mathbf{1}]$$

where AER = Measured drug wt / HM wt and TER = Drug wt. /drug wt. & polymer wt.

Swelling behavior of (PVA-co-PAA)/NaCl hydrogel microspheres

The pH-dependent equilibrium swelling of the HM were studied both in the simulated gastric fluid (SGF, 3.2 mg/ml pepsin in 0.05 M hydrochloric acid, pH 1.2) and simulated intestinal fluid (SIF, 10 mg/ml pancreatin in Sorensen's phosphate buffer, pH 7.4).HM were allowed to swell completely for about 24 h to attain equilibrium at 37 °C. Adhered liquid droplets on the surface of the particles were removed by blotting with tissue papers. The swollen HM were weighed and dried in an oven at 60 °C for 6 h until there was no change in the dry mass of the samples. The swelling behavior of the HM were computed by calculating the percentage swelling (% S) as per equation [**2**]:

$$\%S = [(Mt - Mo) / Mo] \times 100 \quad [\mathbf{2}]$$

where Mt is the mass of the swollen sample at time "t" and Mo is the mass of the dry sample.

In vitro drug release study in pH progressive media

The *in vitro* drug release was carried out by filling the calculated amount of DL loaded (PVA-co-PAA)/NaCl H and (PVA-co-PAA)/NaCl HM in capsule (size 2), analyzed using USP-I basket dissolution apparatus and proper simulation of gastro intestinal (GIT) condition was maintained by altering the pH of dissolution medium at different time intervals following two step-dissolution conditions. To simulate the physiological conditions of GIT, first 2 h of dissolution were carried out in 250 ml of simulated gastric fluid (SGF, 3.2 mg/ml pepsin in 0.05M hydrochloric acid, pH 1.2) and the rest of the time in 250 ml of simulated intestinal fluid (SIF, 10 mg/ml pancreatin in Sorensen's phosphate buffer, pH 7.4). The media was stirred at 100 rpm at 37 ± 0.5 °C. At predetermined time intervals, specified amount of dissolution medium was removed; filtered through 0.22 µm microfilter and analyzed in UV spectrophotometer at 237 nm. After each sampling an equal volume of fresh dissolution media was added to the dissolution medium. All the dissolution studies were repeated six times.

In vitro drug release kinetic mechanism

Different mathematical models may be applied for describing the kinetics of the drug release process from the hydrogel matrix; the most suited being the one which best fits the experimental results. The kinetics of DL release from HM formulations was determined by finding the best fit of the dissolution data (drug release *vs.* time) to distinct models: first-order [eq. **3**] and Higuchi [eq. **4**] [26,27].

$$Q_t = Q_\infty (1-e^{-k1t}) \quad [\mathbf{3}]$$

$$Q_t = k_H t^{1/2} \quad [\mathbf{4}]$$

where Q_∞ being the total amount of drug in the matrix, k_1 the first order kinetic constant and k_H representing the Higuchi rate constant.

Furthermore, in order to better characterize the drug release behavior for the polymeric systems studied, namely to understand the corresponding mechanism, the Korsmeyer–Peppas [eq. **5**] semi-empirical model was applied [28].

$$Q_t / Q_\infty = k\ t^n \qquad [5]$$

where Q_t / Q_∞ is the fraction of drug released at time t; k a constant comprising the structural and geometric characteristics of the tablet, and n; the release exponent, is a parameter which depends on the release mechanism and is thus used to characterize it [29].

Stability studies

The physical stability of the prepared HM of different batches was evaluated after storing for two months under different temperature and humidity conditions. Particular amount of dried HM from each batch were packed in amber glass vials and stored in the stability chamber at 40 °C /75% relative humidity condition as per I.C.H. guidelines [30]. Samples of definite amount from each batch were withdrawn after 2 months to see the effect of DL release from the polymers on storage.

In vivo activity studies

For *in vivo* studies of DL loaded (PVA-co-PAA)/NaCl HM system, male albino rats (8 weeks old; 220-260 g) were supplied by Institutional central animal house facility and kept under standard laboratory conditions in 12 h light/dark cycle at 25 °C.The central animal house facility of Sri Jayadev college of Pharmaceutical Sciences, India (Regd. No. 678/02/Q/CPCSEA) approved this study. Animals were provided with standard diet and water. They were marked with picric acid solution for easy identification. For conducting the blood pressure (BP) measurement studies, the animals were kept in a restrainer (rat holder) which had only one side open with proper ventilation at all other sides. The rats were trained for their stay in the restrainer as slight movement would have led to variation in BP reading. The initial BP of all the rats was recorded using blood pressure measuring instrument (Digtal 151controller, Stoctling).The restrainer carrying the rat was placed in the BP instrument with tail protruding out. The tail of rat was connected to the transducer membrane of the instrument. The instrument was then turned on and allowed to stabilize until steady pulse rate was observed. After stabilizing the steady pulse rate, the mean arterial BP was recorded. For induction of hypertension, the rats were divided into 11 groups of 6 animals each. Group 1 was taken as control. Hypertension was induced in the remaining 9 groups by subcutaneous injection of Methyl prednisolone acetate (MPA) (20 mg/kg/wk.) for 2 weeks as per method [31]. After MPA treatment, groups 4,5,6,7 were subjected to (PVA-co-PAA)/NaCl HM formulations (A1,B1,C1,D1 respectively) and groups 8,9,10,11 were subjected to (PVA-co-PAA)/NaCl H (A,B,C,D respectively) orally containing identical dose of DL. Group 2 was treated with pure DL drug orally, Group 3 served as toxic control and received no further treatment. The rats were placed in the restrainer and BP was recorded at regular time interval up to 26 h.

Statistical analysis

The statistcal analysis was performed in INSTAT software and results were expressed as arithmetic mean±SEM. The pre and post treatment values within a group were compared using paired *t* –test. The comparison between various groups was performed by one-way analysis of variance (ANOVA).The percentage reduction in BP for all the treatment groups were also compared.

RESULTS AND DISCUSSION

In the present study, IPN hydrogel of (PVA-co-PAA)/NaCl has been synthesized and the reaction mechanism of IPN formation between PVA and AA is proposed to occur in two ways: at first the AA is grafted onto the PVA backbone by radical copolymerization. Secondly, the reaction between some of the hydroxyl groups of PVA and some of the carboxylic groups of PAA formed by homopolymerization of AA occurs to form an ester linkage resulting in the formation of a crosslinked IPN hydrogel as shown in Figure 1.

Fourier transformed infrared (FTIR) spectrophotometry

Comparative FTIR spectra of PVA, PAA, and (PVA-co-PAA)/NaCl are shown in Figure 2. The characterstics peak of PVA is located at 3340 cm^{-1} for hydroxyl group and others are due to C-H stretching vibration. In PAA, peak at 1715 cm^{-1} is due to –COOH acid group. In (PVA-co-

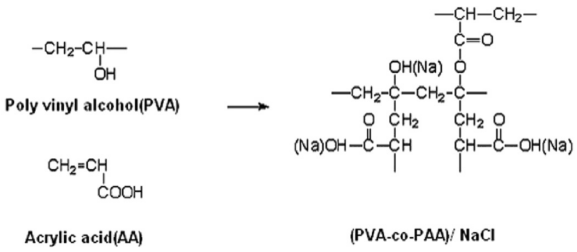

Figure 1. Formation of (PVA-co-PAA)/NaCl IPN hydrogel.

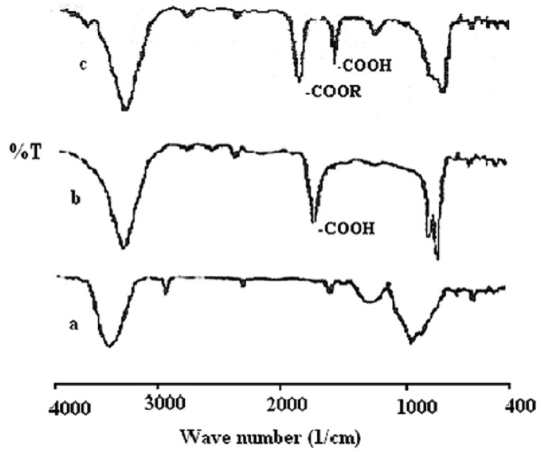

Figure 2. FT-IR spectra of **a)** PVA. **b)** PAA. **c)** (PVA-co-PAA)/NaCl IPN hydrogel.

PAA)/NaCl hydrogel, in addition to the –COOH group peak at 1715 cm^{-1}, another peak at 1780 cm^{-1} appeared for ester group indicating reaction between the –OH group of PVA with the –COOH group of PAA resulting in network formation.

Thermogravimetric analysis (T.G.A) studies

The comparative thermal behavior of PVA, PAA, and (PVA-co-PAA)/NaCl are shown in Figure 3. From the curves, the temperature of decomposition were found to be 230 °C for PVA,

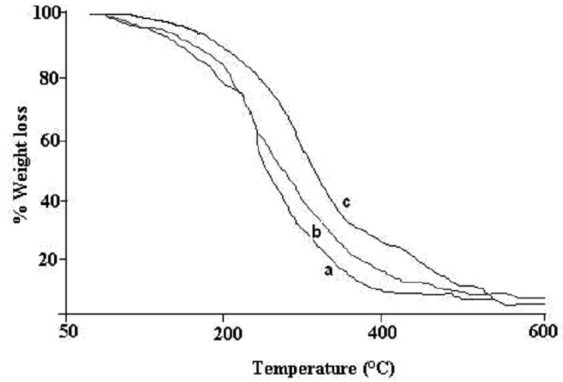

Figure 3. TGA thermogram of **a)** PVA. **b)** PAA. **c)** (PVA-co-PAA)/NaCl IPN hydrogel.

170 °C for PAA and 190 °C for (PVA-co-PAA)/NaCl. The results indicate a decrease in thermal behavior by network formation. This might be due to the increasing porosity of the hydrogel network.

Scanning electron microscopy (S.E.M.) studies

The morphology of the (PVA-co-PAA)/NaCl HM and (PVA-co-PAA)/NaCl H were analyzed using Scanning Electron Microscopy (SEM). (PVA-co-PAA)/NaCl H indicated the presence of many fragments of the residues on the pore walls (Fig. 4a) with size 500 µm. From SEM studies it was found that the prepared HM were spherical (Fig. 4b) with average particle size 80 µm.

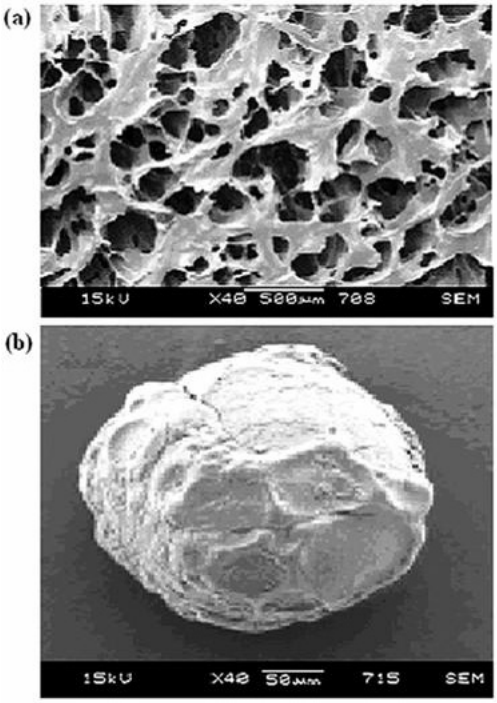

Figure 4. (**a**)Scanning electron microscopy of (PVA-co-PAA)/NaCl IPN hydrogel with 25:75 (PVA: AA) monomer feed (wt %) ratio. (**b**) Scanning electron microscopy of (PVA-co-PAA)/NaCl IPN hydrogel microspheres with 25:75 (PVA : AA) monomer feed (wt %) ratio; GA, 2.5ml.

Swelling behavior of (PVA-co-PAA)/NaCl hydrogel microspheres

Swelling studies of the prepared IPN HM performed in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) are shown in Figure 5 (a) and (b). Different amounts of GA as crosslinking agent were added to prepare HM.

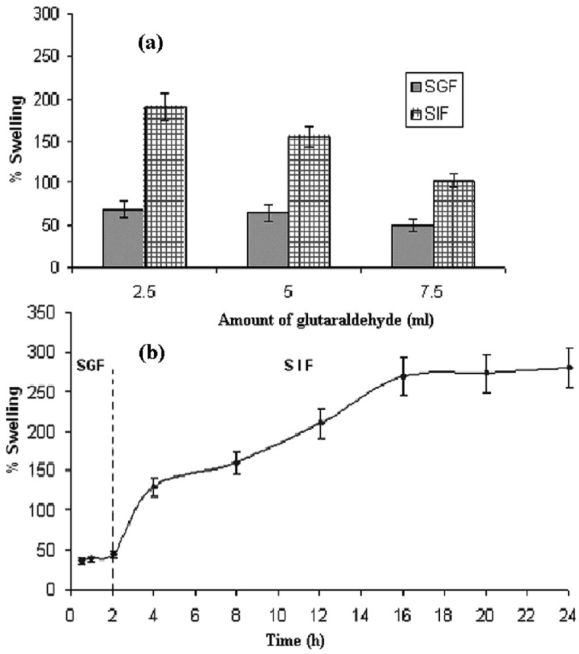

Figure 5. (**a**) Influence of amount of glutaraldehyde (GA) on the swelling behavior of (PVA-co-PAA)/NaCl IPN hydrogel microspheres in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF); (**b**) Time and pH dependent swelling studies of (PVA-co-PAA)/NaCl IPN hydrogel microspheres with 2.5 ml GA in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).

The extent of crosslinking had shown much difference in equilibrium swelling, only in SIF, whereas for SGF, the difference was not considerable. A swelling of 68% was observed for HM containing 2.5 ml of GA in SGF, which decreased as the extent of crosslinking increased, *i.e.,* for 5 and 7.5 ml of GA containing HM, swelling was found to be 64% and 50% respectively. In SIF, swelling decreased abruptly with an increase in crosslinking, *i.e.,* for 2.5, 5 and 7.5 ml GA containing HM, observed swelling was 191%, 156% and 102%, respectively (Fig. 4a). pH dependent swelling studies of prepared HM containing 2.5 ml of GA were carried out in SGF for 2 h and then with SIF up to 24 h. Swelling of HM in SIF media was found to be much higher than that of SGF (Figure 4b).

Drug entrapment efficiency (%)

Entrapment efficiency is the amount of added drug (in percent) that is encapsulated in the microsphere formulation. Entrapment efficiency was calculated as the ratio of the weight of DL content in the final microspheres and the DL introduced in the process. DL entrapment in the (PVA-co-PAA)/NaCl H and (PVA-co-PAA)/NaCl HM at different monomer ratio was compared. Entrapment of DL was found to be higher in (PVA-co-PAA)/NaCl HM than (PVA-co-PAA)/NaCl H. In both cases, (PVA:AA) monomer ratio 25:75 showed comparatively higher DL entrapment. Low coefficient of variance (<2.2 %) in % DL entrapment indicates uniformity of drug entrapment in different batches. % Yield from all the batches of HM was found to be in the range of 70-88 %.

In vitro drug release study

In vitro DL release from (PVA-co-PAA)/NaCl HM and normal hydrogel were evaluated in pH progressive media *i.e.* SGF as well as in SGF as the dissolution medium to see the release behavior in different pH conditions. The applied formulative variable *i.e.* change in monomer ratio in the crosslinked polymer were compared for their influence on drug release rate. By increasing the PVA: AA monomer ratio to 90:10 in hydrogel, reducing the release rate of DL in both the media. Formulation D1 with PVA: AA monomer ratio 90:10 showed slowest drug release profile in both the medium than other formulations and formulation A1 with PVA:AA monomer ratio 25:75 showed relatively more release in both the media (Fig. 6a and 6b) .It indicated that PVA was found to be playing the major role in controlling the DL release. Comparatively much higher DL release was observed in SIF than that of SGF. This may be due to the higher swelling capacity of the system in SIF (Fig. 5b).DL release from HM was found to be controlled up to 24 h, where as normal hydrogel (Fig. 6a) could control the release upto 20 h.

In vitro drug release kinetic mechanism

The drug release mechanism from swellable hydrogel matrices is complex. Although some processes may be classified as either purely diffusional or purely erosion controlled, many others can only be interpreted as being governed by both. The analysis of experimental data in the light of the Korsmeyer–Peppas equation [**5**], as well as the interpretation of the corresponding release exponent values (n); leads to a better understanding of the balance between these mechanisms. For A1 formulation, (n) was determined to be equal to 0.811. Notwithstanding this value is pointing to an anomalous (non-Fickian) diffusional mechanism; both Higuchi's model (Fickian) and first-order kinetics yielded similarly good quality adjustments. For formula-

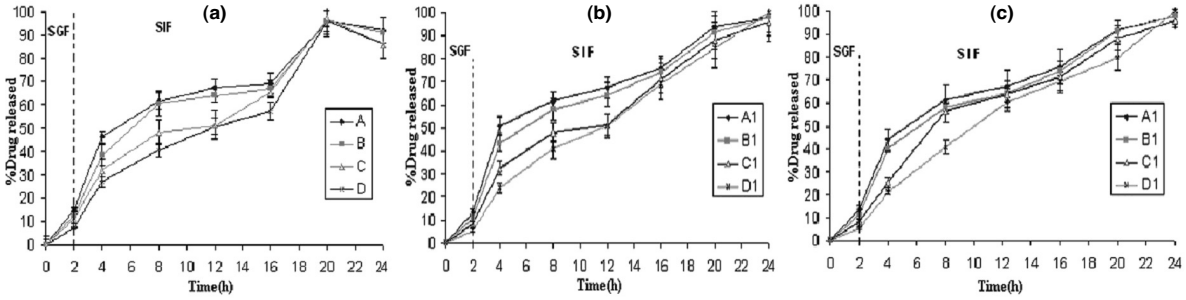

Figure 6. (**a**) Comparison of *in vitro* release study of DL from (PVA-co-PAA)/NaCl IPN hydrogel in pH progressive media at different (PVA:AA) monomer feed (wt %) ratio; GA, 2.5 ml. (**b**) Comparison of *in vitro* release study of DL from (PVA-co-PAA)/NaCl IPN hydrogel microspheres in pH progressive media at different (PVA:AA) monomer feed (wt%) ratio; GA, 2.5 ml. (**c**) Comparison of *in vitro* release study of DL loaded (PVA-co-PAA)/NaCl IPN hydrogel microspheres in at different (PVA:AA) monomer feed (wt%) ratio after storing for two months at accelerated stability condition.

tions, B1-D1, the diffusional exponent value (n) ranged from 0.674 to 0.617 (Table 2), indicating that the release mechanism of DL from these hydrogel matrices is an anomalous (non- Fickian) transport, which suggests that both diffusion of the drug in the hydrated matrix and its own erosion modulate drug release. For these systems, the Higuchi- kinetic model yielded remarkably good adjustment $R^2 > 0.999$. After storing the formulations for two months at accelerated stability condition *i.e* 40 °C/75%RH as per I.C.H. guidelines, the DL loaded HM showed similar type of DL release profile in both the medium (Fig. 6c) and retained the % DL entrapment with minor deviations.

In vivo activity studies

Hypertension was successfully induced in the normotensive rats by MPA administration as highly significant difference (paired t test, *P< 0.005)* was found in the pre and post treatment values (Group 3, Table 3). The rats remained hypertensive (with a minimum mean BP of 150 mm Hg) for 3 days. On treating DL loaded (PVA-co-PAA)/NaCl HM system and normal hydrogel, a significant fall in BP *(P < 0.01)* was observed in the treatment groups (Table 3).The effect in group 4 was even more pronounced (P < 0.005). However post treatment BP values in control and treatment group 3 were comparable and not significant *(P > 0.01)*. Group 2 containing pure DL showed 38.6 % reduction in BP after 8 h. On comparing the effects of all the systems, the percentage reduction in mean BP in rat by formulations, formulation A1 prepared with PVA:AA ratio 25:75 was found to be more effective in reducing BP to 40.1% (Table 3) controlling the release of DL upto 24 h. Although there was significant fall in BP by formulations B1, C1 and D1, these formulations failed to restore the normotensive BP values.(PVA-co-PAA)/NaClnormal hydrogel system showed comparatively lower antihypertensive activity (26.7%) than (PVA-co-PAA)/NaCl HM (40.1%)

Formulation	First order ($K1h^{-1}$)	R^2	Higuchi ($kH\ \%\ h^{-1}$)	R^2	Korsemeyer-Peppas		
					($k_{KP}\%\ h^{-n}$)	N	R^2
A1	0.416 (0.039)	0.9920 (0.004)	49.721 (1.116)	**0.9937 (0.0009)**	21.681 (1.216)	0.811 (0.106)	0.9961 (0.0011)
B1	0.061 (0.068)	0.9938 (0.0013)	19.571 (1.261)	**0.9991 (0.0001)**	15.113 (1.214)	0.674 (0.009)	0.9993 (0.0026)
C1	0.066 (0.114)	0.9951 (0.0004)	21.061 (1.174)	**0.9979 (0.001)**	9.896 (0.141)	0.619 (0.06)	0.9978 (0.0003)
D1	0.038 (0.009)	0.9926 (0.006)	17.161 (0.888)	**0.9986 (0.0007)**	9.919 (0.128)	0.617 (0.021)	0.9971 (0.0006)

Table 2. Fitting results of experimental DL release data of hydrogel microspheres (HM) formulation A1-D1 to different kinetic equations. Values in parenthesis mean S.D., R^2 is the coefficient of determination. Best results in bold.

Group	Treatment	* Mean BP (mm Hg) ± SEM			%BP reduction
		Before Treatment	After MPA Treatment	After formulation Treatment	
1	Control #	96.8 ± 4.3	-	94.3 ± 8.6 ns	-
2	DL+	94.1 ± 2.9	168.9 ± 4.2 s	112.9 ± 5.1 ∞s	38.6
3	MPA+Placebo HM &	93.5 ± 6.1	162.5 ± 5.2 s	165.6 ± 3.8 ns	-
4	MPA+A1 HM α	96.7 ± 3.6	179.4 ± 5.9 s	110.1 ± 5.3 s	40.1
5	MPA+B1 HM α	94.9 ± 3.7	163.7 ± 4.6 s	111.8 ± 4.3 s	31.7
6	MPA+C1 HM α	91.6 ± 6.8	161.8 ± 3.8 s	119.9 ± 5.1 s	25.9
7	MPA+D1 HM α	92.5 ± 5.1	159.7 ± 7.1 s	121.5 ± 3.7 s	24.3
8	MPA+A H ¥	95.6 ± 3.8	159.7 ± 5.6 s	118.3 ± 4.2 s	26.7
9	MPA+B H ¥	95.3 ± 4.6	160.4 ± 3.6 s	122.1 ± 3.9 s	24.5
10	MPA+C H ¥	92.8 ± 5.5	156.9 ± 5.1 s	124.4 ± 5.1 s	21.3
11	MPA+D H ¥	92.3 ± 5.6	160.5 ± 4.4 s	114.6 ± 4.2 s	19.8

Table 3. Comparison of blood pressure reduction of hydrogel microsphere system with normal hydrogel system in hypertensive rats. *BP indicates blood pressure; HM, hydrogel microspheres ; H,normal hydrogel; MPA, methyl prednisolone acetate; ns, not significant; s, significant *($p < 0.01$).* # Control group: received no treatment. After treatment value represents final pressure at 24 h. DL+ indicates Diltiazem hydrochloride drug treatment orally in identical dose. ∞ Final blood pressure recorded for DL treatment group at 8 h. & Toxic control group: received methyl prednisolone acetate followed by placebo hydrogel micrsphere for 24 h. α Treatment groups: received methyl prednisolone acetate followed by hydrogel microsphere formulations for 24 h. ¥ Treatment groups: received methyl prednisolone acetate followed by normal hydrogel for 24 h.

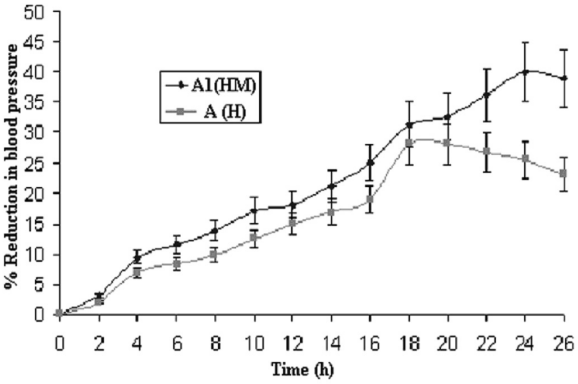

Figure 7. Comparison of blood pressure reduction of (PVA-co-PAA)/NaCl IPN hydrogel microspheres with normal hydrogel in hypertensive rats.

after 24 h as per Figure 7. This might be due to better controlled release capacity of (PVA-co-PAA)/NaCl HM which can control DL release upto 24 h where as normal hydrogel could control DL release upto 20 h, which could be predicted from *in vivo* studies. The results of *in vivo* studies were in conformity with *in vitro* drug release data.

CONCLUSION

The IPN hydrogel (PVA-co-PAA)/NaCl was successfully synthesized. From SEM studies it was found that the prepared (PVA-co-PAA)/NaCl HM were spherical with average particle size comparatively less than (PVA-co-PAA)/NaCl H. Swelling of HM in SIF media was found to be much higher than that of SGF. Entrapment of DL was found to be higher in (PVA-co-PAA)/NaCl HM than (PVA-co-PAA)/NaCl H. In both cases, (PVA:AA) monomer ratio 25:75 showed comparatively higher DL entrapment. From *in vitro* release study, PVA was found to be playing the major role in controlling the DL release. Comparatively much higher DL release was observed in SIF than that of SGF. This may be due to the higher swelling capacity of the system in SIF.DL release from HM was found to be controlled upto 24 h, where as normal hydrogel could control the release upto 20 h. Better control on release rate in case of HM might be due to it's size and better cross-linking than normal hydrogel. DL release mechanisms from the HM were fitted to different kinetic models and Higuchi–kinetic model yielded remarkably good adjustment. The release mechanism of DL from hydrogel matrices is an anomalous (non- Fickian) transport, which suggests that both diffusion of the drug in the hydrated matrix and its own erosion modulate drug release. From *in vivo* studies, (PVA-co-PAA)/NaCl normal hydrogel system showed comparatively lower antihypertensive activity (26.7%) than (PVA-co-PAA)/NaCl HM (40.1%) after 24 h. This might be due to better controlled release capacity of (PVA-co-PAA)/NaCl HM than normal hydrogel. The stability studies were conducted and the HM was found to retain their drug release profile.

The experimental results demonstrated that

(PVA-co-PAA)/NaCl hydrogel microspheres had the greater potential than (PVA-co-PAA)/NaCl normal hydrogel to be used as a drug carrier for an effective antihypertensive activity. So it was concluded that a single use of the prepared hydrogel microsphere system of DL can effectively control hypertension in rats. The system holds promise for clinical studies.

Acknowledgements. The authors acknowledge Dr. Reddy's laboratories, Hyderabad for supporting the study.

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