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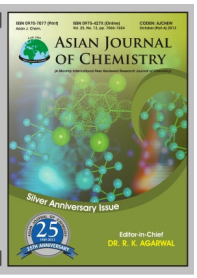

Preparation of Nanocomposite Hydrogels and Its *in vitro* Release Behaviour on Captopril

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Nanocomposite hydrogels are interesting for biomedical applications due to their bio compatibility and good water sorption diffusion properties. The pH sensitive interpenetrating polymer network based nanocomposite hydrogels was prepared from montmorillonite nanoclays by free radical polymerization and solvent sorption method was used for drug loading. Drug used was captopril an angiotensin converting enzyme inhibitor. Interactions and thermal stabilities were studied using fourier transform infrared spectroscopy, differential scanning calorimerty, thermogravimetric analysis. The morphology of the nanocomposite hydrogels was characterized by scanning electron microscopy (SEM), swelling study and per cent drug loading reveals the formation of compatible nanocomposite hydrogels. *In vitro* drug release in the pH of 1.2 and 7.4 fitted with kinetic equation models. This shows that the swelling ratios of the nanocomposite hydrogels has been influenced by the pH of the external media and also increased with an increasing order of clay addition, Drug loading of the gels increased with an increasing concentration of captopril. The fabricated formulation shows targeted release of the drug in intestine (pH 7.4).

Key Words: Free radical polymerization, Solvent sorption method, Montmorillonite nanoclays, Captopril.

INTRODUCTION

Hydrogels are three-dimensional networks of hydrophilic polymer chains, which can absorb and retain a large amount of water[1]. The reasons of using nanoparticles in hydrogels for drug delivery result from their basic properties. Foremost is due to their small size, penetrate within even small capillaries and are taken up within cells, which allows for efficient drug accumulation at the targeted sites in the body[2-4]. Use of biodegradable materials for nanoparticles preparation allows for sustained drug release within the target site over a period of days or even weeks after injection[5]. Organic/inorganic nanocomposites (NCs) are functional materials consisting of immiscible organic and inorganic components and complex nanometer-scale structures can be fabricated there from. As a typical example, polymer/clay nanocomposites have been extensively studied and successfully developed for many applications[6]. Haraguchi and Takehisa[7] reported the creation of a novel nanocomposite hydrogel with a unique organic-inorganic network structure by extending the concept of nanocomposite to the field of hydrogel materials. The nanocomposite hydrogels (NCHs) exhibited extraordinary mechanical, optical, swelling/deswelling properties which could simultaneously overcome the restrictions of conventional chemically crosslinked hydrogels. The formation of NCHs was achieved, not by the mere incorporation of clay nano-particles into a chemically crosslinked network, but by allowing the clay platelets to act as multifunctional cross linkers in the formation of polymer/clay networks[6]. To improve the mechanical properties and thermal stability of polymers, various clay minerals were widely added to thermosetting plastics, ultra high molecular weight polyethylene, rubber and thermoplastic resins and sodium montmorillonite (NaMM) or attapulgite were used to be served as reinforcing filler in the preparation of hydrogels to improve mechanical properties or swelling ability[8-16]. Captopril is an angiotensin-converting enzyme (ACE) inhibitor has poor oral bioavailability. Consequently NCH are attractive for a wide range of relevance in the field of controlled drug release, it's used to increase the bioavailability by releasing the drug at intestine (pH 7.4)[17-31]. Owing to their superior properties, NCHs have attracted much attention and are believed to be a revolutionary type of hydrogel[6].

EXPERIMENTAL

Captopril received as gift sample from M/s Bafna Pharmaceuticals (Chennai, India), poly(vinyl alcohol) (PVA) having mol. wt. of 14,000 from S.D. Fine Chemicals, Mumbai, India. Sodium acrylate (SA), potassium persulphate (KPS) and montmorillonite (MMT) nanoclays were from Sigma Aldrich. Double distilled water was used throughout the study.

Preparation of nanocomposite hydrogels: Hydrogels were prepared by solution polymerization method based on

free radical polymerization mechanism; potassium persulphate was used as a redox initiator. Poly vinyl alcohol and sodium acrylate were used in the formulation of semi interpenetrating polymer network of different ratios between polymer/monomer (N1, N2 and N3) systems may be due to the physical cross linking between polymers. Based thermal stability of (N1) system of inter penetrating polymer network hydrogels optimized for the preparation of nanocomposite hydrogels optimized for the preparation of nanocomposite hydrogels (N4, N5, N6 and N7) by the addition of different concentration of nanoclays as a modified montmorillonite with the particle size range of 14 μm and strong intercalating property. These processes were carried out under the temperature range of 60-70 ºC for 6 h. After completion of the process, collection the hydrogels and before characterizing them, it should be washed with distilled water and dried under vacuum oven at 50 ºC for 24 h. Composition of polymer/monomer and nanoclays used in interpenetrating polymer network (IPN) and nanocomposite hydrogels preparation are shown in Table-1.

TABLE-1
COMPOSITION IN IPN HYDROGELS AND NANOCOMPOSITE HYDROGELS PREPARATION

Sample code	PVA (%)	SA (%)	KPS (%)	Nanoclay (%)
N1	98	2	1	–
N2	95	5	1	–
N3	93	7	1	–
N4	98	2	1	0.1
N5	98	2	1	0.5
N6	98	2	1	1.0
N7	98	2	1	2.0

PVA- Poly vinyl alcohol, SA-sodium acrylate, KPS- potassium persulphate.

Drug loading to the nanocomposite hydrogels: The loading of a drug onto nanocomposite hydrogels was carried out in triplicate by swelling equilibrium (solvent sorption) method. The method has the advantages over the simultaneous method in which the drug is incorporated during the polymerization or process. The unreacted material can be removed before drug loading and the loading amount of the nanocomposite hydrogel can be adjusted by controlling the concentration of drug solution and degree of swelling. The prepared and optimized composition of nanocomposite hydrogels to N4, were allowed to swell in the drug solutions of different concentration (0.05, 0.10 and 0.15 %) as known as CPA, CPB, CPC respectively, for 24 h at 37 ºC and then dried. Where CPA-captopril loaded percentage 0.05, CPB-captopril loaded percentage 0.10, CPC-captopril loaded percentage 0.15.

Fourier transforms infrared spectroscopic (FTIR) studies[32]: The FTIR spectra of the copolymer membrane were recorded with an Equnox 55 Fourier-transform infrared spectrometer (Bruker, Germany) by a direct transmission The drug loaded NCH was placed on a crystal sample holder and scanning from 4000-400 cm^{-1} at a resolution of 2 cm^{-1}.

Thermo gravimetric (TGA) analysis[33]: The thermal stability of IPNs and nanocomposite hydrogels was examined using thermo gravimetric analyzer (TA Instruments, Model Q50). The TGA thermo gram was obtained during the heating from 30-800 ºC at a heating rate of 20 ºC/min under nitrogen atmosphere (200 mL/min).

Differential scanning calorimetric (DSC) studies[33]: The differential scanning calorimeter (DSC, TA Instruments and Model Q 200) was used to investigate the glass transition temperature (T_g) and melting point of the drug and drug loaded nanocomposite hydrogels. The DSC thermogram (equilibrated with an indium standard; each sample weighed 3-5 mg) was obtained during the heating from room temperature to 300 ºC at a heating rate of 15 ºC/min under nitrogen purge (60 mL/min).

Scanning electron microscopic (SEM) studies[34]: The morphology of IPNs and nanocomposite hydrogels with and without drug loaded were coated with a thin layer of palladium gold alloy and studied by scanning electron microscopy (Instrument model TS5136MM) at the magnification range of 1000x and 2000x.

Swelling study[34]: Swelling experiments were performed by immersing a known amount (0.03 g) of dried hydrogel in three different aqueous media, *i.e.*, water, PBS (pH 7.4) and a buffer pH solution (pH 1.2), respectively. The pH of buffer solutions was measured on LI614 pH Analyzer. The swelling ratios of hydrogel with time from the beginning to the equilibrium state was also studied and weight of swollen samples was measured after the surface solution removed by filter paper. The SR was calculated as

$$SR = \left(\frac{W_t - W_0)}{W_0} \right) \quad (1)$$

where W_0 and W_t are the weight of dried and swollen hydrogel, respectively.

Per cent drug loading[35]: The percentage of drug loading was estimated by using the formula

$$\text{Drug loading (\%)} = \frac{(M_i - M_d)}{M_i} \times 100 \quad (2)$$

where, M_i is the initial weight of the drug dissolved in the drug solution and M_d is the weight of the drug in the aqueous media measured immediately after preparing the drug loaded nanocomposite hydrogels by using the absorbance of UV spectroscopy.

***In vitro* release studies[36]:** *In vitro* release studies of the drug have been carried out in triplicate by placing dried and loaded hydrogel in definite volume of releasing medium at 37 ºC both in the simulated gastric and intestinal pH conditions using 0.1 N HCl and 7.4 pH phosphate buffer, respectively under gentle stirring. The amount of captopril released in 1.2 and 7.4 pH buffer solutions was measured by UV spectrophotometer, at the wavelength of 202 and 225 nm. The drug release was measured after a fixed interval of time and release dynamics were studied.

Release kinetics: To study the captopril transport mechanism from the nanocomposite hydrogels, various diffusion models are considered to fit the experimental data.

The model is described by the Ritger-Peppas equation:

$$\frac{M_t}{M_\alpha} = k_1 t^{1/2} \quad (3)$$

where M_t/M_α is the fractional drug release, k_1 is a kinetic constant, t is the release time and n is the scaling exponent

which is related dependent on the drug transport mechanisms. For a thin hydrogel film, when n = 0.5, the drug release mechanism is the Case I or the Fickian diffusion. Case II transport occurs when n = 1 corresponding to the zero-order release or the linear release. When the value of n is between 0.5 and 1.0, the non-Fickian or anomalous transport is observed.

RESULTS AND DISCUSSION

The absorption infrared spectra of polymeric interpenetrating polymer network of polyvinyl alcohol and poly sodium acrylate were confirmed by the C=O, C-O, CH_2 stretching at 1725, 1330, 2941 cm^{-1} and the broad peak of OH stretching at 3280-3400 cm^{-1}. The clay based NCH were confirmed by the absorption peaks of Si-O stretching (out of plane) at 1115 cm^{-1}. Captopril was confirmed by the peaks at 2981 and 2949 cm^{-1} were assigned to the asymmetric CH_3 and CH_2 stretching vibration and the peak at 2877 cm^{-1} was due to the symmetric CH_2 stretching mode. The peak at 2567 cm^{-1} corresponded to the SH stretching vibration. The peaks at 1747 and 1591 cm^{-1} were assigned to the C=O stretching vibration of carboxylic acid and amide band, respectively. The peaks at 1469 and 1385 cm^{-1} were due to the asymmetric and symmetric CH_3 bending vibrations, respectively and the peak at 1333 cm^{-1} was assigned to the OH bending vibration. The peaks at 1228-1200 cm^{-1} also corresponded to the C-O and CN stretching vibrations[37]. In Fig. 1 almost identical absorption bands were obtained from captopril loaded NCH but with lower intensity were confirms the no polymer/clay/drug interaction.

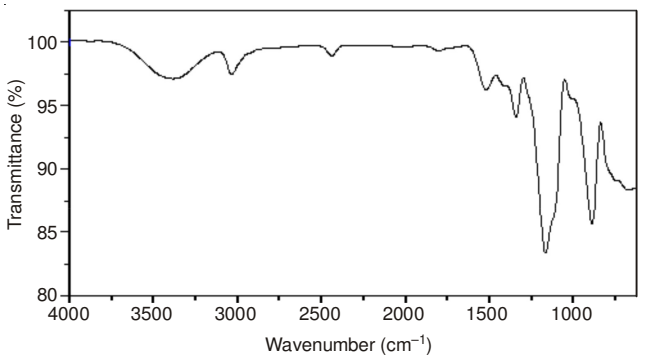

Fig. 1. FTIR spectroscopy of captopril loaded nanocomposite hydrogel

Thermogravimetric analysis: TGA thermograms of IPNs and NCH were seen. There are three transitions of NCH shifted from the degradations of IPNs. The first decomposition transition in the temperature range of *ca.* 50-100 ºC, corresponding to the loss of water molecule, the second and third decomposition transitions covers the temperature ranges of 240 and 350 ºC, corresponding to the thermal decomposition of nanocomposite hydrogels (NCH) are shown in Fig. 2. Thermal stability of nanocomposite hydrogel shows increase due to the intercalation on nanoclay material in to the polymer matrix.

Differential scanning calorimetric study: The DSC thermogram of pure captopril and captopril loaded NCH were shown in Fig. 3. Captopril exhibited a sharp endothermic peak at 107 ºC corresponding to its melting point. The peak of the captopril did not appear in the thermogram of captopril loaded NCH. It may indicate the drug was uniformly dispersed

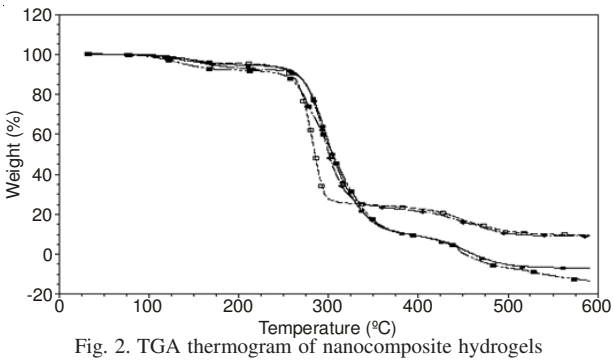

Fig. 2. TGA thermogram of nanocomposite hydrogels

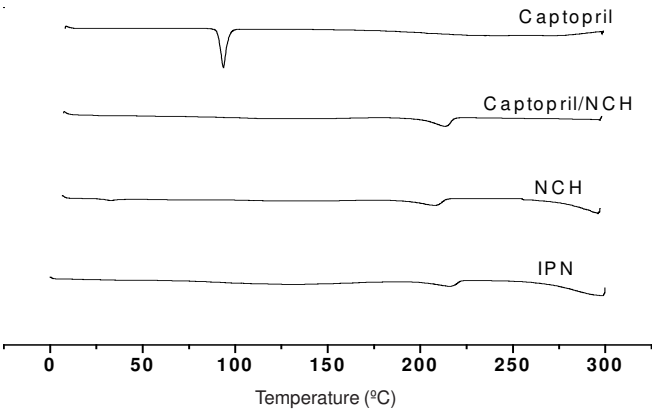

Fig. 3. DSC thermograms of Captopril, IPN, NCH and captopril loaded NCH

at the molecular level in NCH. The intercalation of IPNs with nanoclays were improves the thermal stability of the NCH and captopril loaded NCH.

Swelling study: The swelling kinetics of IPNs hydrogels and nanocomposite hydrogels in deionized water simulated gastric fluid (pH 1.2) and intestinal fluid (pH 7.4) were studied (Table-2). In these case free water penetrates the hydrogels in order to fill the inert pores that developed within the gel matrices, contribute to be a greater degree of swelling. Time required to reach the swelling equilibrium of IPNs and NCHs in different external medias. The NCH takes more time (7-9 h) to reach the equilibrium swelling than the IPN hydrogels (4-6 h) (Table-3). The IPNs and NCHs tend to swell more in pH 7.4 buffer than water and 1.2 buffer solution. At pH below the pK_a value of carboxylate group (pH 1.2 buffers) the COO^- groups of poly sodium acrylate on the gels will be protonized and the electrostatic repulsion between these groups will be reduced and favours to less selling ratios. On the other hand at a pH above the pK_a value of carboxylate (pH 7.4 buffer), the groups will mostly remains ionized to absorb more water. This was the reason behinds the higher swelling ratio of Nano-cmposite hydrogel in pH-7.4 buffer than the water and pH-1.2 buffer solutions.

Scanning electron microscopic studies: The morphology of IPNs and drug loaded NCHs shows the clean and smooth surface of the IPNs, confirms that the formation of homogeneous phase of IPNs. The porous nature of nanocomposite hydrogel were conformed from the SEM photographs. The drug loaded NCH were clearly evidenced by the dispersion of drug onto the gels.

TABLE-2
EQUILIBRIUM SWELLING OF INTERPENETRATING POLYMER NETWORK HYDROGELS IN DIFFERENT EXTERNAL MEDIAS

Sample codes	Equilibrium swelling in pH 1.2		Equilibrium swelling in water		Equilibrium swelling in pH 7.4	
	Time (min)	Swelling ratio (g/g)	Time (min)	Swelling ratio (g/g)	Time (min)	Swelling ratio (g/g)
N1	180	1.34	210	1.54	270	1.75
N2	210	1.39	240	1.60	300	1.80
N3	240	1.42	270	1.70	330	1.86

TABLE-3
EQUILIBRIUM SWELLING OF NANOCOMPOSITE HYDROGELS IN DIFFERENT EXTERNAL MEDIAS

Sample codes	Equilibrium swelling in pH 1.2		Equilibrium swelling in water		Equilibrium swelling in pH 7.4	
	Time (min)	Swelling ratio (g/g)	Time (min)	Swelling ratio (g/g)	Time (min)	Swelling ratio (g/g)
N4	360	2.18	420	2.25	480	2.34
N5	375	2.24	435	2.28	495	2.36
N6	390	2.28	450	2.32	510	2.39
N7	405	2.30	465	2.34	525	2.41

Drug loading (%): Three different concentrations (0.05, 0.10 and 0.15 %) of drug loaded into the nanocomposite hydrogel (N4) as represented as CPA, CPB and CPC was measured at the wavelength of 210 nm by UV-spectrophotometer. The results of percent drug loading were calculated by using the eqn. 2 and data's presented in Table-4. These studies confirms the drug loading efficiency of known weight (0.3 g) of nanocomposite hydrogel were increased by increasing the drug concentrations.

TABLE-4
DRUG LOADING EFFICIENCY OF NANOCOMPOSITE HYDROGELS

Sample codes	Concentration of drug (%)	Percent drug loaded in 0.3 g of NCH (%)	Amount of drug (mg/g of NCH)
CPA	0.05	54.48	9.08
CPB	0.10	75.72	25.24
CPC	0.15	85.95	42.975

***in vitro* release studies:** *In vitro* release profile of different concentrations (0.05, 0.10 and 0.15 %) of captopril loaded nanocomposite hydrogels as represented as CPA, CPB and CPC in buffer solutions of simulated gastric fluid (pH 1.2) and intestinal fluid (pH 7.4) were studied at 37 ± 0.5 ºC, under constant stirring. The maximum cumulative release profiles of captopril loaded NCHs of CPA, CPB and CPC, were reaches within the range of 3 and 6 h at 1.2 and 7.4 pH media. At pH-1.2, the maximum cumulative release of CPA, CPB and CPC reaches within 45 min, 2 and 3 h, respectively. At pH 7.4 buffer, the maximum cumulative release of CPA, CPB and CPC reaches within 1, 4 and 6 h. At the level of higher amount of drug loading (CPC), the time required for reaching maximum cumulative % release in pH 7.4 was high, compared to that of pH 1.2, then the lower amount of drug loaded NCHs of CPB and CPA, release studies were shown in Figs. 4-6. From the above release studies, it was clearly evident that the release of captopril, highly pH dependent and amount of drug loading.

***in vitro* release kinetics:** The amount of drug present in each captopril loaded NCHs were determined from standard calibration curve. The data obtained from *in vitro* release rate studies were fitted with various kinetic equations like Zero order, First order, Higuchi and Korsemeyer Peppas model and the (R^2) were shown in the Table-5. For (CPA, CPB and CPC)

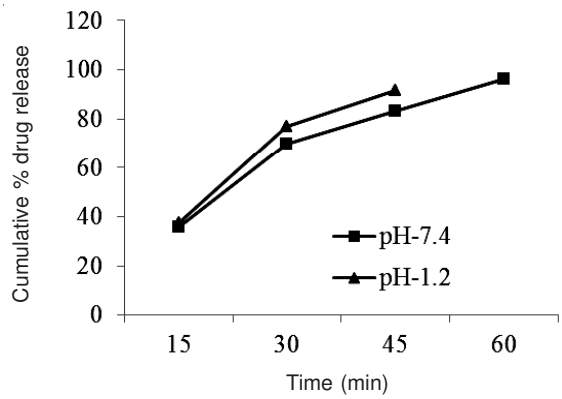

Fig. 4. Effect of pH on cumulative % drug release of 0.05 % captopril loaded nanocomposite hydrogel

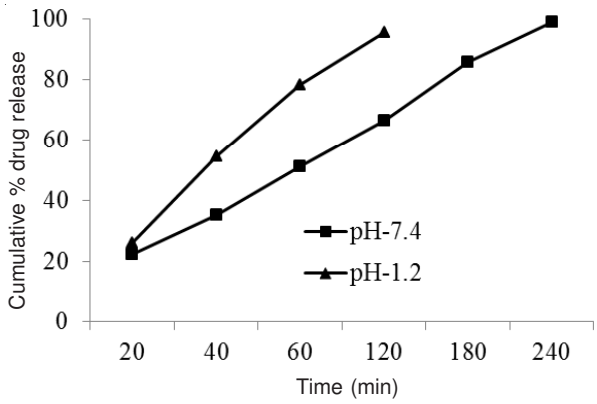

Fig. 5. Effect of pH on cumulative % drug release of 0.10 % captopril loaded nanocomposite hydrogel

at pH 1.2 and pH 7.4 media it suggests that the release kinetics of captopril loaded nanocomposite hydrogels mostly follows zero order and Higuchi model kinetics.

Conclusion

It has been shown that the solution polymerization method for nanocomposite hydrogels preparation and solvent sorption method used for drug loading. Formulation was developed and optimized with increased swelling and drug loading capacity. Captopril absorbed by both gastric and intestinal sites. By using drug loaded pH sensitive hydrogels seen that the maximum release at the pH 7.4 (intestinal fluid) up to 6 h and

TABLE-5
DRUG RELEASE KINETICS OF CAPTOPRIL LOADED NCHS (THE LINEAR REGRESSION CO-EFFICIENT (R^2) VALUES OF ALL KINETICS MODELS)

Release kinetic models	CPA		CPB		CPC	
	pH 1.2	pH 7.4	pH 1.2	pH 7.4	pH 1.2	pH 7.4
Zero order kinetics	0.9345	0.935	0.9887	0.997	0.9969	0.991
First order kinetics	1.000	0.951	0.9285	0.774	0.8255	0.794
Higuchi model	0.9345	0.935	0.9887	0.997	0.9969	0.991
Korsemeyer-peppas model	0.8896	0.861	0.925	0.966	0.918	0.876

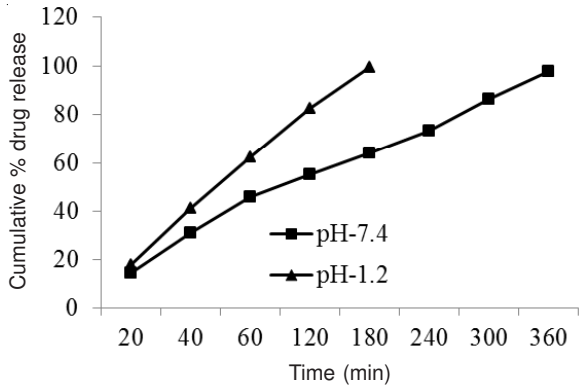

Fig. 6. Effect of pH on cumulative % drug release of 0.15 % captopril loaded nanocomposite hydrogel

at pH 1.2 (gastric fluid) is up to 3 h. This shows that using nanocomposite hydrogels we have target the release of drug. This will increase the bioavailability and half life of the drug. It should be possible to control the release of various drugs, by a proper selection of drug candidates and by the modification of hydrogel matrix.

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