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A novel amphiphilic nano hydrogel using ketene based polyester with polyacrylamide for controlled drug delivery system

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Abstract A ketene based Low molecular weight polymer (LMKP) having ester functional group was prepared using glycine through surface initiated anionic polymerization. NMR, ATR-FTIR & SEC were used to characterize the LMKP. The LMKP and acrylamide (AAm) were co-polymerised in methyl ethyl ketone to yield semi-IPN nanohydrogels (NHG). Benzoyl peroxide (BPO) was used as an initiator and *N*,*N*-methylene bisacrylamide (MBA) as crosslinking agent. Formation of NHG was confirmed through frequency shift in LMKP and poly acrylamide (PAAm) in FTIR spectroscopy. Photon correlation spectroscopy reveals that the sizes of the NHG were in the range of 140–225 nm and Transmission Electron Micrograph (TEM) also confirms the nano dimension of NHG. Biocompatibility of the NHG was confirmed through the cytotoxicity analysis. The swelling and diffusion behaviour of NHG, prepared under various formulations, were evaluated. The swelling pattern of NHG was studied at different pH conditions. The drug delivery capacity of NHG was investigated using ciprofloxacin as a model drug. The drug release kinetics of NHG suggested their anomalous (non-fickian) behaviour.

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1 Introduction

Controlled drug delivery technology (CDD) represents one of the frontier areas of science, which involves multidisciplinary scientific approach, contributing to human health care [1]. One of the challenging approaches to achieve CDD is bio-nanotechnology [2]. As the pharmaceutical industry continues to develop new and effective formulations, there has been a drive to develop efficient and sustained delivery systems. Drug delivery through hydrogels is a widely followed technique in pharmaceutical industry. A hydrogel is defined as a 3-dimensional crosslinked network, which swells but does not dissolve in an aqueous environment. They swell by absorbing water and shrink on drying [3, 4]. This property can be altered by environmental conditions like pH [5] and temperature [6, 7]. The CDD application of hydrogels is fundamentally related to water sorption behaviour of the hydrogels, which has been an area of active and intensive research in the recent past [8, 9]. Bajpai et al. [10] revealed the mechanism of water sorption by semi-IPN hydrogels formed from hydrophilic and hydrophobic polymer, which are considered to be the essential constituents.

Polymers of acrylamide are well known for their hydrophilicity and inertness that make them a material of choice in large number of applications in medical and pharmacy [11]. PAAm based hydrogels have been used for hygienic, biomedical and pharmaceutical applications [12, 13].

In this present investigation semi-interpenetrating network (semi-IPN) hydrogels in nano-dimensions (NHGs) were synthesized using hydrophobic low molecular weight ketene polymer (LMKP) and hydrophilic polyacrylamide (PAAm). The LMKP with narrow poly dispersity index was prepared from glycine through hetero catalytic conversion [14].

The focal theme of the present investigation was (i) preparation of NHG from crosslinked PAAm and LMKP (ii) characterization of NHG (iii) ability of the NHG systems on loading and releasing the model drug, ciprofloxacin HCl (CPH).

2 Experimental section

2.1 Materials

Glycine, methanol, chloroform, Methyl Ethyl Ketone (MEK), *N*,*N*-Methylene Bis acrylamide (MBA) and Benzoyl peroxide (BPO) were purchased from Merck (Germany) and used as received. Acrylamide was purchased from Merck and purified by recrystallization from toluene/n-hexane. Ciprofloxacin hydrochloride was purchased from sigma-aldrich. Free electron rich carbon matrix (MAC_{800}) was prepared in the Department of Environmental Technology, Central Leather Research Institute, India [15].

2.2 Preparation of hydrophobic LMKP

Synthesis of LMKP from glycine (non-toxic source) was carried out in a spiral fixed-bed heterogeneous catalytic reactor. The reactor was packed with MAC_{800} matrix of 12 g, and the reactor was maintained at 30°C. Glycine, at mole concentration 2.66 mmol/l, was dissolved in 2–4 ml of triple distilled water and made up to 1 l with methanol. The above mixture was injected into the spiral packed bed reactor at the flow rate of 1.0 ml/min using peristaltic pump (Watson Marlow, Germany). The solution collected at the outlet was subjected to vacuum distillation, whereby the alcohol escapes leaving behind the LMKP. The LMKP in water was purified using extraction in chloroform.

2.3 Characterization of LMKP

The ^{1}H, ^{13}C and DEPT Nuclear Magnetic Resonance (NMR) spectra of the LMKP were recorded in $CDCl_3$ on Bruker AMX-400 MHz spectrometer. Tetramethyl silane (TMS) was used as the internal standard.

Number and weight-average molecular weights (Mn and Mw) were determined by size exclusion chromatography (SEC) using a Waters 244 gel permeation chromatograph equipped with a refractive index detector. A set of 10^4, 10^3 and 100 Å Waters columns conditioned at 25°C was used to elute samples at 1 ml min^{-1} HPLC grade Dimethyl formamide flow rate. Polystyrene standards were used for calibration.

2.4 Preparation of NHG

Semi-IPN nanohydrogels (NHG) were prepared through free radical polymerization process. To 3 ml of MEK, 1 mmol of acrylamide (AAm) and 0.05 mmol of MBA were added. To this 0.1 mmol of LMKP was added and it was degassed, using nitrogen by bubbling through the solution for 15 min before the addition of 1 mol% (with respect to total monomer concentration) of BPO in 0.5 ml MEK as the initiator. The mixture was then polymerized in a typical polymerization tube, kept in a water bath at 75°C. The polymerization was carried out for 8 h. The precipitated polymer NHGs were washed with substantial MEK and then in water to remove traces of initiator and unreacted monomers. Finally they were dried at 50°C under the vacuum for 24 h. Nine series of NHGs (Table 1) with different crosslinking densities (in the mole ratio of AAm to MBA as 1:0.05, 1:0.1, and 1:0.15) and different LMKP concentrations (0.1, 0.075 & 0.05) mmol were prepared.

Table 1 Composition, equilibrium swelling ratio, equilibrium water content, and swelling characteristics of nhgs at different conditions

Sample	LMKP (mmol)	AAm/MBA (Molar ratio)	BPO (mg)	Equilibrium swelling ratio (g water/ g gel)	EWC (%)	Initial swelling rate constant [(g water/g gel) min]	Swelling rate constant [(g gel/ g water)/min]	Correlation coefficient (R^2)	Swelling exponent
NHG1	0.1	1/0.05	4	4.12	83.54	0.24	1.38	0.97	0.35
NHG2		1/0.1	4	5.08	87.61	0.17	2.10	0.98	0.19
NHG3		1/0.15	4	4.36	81.36	0.22	2.63	0.99	0.13
NHG4	0.075	1/0.05	4	6.37	88.27	0.30	2.05	0.99	0.37
NHG5		1/0.1	4	7.52	90.35	0.23	4.36	0.97	0.15
NHG6		1/0.15	4	6.36	86.40	0.29	3.38	0.97	0.18
NHG7	0.05	1/0.05	4	7.09	89.39	0.42	2.68	0.99	0.29
NHG8		1/0.1	4	8.43	91.45	0.38	2.14	0.99	0.24
NHG9		1/0.15	4	7.25	87.88	0.39	4.45	0.97	0.14

2.5 Characterization of NHG

Fourier Transform Infrared Spectra (FTIR) was recorded for LMKP, AAm and NHGs. The dried AAm and NHG samples were powdered, mixed thoroughly with KBr of spectroscopic grade and pressed into disk of dimensions 10 mm in diameter and 1 mm in thickness. To analyze LMKP and record the spectrum, sodium chloride cell was used. Spectra of the samples were recorded using a Perkin Elmer spectrophotometer in the spectral range of 400–4,000 cm^{-1}.

The mean particle size of the NHGs was measured by Photon Correlation Spectroscopy (PCS) (Malvern Instruments, Malvern, UK). Size of the particle was measured by using a dry sample adapter, kept on the sample tray in an inbuilt vacuum and compressed air system was used to suspend the particles. The analysis was performed in triplicate and average values were used.

Bright-field transmission electron microscope images were taken using a JEOL 3010 high-resolution transmission electron microscope (HR-TEM) operated at 300 keV. Samples for transmission electron microscopy were prepared by dropping a dispersion of the NHGs on copper grid supported Formvar films.

In vitro biocompatibility of the LMKP and NHG were evaluated using MTT assay. Briefly, Hep2 cells were seeded into 96-well microtiter plates (Nunclon™, Nunc, Wieshaden, Germany) at a density of 10,000 cells/well. After 24 h the culture medium was replaced with 100 µl/well of serial dilution of LMKP & dispersed NHG stock solution in DMSO & antibiotic-free DMEM ($n = 5$) respectively. Polymer extracts were prepared under sterile condition.

2.6 Swelling studies

Drug release from the crosslinked hydrogel depends upon the extent of water penetration into the matrix. The conventional gravimetric method was employed for the determination of the swelling ratio (S) and the Equilibrium Water Content (EWC %) of semi-IPN hydrogels [16].

The NHG, equilibrated with distilled-deionized water, were dried in a vacuum oven at room temperature until no detectable weight change was observed. The dried NHG were pelletized in an IR-pelletizer at a pressure of 300 Mpa and placed in 100 ml of distilled water/swelling medium (three different pH values; range 2.7 to 7). The weight of swollen NHG was determined at different time intervals and the swelling experiment was continued to a constant weight. At every measurement, the excess water was removed superficially by filter paper and then weighed accurately [17]. By using the swelling experimental weights of NHGs, their swelling ratios were calculated using the following equations:

$$S = \frac{\text{Weight of swollen } (W_s) - \text{Weight of dry gel } (W_d)}{\text{Weight of dry gel } (W_d)} \quad (1)$$

The swelling ratio at equilibrium is known as equilibrium swelling ratio. The EWC values of NHGs were determined using the gravimetric method by the equation given below:

$$\text{EWC}\,(\%) = \frac{\text{Weight of swollen } (W_s) - \text{Weight of dry gel } (W_d)}{\text{Weight of swollen gel at equilibrium } (W_s)} \times 100 \quad (2)$$

The mechanism of the swelling process of hydrogels was evaluated by using several kinetic models. A simple kinetic analysis is the second order rate equation [18, 19],

$$\frac{dS}{dt} = k_s\left(S_{eq} - S\right)^2 \quad (3)$$

where S_{eq}, S and k_s denote the equilibrium swelling (theoretical), swelling at any time and swelling rate constant, respectively. The integration of the above equation over the limits $S = S_0$ at $t = t_0$ and $S = S$ at $t = t$, gives

$$\frac{t}{S} = A + Bt \quad (4)$$

where $B = 1/S_{eq}$ is the inverse of the maximum or equilibrium swelling, $A = (1/k_s\, S_{eq}^2)$ is the reciprocal of the initial swelling rate of the hydrogel, and k_s is the swelling rate constant. To examine the above kinetic model for these NHGs, t/s versus t graphs were plotted, from which the initial rate of swelling and swelling rate constant were calculated from the slope and intersection of the lines obtained in the graphs.

The dynamics of the water sorption process were investigated by monitoring the change in the amounts of water imbibed by the NHGs at various intervals. For the kinetic analysis, the swelling results obtained were utilized up to 90% of the swelling curves,

$$\frac{M_t}{M_\alpha} = kt^n \quad (5)$$

where M_t/M_α is the fraction swelling ratio at time t, 'k' is the rate constant and 'n' is the release exponent. The n value is used to characterize different release mechanisms. The release is Fickian in nature and diffusion controlled for $n = 0.5$, while the values of n between 0.5 and 1.0 indicate non-Fickian diffusion (anomalous diffusion). In anomalous diffusion, diffusion and relaxation are said to be isochronal effective [20]. If n value is exactly equal to unity, then the diffusion is designed as Case II diffusion. In very few cases, the n value is found

to exceed unity and is called super Case II diffusion ($n > 1$).

To study the effect of pH, different pH (2.7, 4.2 &7.0) solution was prepared by adding hydrochloric acid with constant stirring to obtain a required pH.

2.7 Drug loading/release studies

Drug loading was accomplished by placing the dialysis membrane containing NHG particles of known weight in 200 ml of 0.2 mg/ml CPH solution in water at 10°C for 1 day. The dialysis membrane containing drug-loaded NHG was soaked in chilled water (10°C) for 24 h. They were washed with small amount of water to remove excess drug adhered at the surface of NHG and dried at 40°C in vacuum for overnight.

The Dissolution studies were conducted in a micro-dialysis setup [21]. One end of a hallow tube of 6 cm in length was tied with dialysis membrane and NHG of known weight was added into it. The loaded hallow tube was kept inside a beaker containing 25 ml of phosphate buffer of pH 7.0 such that it just touches the buffer. The buffer (maintained at 37°C) was stirred at 600 rpm using a magnetic stirrer and the aliquots of sample (1 ml) was withdrawn at regular intervals and replaced with equal volume of the buffer. The quantity of the CPH released from the NHG was analyzed using a UV spectrophotometer (Cary 100 Varian) at the absorption maximum of 277 nm through the standard calibration curve obtained previously under the same condition. NHG2 and NHG5 were the two compositions studied for the dissolution studies in order to observe the difference in dissolution pattern with respect to LMKP.

2.8 Release kinetics

Data obtained from in vitro release studies were fitted to the various kinetic equations [22, 23]. The kinetic models used are zero order, first order, Hixson and Crowell and Higuchi equation. The following plots were made: Q_t versus t (zero order kinetic model); log ($Q_0 - Q_t$) versus t (first order kinetic model); Q_t versus square root of t (Higuchi model) and $Q_t^{1/3}$ versus t (Hixson and Crowell model), Where Q_t is the amount of Ciprofloxacin hydrochloride at time t and Q_0 is the initial amount of Ciprofloxacin hydrochloride present in NHG. Further, to find out the mechanism of drug release, drug release was fitted in Korsmeyer–Peppas model.

$$\frac{M_t}{M_\alpha} = kt^n \tag{6}$$

where M_t/M_α is the fraction of drug released at time t, 'k' is the rate constant and 'n' is the release exponent. The n value is used to characterize different release mechanisms.

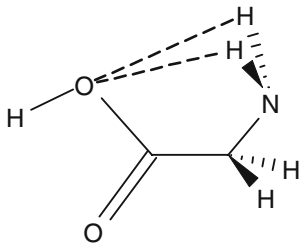
3 Results and discussion

3.1 Preparation of LMKP

The formation of ketene from glycine is shown in the equation 7 [14]

$$NH_2{-}CH_2{-}COOH \rightarrow CH_2{=}C{=}O + NH_2OH \tag{7}$$

The polar groups [15] in the network of catalyst draw the glycine molecules towards themselves and change the orientation of glycine molecule by the free electron cloud in catalyst [24]. This leads to form an isomer having the bi center-three electron interaction between the functional groups NH_2 and OH as follows [14]:



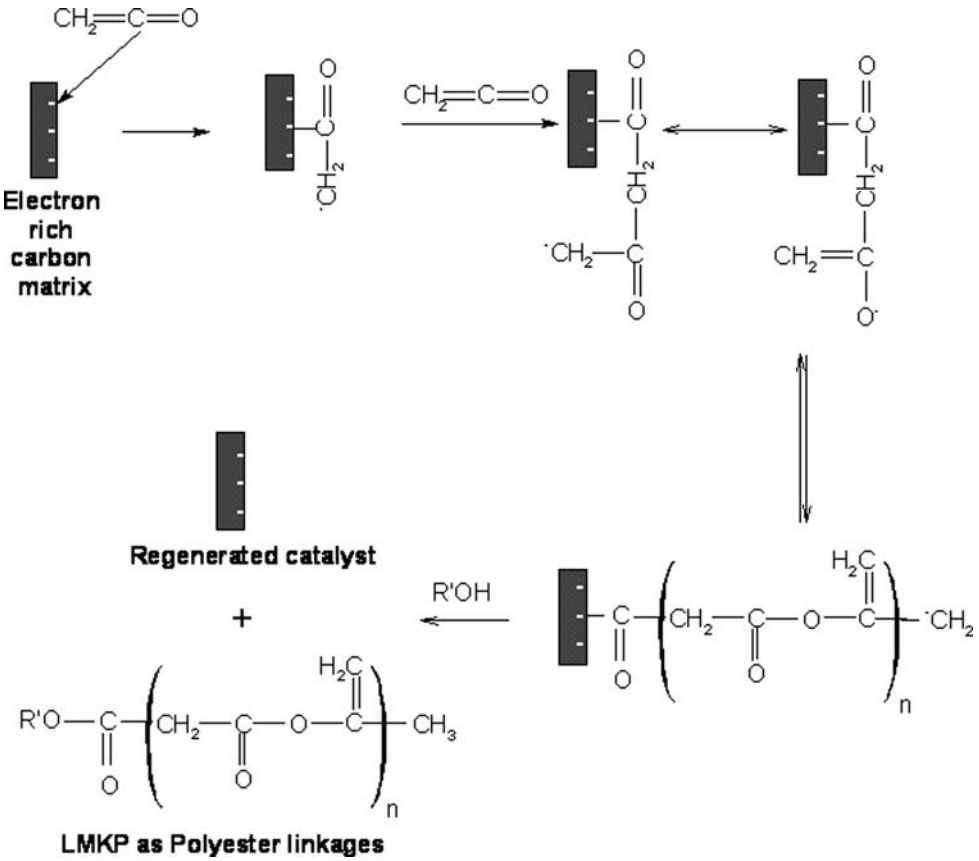
The isomer facilitates the formation of intramolecular hydrogen bond between NH_2 and OH groups, leading to acquire the positive charge at the carbon centre and negative charge at the carbonyl oxygen. The Glycine radical presents a shorter –NH–O hydrogen bond (2.10°A) [25]. As the intramolecular hydrogen bond interaction strengthens, the glycine molecule was strained such that the bond between –C–N and –C–OH was weakened leading to the bond rupture with the elimination of hydroxylamine.

In the explained mechanism the observation of hydroxylamine in the reaction was confirmed by converting it into acetone oxime by addition of acetone, followed by direct injection of the supernatant into the GC-MS, with detection of the oxime by selective-ion-monitoring [26].

Anionic polymerization of ketene into LMKP occurs at the surface of free electron rich carbon as insitu reaction. Alcohols can able to dissociate the possible aggregates in active centers (electron rich matrix) to keep it as an active non-aggregated form [27].

Ketenes are isoelectronic with isocyanates; we can place partial negative charge on both the end carbon & oxygen atoms and partial positive charge on the central carbon atom. The structure of the polymer depends upon the propagating anion in the polymerization reaction. The propagating anion may form either on the carbon or on the oxygen atoms, generating either a carbanion or an enolate anion. Polyketene are obtained by carbon–carbon addition. Acetal structures are formed by carbon–oxygen

Scheme 1 Insitu formation of LMKP in electron rich carbon matrix

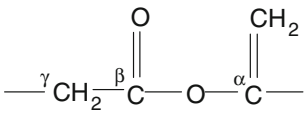


addition, while mixed addition gives the ester structure [28]. The initiation, propagation and termination step of the polymerization of ketene to polyester is represented in Scheme 1.

4 Characterization of LMKP

4.1 NMR studies

The complete assignment of the ^{13}C, ^{1}H and DEPT NMR spectra of the LMKP in $CDCl_3$ is shown in Table 2. The generation of polymers as esters is represented as follows.



4.2 Molecular weight determination

Size exclusion chromatography of LMKP indicates that the weight average molecular weight (Mw) is 1,742 and number average molecular weight (Mn) is 1,514. The synthesized polymer shows poly dispersity of 1.17. This leads us to conclude that LMKP was the polymer with narrow dispersity index.

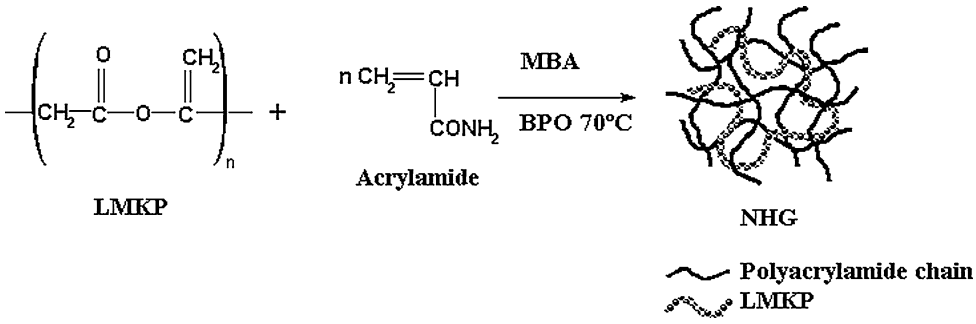
Table 2 Assignments of ^{13}C, ^{1}H & DEPT NMR

Structure	NMR—assignments (ppm)		
	^{13}C	^{1}H	DEPT
α-quaternary carbon	δ 132		
Methylene group attached to α-quaternary carbon	δ 128–130	δ 7.4–7.8	δ 128–130 (negative phase)
Ester carbonyl	δ 167		
γ-methylene carbon	δ 27.8	δ 2.81	δ 27.8 (negative phase)

4.3 Preparation of NHGs

NHG was generated with amphiphilic property. The existence of hydrophilic functional group amide traits the swelling characteristics of hydrogels and inclusion of ketene-based LMKP, hydrophobic in nature, will distress the water-gain property of hydrogels. Therefore, acrylamide and the synthesized LMKP may be considered as better raw materials for sustained drug delivery system due to their amphiphilic behaviour.

Scheme 2 Synthesis of nanohydrogel



In this study, NHG composed of crosslinked polyacrylamide and a physically entangled hydrophobic LMKP were synthesized (Scheme 2). Thus, prepared semi-IPNs were purified and subsequently used for characterization and drug delivery application.

5 Characterization of NHG

5.1 FTIR spectra

The FTIR spectrum of LMKP, polyacrylamide and NHGs are shown in the Fig. 1a–e respectively.

The FT-IR spectrum of the LMKP (Fig. 1a) showed the asymmetrical and symmetrical stretching of the methylene groups which were present as the backbone of the polymer occur near 2929.97 and 2860.41 cm^{-1} respectively. The band appears at 1458.24 cm^{-1} is the scissoring vibration of methylene groups and its twisting & wagging vibrations appear at 1380.02 cm^{-1}. The C=O stretching vibration of ester group is centered at 1733.45 cm^{-1}. The band centered at 1271.23 cm^{-1} can be attributed to the C–O stretching vibration of the ester group.

The FT-IR spectrum of polyarylamide (Fig. 1b) also shows the asymmetrical and symmetrical stretching of the methylene groups at 2930.68 and 2870.84 cm^{-1} respectively. The band centered at 3432.18 cm^{-1} is due to the N–H stretching of amide group. The C=O stretching of amide group is represented by the band at 1,666 cm^{-1}, whereas N–H bending vibration of amide is present at 1612.90 cm^{-1}. The C–N stretching vibration of polyacrylamide is shown around 1400 cm^{-1}.

The FTIR spectra of semi-IPNs with different cross linking densities, i.e. different MBA concentrations (0.05, 0.1 & 0.15) are shown in the Fig. 1c–e. It shows the presence of the asymmetrical and symmetrical stretching of the methylene groups of polyacrylamide/LMKP/MBA around 2932.61 and 2863.74 cm^{-1}. It reveals the shift in the C=O stretching in the LMKP from 1733.45 cm^{-1} to 1,723 cm^{-1}, 1,727 cm^{-1}, 1,728 cm^{-1} corresponding to partial loss in its double bond character due to the intermolecular hydrogen bonding between the ester group of LMKP and amide group of acrylamide. The N–H stretching of polyacrylmide is overlapped with MBA and exhibited as a broad envelope around 3,380–3,400 cm^{-1}. A band at 1,026 cm^{-1} represents the C–O stretching vibration. In curves (c), (d), (e) a band at 1,664 cm^{-1}, 1,666 cm^{-1}, 1,668 cm^{-1} respectively confirms the presence of N–H bending vibrations of acrylamide which is not observed in LMKP.

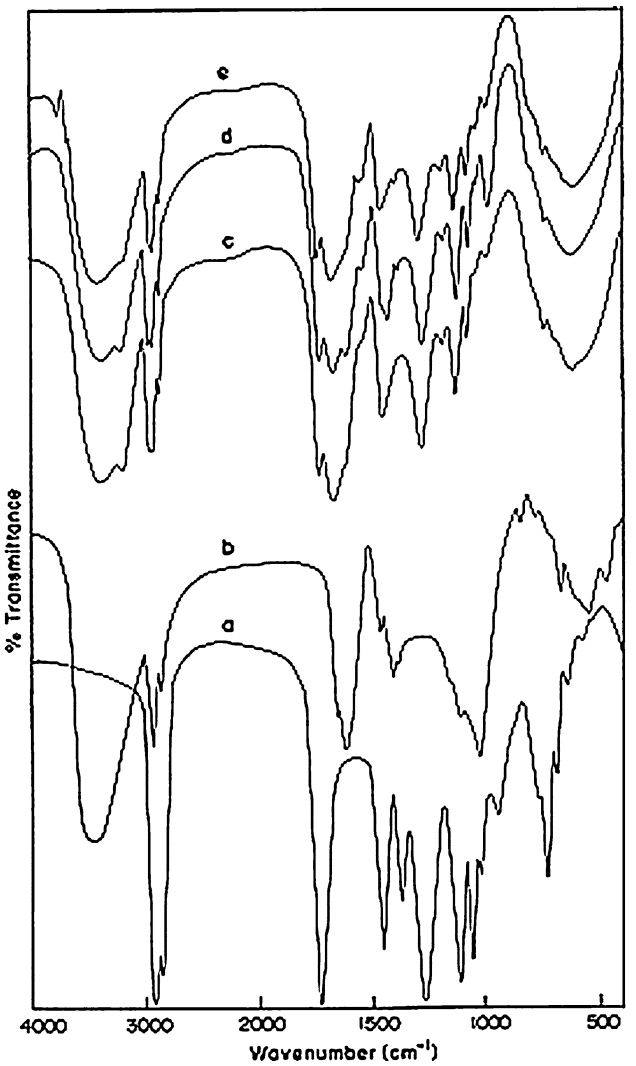


Fig. 1 FTIR spectra of (**a**) LMKP (**b**) Polyacrylamide (**c**) NHG with 0.05 mmol MBA (**d**) NHG with 0.1 mmol MBA (**e**) NHG with 0.15 mmol MBA

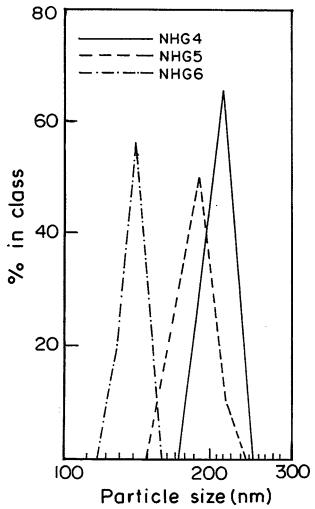


Fig. 2 Particle size distribution curves

5.2 Nanosize measurement

The size of NHG decreased as 203.1 ± 19.8 nm, 181.2 ± 35.5 nm, 145.9 ± 14.1 nm with increase in amount of crosslinker (MBA) of 0.05 mmol, 0.1 mmol and 0.15 mmol respectively (Fig. 2). This can be attributed to the decreasing nature of elasticity of the synthesized networks while increasing the amount of crosslinking agent. This result has proved the dependent nature of size over crosslink density.

6 HR-TEM

The surface morphology of the NHG1 was studied using HRTEM and presented in the Fig. 3. The Figure reveals that the NHG appears as irregular spherical shape in nano dimensional ranges.

6.1 Swelling studies

Swelling behavior of NHGs is attributed to the absorption mechanism which in turn is caused by diffusion process. The diffusion process is as a result of the affinity between polymeric network and external bulk solution. The swelling capacity of the NHG is controlled by the degree of hydrophilicity of PAAm, hydrophobic behaviour of LMKP and crosslink density or close packing density [29, 30]. Further, the swelling behaviour also depends on pH of swelling medium which plays a key role to ionize the functional groups in NHG. In the present investigation, the influence of crosslink density (MBA), hydrophobic LMKP and pH of swelling medium on swelling capacity were studied. The swelling kinetic parameters such as initial swelling rate, equilibrium swelling, swelling dynamics that include swelling constant, swelling exponent and diffusion mechanism were determined by Eq. 4 (second order rate kinetics) & 5 and the results are tabulated in Table 1.

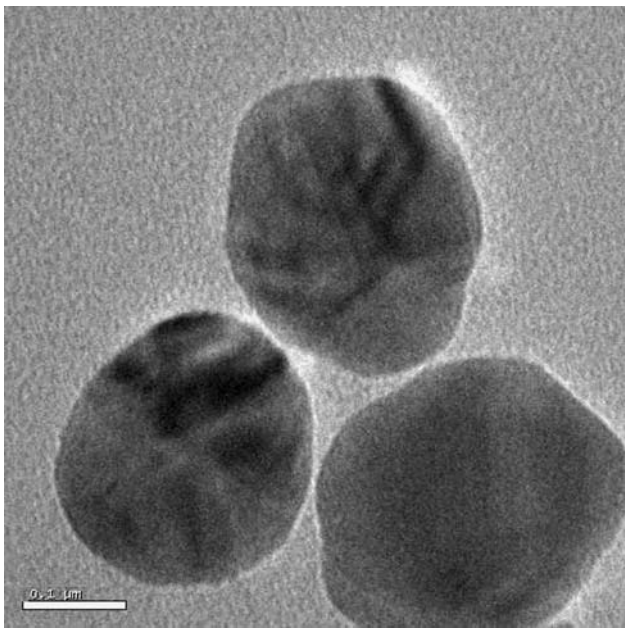


Fig. 3 HR-TEM of NHG1

6.2 Influence of crosslink density on swelling capacity

The swelling behaviour of the NHG is greatly influenced by the nature and type of crosslinking agent [31]. The swelling properties of NHG having hydrophilic crosslinker (MBA) were evaluated and the results are presented in Fig. 4. It is evident from the figure that as the concentration of MBA increased from 0.05 to 0.1 mmol, the equilibrium-swelling ratio also increased and further increase in concentration to 0.15 mmol, the value decreased slightly. This is due to the fact that at low concentrations the hydrophilicity of crosslinker is responsible for water uptake. But at very concentration the effect of hydrophilic is offset by crosslink density, which restrains the diffusion of water molecules into

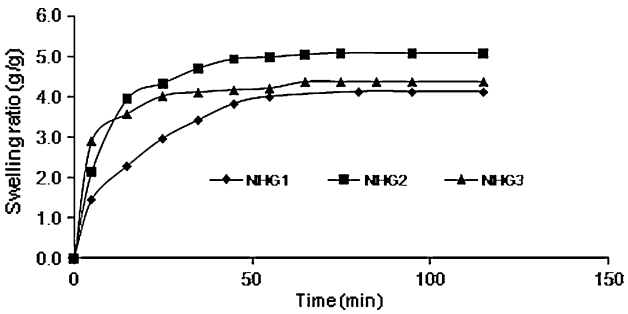


Fig. 4 Effect of concentration of crosslinker on swelling ratio of NHGs

the hydrogel matrix. This corroborates with the observations recorded by some of the previous researchers [10].

6.3 Influence of hydrophobic behaviour of LMKP on swelling capacity

The influence of LMKP in NHG on its swelling capacity has been studied by varying its concentration from 0.1 to 0.05 mmol and the results are depicted in Fig. 5a–c. The results indicate that with decreasing proportion of LMKP in the NHG results in there occurs an increase in swelling ratio and EWC. The reason is quite obvious that at low concentration of LMKP hydrophobic domain is considerably decreased which eases the swelling behaviour of gels [10].

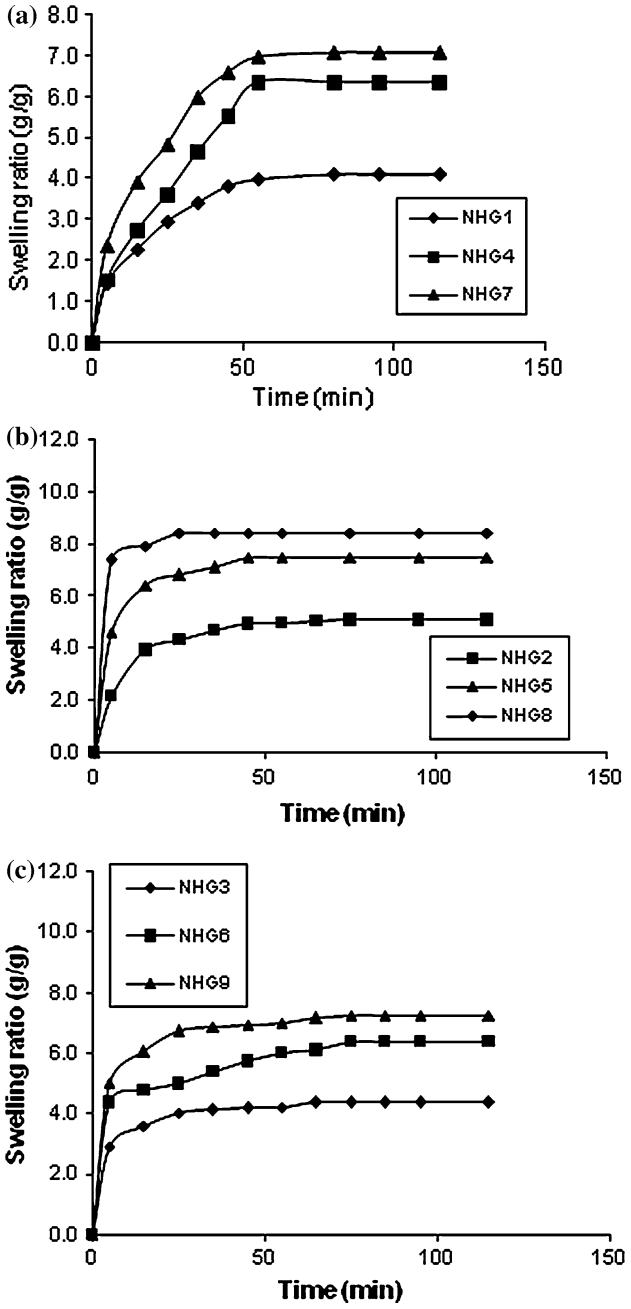


Fig. 5 Change in swelling ratio of NHG with respect to concentration of LMKP in presence of (**a**) 0.05 mmol of MBA (**b**) 0.1 mmol of MBA (**c**) 0.15 mmol of MBA

6.4 Influence of pH of swelling medium on swelling capacity

The pH sensitivity has gained much importance in sustained drug delivery application. The role of pH in regulating water sorption by NHG is of greater significance for its swelling property. The change in pH of the swelling medium often causes fluctuation in flux of solvent molecules to penetrate the hydrogel matrix. This may be attributed to widening of interstial volume within NHGs at higher pH due to PAAm which produces anionic charged centers, make repulsive action with linear chain LMKP [10]. The results show that despite the LMKP at higher concentration the equilibrium-swelling ratio was increased with increase in pH from 2.7 to 7. From the Fig. 6, it is evident that the EWC was decreased by 5–8% with decrease in pH.

7 Drug delivery studies

7.1 Cytocompatibility studies

The invitro cytocompatibility of LMKP and NHG were investigated with Hep-2- cell culture medium. There was no decrease in the cell viability percentage at concentrations of LMKP & NHG but there is slight decrease (14%) in cell viability at a very high concentration (80 mg) of the NHG (Fig. 7).

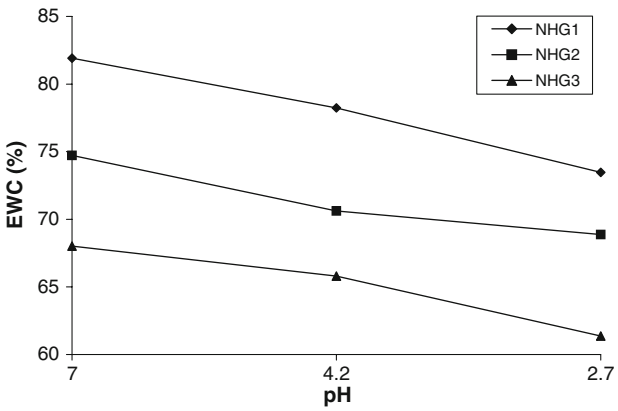


Fig. 6 Effect of pH on equilibrium water content of NHGs

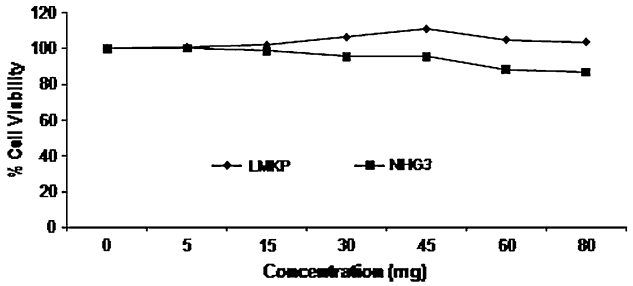


Fig. 7 Cell viability (%) of invitro cytotoxicity study of LMKP and nanogel

7.2 Drug release kinetics

The absorption and release behaviors of NHG for CPH in aqueous phase were investigated at pH 7.0. The release study was accomplished at temperature 37°C, monitored using UV spectrophotometer at the λ_{max} of CPH at 277 nm

The CPH from encapsulated NHG was released linearly with time in the initial period followed by a non linear increase and reached a plateau value after 18 h in each gels (Fig. 8). This experiment supports the view that NHG acts as good matrix for controlled drug release.

The data obtained from the drug release were fitted to various kinetic equations to determine the release rate and thereby mechanism of drug release was arrived. As indicated Table 3 by higher correlation coefficient (R^2), the drug release from NHG followed Higuchi model (diffusion controlled) than the first order and zero order equations. Hixson–Crowell equations (dissolution controlled) showed poor correlation. To confirm the release mechanism, the data were applied to Korsmeyer–Peppas equation to find out the release exponent n, which indicates the mechanism of drug diffusion. The data were well fitted with the equation as indicated by high correlation (R^2) coefficient and the mechanism of Ciprofloxacin HCl from NHG was found to be non-Fickian diffusion (anomalous transport, since the n value was between 0.5 and 1).

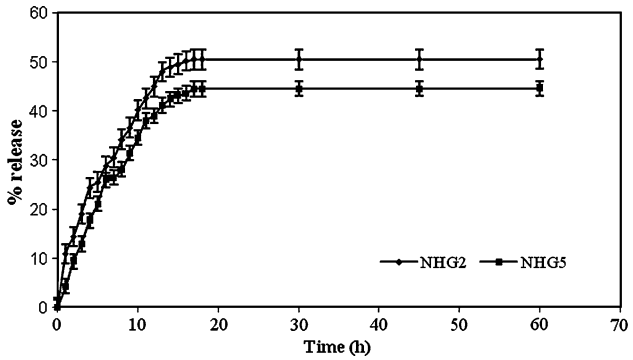


Fig. 8 Drug release profile of NHG

8 Conclusion

In this study, a novel ketene-based low molecular weight polymer having ester functional group was synthesized from glycine through elimination of hydroxylamine and surface initiated anionic polymerization using free electron rich carbon as catalyst and it is characterized for its structure through NMR, FTIR & SEC. Thus prepared hydrophobic LMKP and Acrylamide were utilized to prepare semi-IPN nanohydrogels using BPO as initiating system with MBA as crosslinking agent. The particle size analysis showed that the synthesized hydrogel is in the nano particle range (140–225 nm) and the size decreases with increase in amount of crosslinker. The biocompatibility studies shows that only 14% of the cells were dead at the concentration of 80 mg of the NHG.

EWC was decreased by 5 to 8% with decrease in pH from 7 to 2.7. As the hydrophobicity of the gel decreases by decreasing LMKP from 0.1 to 0.05 mmol, equilibrium water content and swelling rate constant increases from 87–91% and 1.40–2.71 g gel/g water respectively. These studies reveals that the swelling properties of the gel can be altered by varying the hydrophobic and hydrophilic constituents, thus it is more suitable for biomedical application.

In order to prove the applicability of these nanohydrogels in controlled drug delivery (CDD), drug loading and release studies were investigated for NHG1 and NHG2 (NHGs having maximum concentration of LMKP with 0.05, 0.1 mmol of MBA concentration) using ciprofloxacin hydrochloride as a model drug and the drug transport data were fitted to the different kinetic models. The mechanism of drug transports was found to be non-fickian (anomalous) in nature.

Table 3 Fitting of drug release to various equations

Sample	Zero order equation		First order equation		Higuchi model		Hixson and crowell model		Korsmeyer-Peppas model	
	R^2	K_0	R^2	K_1	R^2	K_H	R^2	K_{HC}	R^2	n
NHG2	0.99	2.89	0.99	0.0013	0.99	14.30	0.94	0.1036	0.99	0.61
NHG5	0.97	2.73	0.97	0.0012	0.99	14.09	0.88	0.1163	0.99	0.71

The results from this study confirm that the synthesized LMKP / PAAm NHG are potential drug delivery system with sustained release properties.

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