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International Journal of Pharmaceutics 209 (2000) 1–14

international journal of pharmaceutics

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Ketorolac entrapped in polymeric micelles: preparation, characterisation and ocular anti-inflammatory studies☆

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Received 4 February 2000; received in revised form 22 May 2000; accepted 30 May 2000

Abstract

Polymeric micelles made of copolymer of *N*-isopropylacrylamide (NIPAAM), vinyl pyrrolidone (VP) and acrylic acid (AA) having cross-linkage with *N*,*N*′-methylene *bis*-acrylamide (MBA) were used as host carrier in which up to 30%w/w ketorolac (free acid) was entrapped to make the formulation. The lyophilised powder was used for physical characterisation. The drug entrapment was found to be about 80% and the formulation was stable for 8–10 days at room temperature. The smaller the amount of ketorolac dissolved into the micelles, the longer was the formulation shelf life. The size of the particles as measured by dynamic light scattering was found to be around 35 nm diameter at 25°C. TEM picture showed spherical particles. The structure of the polymer and its morphology were characterised by FTIR, NMR and XRD measurements. IR data indicated weak interaction between polymer and ketorolac in the encapsulated system. NMR spectra indicated rigid polymer backbone with intermittent *iso*-propyl group in the chain. XRD spectra showed significant loss of crystallinity of the drug while being entrapped in the polymeric micelles. The release of drug in aqueous buffer (pH 7.2) from the polymeric micelles at 25°C were 20 and 60% after 2 and 8 h respectively and is temperature and pH dependent. In vitro corneal permeation studies through excised rabbit cornea indicated two fold increase in ocular availability with no corneal damage compared to an aqueous suspension containing same amount of drug as in nanoparticles. The formulation showed significant inhibition of lid closure up to 3 h and PMN migration up to 5 h compared to the suspension containing non-entrapped drug, which did not show any significant effect.

Keywords: Ketorolac; NSAID; Ocular delivery; Polymeric micelles; NIPAAM

1. Introduction

Ketorolac is a non-steroidal anti-inflammatory drug (NSAID), which has potent analgesic and anti-inflammatory activity due to prostaglandin related inhibitory effect of drug. Ketorolac (free acid) is sparingly soluble in water and, therefore,

☆ Indian patent application No. 845/Del/2000 dated 25th September, 2000.

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0378-5173/00/$ - see front matter
PII: S0378-5173(00)00508-1

it is marketed in the form of tromethamine salt (KT), which increases its solubility in water. KT is effective in inhibiting postoperative inflammation of eyes. It is also effective in reducing conjunctivitis with no alteration of corneal opacity (Fraser-Smith and Mathews, 1988). It does not facilitate Herpes Simplex, bacterial or fungal infection of the eye (Buckley and Brogden, 1990). KT (0.5%w/v) eye drops are available in the market. A solution of KT (0.5%w/v) applied topically to eyes is non-irritant and does not increase intraocular pressure (Fu and Lidgate, 1986). Only a small amount of instilled dose (1–3%) from such a formulation penetrates the cornea and reaches intraocular tissues (Schoenwald, 1985). This is due to lachrymal drainage and drug dilution by tears.

To overcome the problems, several new approaches have been tried including the use of bioadhesive polymers (Duchene et al., 1988; Saettone et al., 1989; Krishnamoorty and Mitra, 1993; Slovin and Robinson, 1993; Saettone et al., 1994; Das et al., 1995), liposomes (Fitzgerald et al., 1987; Lee, 1995) and nanoparticles (Li et al., 1986; Fitzgerald et al., 1987; Losa et al., 1991; Marchal-Heussler et al., 1991; Calvo et al., 1996a,b; Bourlias et al., 1998), that improve the ocular bioavailability of the drug. Substantial efforts have been directed towards the development of ocular drug delivery systems that would prolong the drug retention, allowing the drug to remain in contact with the cornea for longer duration and thus increases bioavailability (Lee and Robinson, 1986; Lee, 1990; Keiser et al., 1991; Slovin and Robinson, 1993; Urtti and Salminen, 1993; Sasaki et al., 1999). Nanoparticulate technology is advocated as an ophthalmic drug delivery approach that may enhance dosage form acceptability while providing sustained release in the ocular milieu (Zimmer and Kreuter, 1995). There have been some studies over the years examining the mechanism of drug release and reporting the ocular therapeutic action of drug from nanoparticles (Harmia et al., 1986b; Deshpande et al., 1998). The particles utilised in these studies were made of mucoadhesive polymers, which impart increased precorneal retention time. The delivery of drug through these nanoparticles does not increase the ocular bioavailability to a considerable extent due to larger particle size resulting in a smaller retention time. To overcome the same further work needs to be done particularly on the use of ultra small size nanoparticles (< 100 nm diameter) with mucoadhesiveness so that these are not washed away with tears quickly and having sustained release characteristics.

This paper describes the preparation of crosslinked copolymeric micelles made of *N*-isopropylacrylamide, *N*-vinylpyrrolidone and acrylic acid containing ketorolac entrapped into the polymeric network, their characterisation, in vitro release behaviour, in vitro transcorneal permeation characteristics and in vivo ocular anti-inflammatory effect. *N*-isopropylacrylamide was used to form stable micellar aggregates with hydrophobic core mainly composed of isopropyl moiety. Vinylpyrrolidone renders the hydrogel behaviour of the polymer while acrylic acid was added to have copolymer mucoadhesive (Robert et al., 1988).

2. Experimental

2.1. Materials

N-Isopropylacrylamide (NIPAAM) was purchased from Ranbaxy Acros and was crystallised from *n*-hexane before polymerisation. *N*,*N*′-Methylene *bis*-acrylamide (MBA) was product of Sigma, USA and was used directly without further purification. Acrylic acid (AA), *n*-hexane, sodium monohydrogen phosphate and dihydrogen phosphate, ferrous ammonium sulphate (FAS) were procured from SRL (India). *N*-Vinylpyrrolidone (VP) was purchased from Fluka. Absolute ethanol (99.8%) was purchased from Merck (Germany). AA and VP were used freshly distilled before polymerisation. Ketorolac tromethamine was a gift from Ranbaxy Laboratories. The free acid was prepared from the tromethamine salt following the method of Malhotra and Majumdar (1997).

2.2. Synthesis of polymer

A copolymer of NIPAAM + VP + AA was synthesised through free radical mechanism. Water-soluble monomers, NIPAAM, VP and AA, were

used in 85.7:9.5:4.8 molar ratio and the polymer was cross-linked with MBA. FAS was added to activate the polymerisation reaction and also to ensure complete polymerisation of the monomers so that polymer is obtained in good yield. In a typical experimental protocol, we took 900 mg NIPAAM, 100 μl freshly distilled VP and 50 μl AA (also freshly distilled) in 100 ml of water. To cross-link the polymer chain, 300 μl of MBA (0.049 g/ml) was added in the aqueous solution of monomers. The dissolved oxygen was removed by passing nitrogen gas for 30 min. Fifty μl of FAS and 50 μl of saturated ammonium persulphate (APS) solutions were, then, added to initiate the polymerisation reaction. The polymerisation was done at 30°C for 24 h in nitrogen atmosphere. Total aqueous solution of polymer was then dialysed for overnight using a spectrapore membrane dialysis bag (12-kD cut off). The dialysed aqueous solution of polymeric micelles was frozen in liquid nitrogen and was lyophilised immediately to obtain dry powder for subsequent use. The yield of micelle nanoparticles was more than 80%. Lyophilised powder is easily redispersible in aqueous buffer.

2.3. Loading of ketorolac

The physical entrapment of ketorolac (free acid) in NIPAAM-VP-AA copolymeric micelles was carried out as follows: 100 mg lyophilised powder of polymeric micelles was dispersed in 10 ml of water and was stirred well to constitute the micelles. The free acid form of ketorolac was dissolved in absolute ethanol (50 mg/ml) and the alcoholic solution was added in polymeric solution slowly with constant stirring. Ketorolac got directly loaded into the hydrophobic core of micelles. The drug loaded polymeric micelles were then lyophilised to get dry powder for subsequent use. The shelf life of ketorolac loaded polymeric micelles was determined by dissolving certain amount of ketorolac loaded lyophilised powder in water and the solution was kept at 25°C. The solution was clear at the time of loading (zero time). The time when the solution became just turbid was noted.

2.4. Entrapment efficiency (E%)

The $E\%$ of NIPAAM-VP-AA copolymeric micelles loaded with ketorolac was determined as follows. The nanoparticles of polymeric micelles were separated from unentrapped free ketorolac using Millipore UFP2THK24 (100-kD cut off) membrane filter and measuring the amount of free ketorolac in the filtrate spectrophotometrically using a UVIKON 930 KONTRON spectrophotometer at 313 nm. The $E\%$ was calculated as

$$E\% = ([\text{Ketorolac}]_{\text{total}} - [\text{ketorolac}]_{\text{free}}) \times /[\text{Ketorolac}]_{\text{total}} \times 100.$$

2.5. Characterisation of polymeric micelles

2.5.1. FT-IR studies

Mid IR spectra of NIPAAM, VP and AA as well as void and ketorolac loaded polymeric micelles were taken in KBr pellet using Perkin Elmer Fourier Transformed Infrared(FT-IR) spectrophotometer (spectrum 2000) instrument.

2.5.2. ^1H NMR studies

The NMR spectra of monomers like NIPAAM, VP and AA, void polymeric micelles and ketorolac loaded micelles were taken by dissolving the samples in D_2O as solvent using Bruker Spectrospin Avance 300 MHz spectrometer.

2.5.3. X-ray diffraction studies

The X-ray diffraction patterns of void polymeric micelles and micelles loaded with ketorolac were studied using Philips X'pert PW 1830/98 model. X-ray pattern of ketorolac free acid was also included as a control.

2.6. Measurement of size and size distribution

2.6.1. Dynamic light scattering (DLS) measurements

DLS measurements for determining the average size and size distribution of the polymeric micelles were performed using a Brookhaven 9000 instrument with a BI 200 SM Goniometer. An air-cooled argon ion laser operated at 488 nm was used as light source. The intensity of scattered

light was detected at 90° to an incident beam. The freeze-dried powder was dispersed in aqueous buffer and measurements were done, after the aqueous micellar solution was filtered with a microfilter having an average pore size of 0.2 μm (millipore, USA), using a 128-channel digital correlator, which derived the time-dependent autocorrelation function of the scattered intensity. The size of the particles was calculated from diffusion of the particles using Stoke–Einstein equation.

2.6.2. Transmission electron microscopy (TEM) study of the polymeric micelles

TEM picture of the micellar nanoparticles was taken in a Philips EM300 instruments using 14 000 times magnification (80 kV). A drop of aqueous solution of lyophilised powder ($\approx$ 5 mg/ml) was placed on a membrane coated grid surface with a filter paper (Whatman No. 1). A drop of 1% phosphotungstic acid was immediately added to the surface of the grid. After 1 min excess fluid was removed and the grid surface was air dried at room temperature before loaded in the microscope.

2.7. In vitro release behaviour in aqueous buffer

A known amount of lyophilised powder of copoly [NIPAAM-VP-AA] micelles loaded with ketorolac was dispersed in 10 ml buffer of desired pH at which release kinetics was to be studied. The solution was then distributed 500 μl each in 20 Eppendorf tubes and was kept at constant temperature. At a predetermined interval of time the solution was filtered through a Millipore filter of 100 kD cut off as described above. Free ketorolac present as dissolved in aqueous buffer passed through the filter and its concentration was determined spectrophotometrically at 313 nm. The release kinetics was studied in pH 5.0, 7.2 and 10.0 buffers each at 25 and 37°C temperatures.

2.8. In vitro transcorneal permeation studies

In vitro transcorneal permeation studies were carried out using freshly excised rabbit corneas. Albino rabbits weighing 1.5–2.0 kg (Lucky Zoological House, New Delhi), were sacrificed with an intravenous lethal dose of phenobarbitone sodium injection (200 mg/ml) and eyeballs were removed. The corneas were dissected along with 2–4 mm of surrounding scleral tissue and washed with cold (4°C) normal saline. Cornea was mounted between the donor and receptor chambers of a modified version of Franz all-glass diffusion cell developed by Fu and Lidgate (1986). Corneal area available for permeation was 0.64 cm^2. The receptor was filled with 12 ml of bicarbonate ringer and all air bubbles were removed. One ml of formulation was placed in the donor chamber, i.e. on the top of cornea. Sealing a glass cover slip over the opening of the donor cell with silicone grease prevented evaporation of liquid from donor chamber. Water at 37°C was circulated through the water jacket surrounding the receptor cell and the liquid in the receptor cell was stirred continuously to keep it homogeneous. Sample (2 ml) was withdrawn at 60 and 120 min from the receptor chamber through the sampling port and was diluted with 0.1 N HCl, and the ketorolac content was determined spectrophotometrically by measuring the absorbance at 313 nm. Bicarbonate ringer replaced the quantity of samples withdrawn. At the end of the experiment, cornea was freed of scleral ring and weighed. The cornea was then dried at 80°C to constant weight to determine the corneal hydration.

2.9. In vivo studies of ketorolac formulation on PGE_2 induced ocular inflammation in rabbits

Eight albino rabbits of either sex were divided randomly into two groups of four each. Each rabbit received 50 μl of ketorolac formulation in left eye and 50 μl of control vehicle (distilled water) in the contralateral eye. Ten min later 50 μl of PGE_2 (1 μg/ml normal saline, Dinoprostone, Astra IDL Ltd, India) was instilled in both eyes. All eyes were then evaluated for parameters of inflammation, i.e. lid closure and polymorphonuclear leukocyte (PMN) migration. Lid closure parameter was scored as follows: 0, fully open; 1, one third open; 2, two thirds open; 3, fully closed.

Normal saline (100 μl) was instilled in the inferior cul de sac of the rabbit eye and after quick

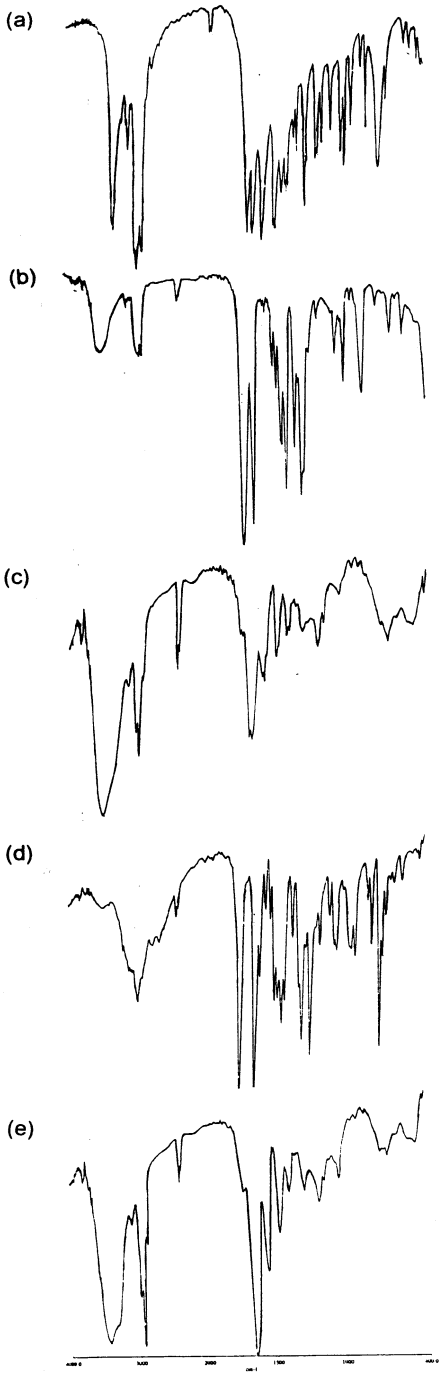

Fig. 1. FTIR spectra of (a) *N*-isopropylacrylamide, (b) *N*-vinyl pyrrolidone, (c) NIPAAM-VP-AA copolymeric micelles, (d) ketorolac (free acid) drug, (e) ketorolac loaded polymeric micelles.

and gentle mixing 50 μl of the tear fluid was withdrawn at 1, 2, 3, 4 and 5 h following PGE_2 instillation. Tear fluid PMN was counted in a Neubaner haemocytometer (Sood, 1994).

3. Results and discussion

3.1. Synthesis and characterisation of NIPAAM-VP-AA copolymeric micelles

Random copolymerisation of NIPAAM with VP and AA was done by radical polymerisation process of the micellar aggregates of the monomers. Polymer formed in this way has amphiphilic character with a hydrophobic core inside the micelles and hydrophilic outer shell composed of hydrated amides, pyrrolidone and carboxylic groups projected from the monomeric units. Water insoluble drug like ketorolac free acid was dissolved into the hydrophobic core of the polymeric micelles.

3.1.1. FTIR study of NIPAAM-VP-AA copolymeric nanoparticles

The copolymer in the form of micelle nanoparticles was prepared from the vinyl polymerisation of monomers in presence of persulphate as catalyst. Fig. 1 exhibits the FTIR spectra of the copolymeric micelles along with those of NIPAAM and VP monomers. As shown in the figure, strong peaks in the range of 800–1000 cm^{-1} corresponding to the stretching mode of vinyl double bonds (Sun, 1994) disappeared in the spectrum of polymer indicating that polymerisation has taken place. Being hydrogel polymer, the water of hydration attached to the polymer gives rise to a broad and intense peak at 3435 cm^{-1}. The C–H stretching vibration of the polymer backbone is manifested through strong peak at 2928 cm^{-1}. Peaks at 1640–1720 cm^{-1} are corresponding to >C=O stretching from all three monomer units. Ketorolac free acid exhibits a strong peak at 1720 cm^{-1} due to carbonyl stretching and at 1614 cm^{-1} due to aromatic C–C stretch. In the loaded polymeric micelles, the band at 1640 cm^{-1} corresponding to carbonyl stretching of the polymer has been slightly shifted to

1649 cm^{-1}, which may be attributed to be due to a weak interaction between the drug and the polymeric chains.

3.1.2. Composition of copolymer

The composition and molecular weight of NIPAAM-VP-AA copolymer nanoparticles were determined by ^1H NMR spectroscopy. Fig. 2 shows a typical ^1H NMR spectrum and ^1H chemical shift assignments of the copolymer. The spectrum shows the resonance of both NIPAAM and VP while those of AA, being present in very small amount in the composition, are lost in the noise. Polymerisation is indicated by the absence of resonance attributable to the vinyl end group protons of the monomers. Rather these protons have shown resonance at sufficiently upfield region in the spectrum attributing to the saturated protons of the polymeric network. The unit ratio of NIPAAM and VP monomers in the copolymer was obtained from the ratio of the peak intensities of the methine proton, (>CH–), of *N*-isopropylacrylamine group to the half of the intensity of β-methylene protons, (-CH_2-), of pyrrolidone group at 3.84 and 3.21 ppm respectively. The results indicate that for each vinyl pyrrolidone unit there are about 8–9 NIPAAM units present in the polymer, which is more or less the added molar ratio of NIPAAM and VP monomers in the reaction mixture. The results from ^1H NMR measurements exhibited nearly similar value to the calculated molecular weight of copolymer (23.1×10^3). This value is quite close to the molecular weight determined by gel permeation chromatography (not shown) using Sephadex G-200 gel ($\approx 18.9 \times 10^3$).

3.1.3. Crystallinity of the copolymeric micelles

Powder X-ray diffraction measurements were carried out to investigate the crystallinity of NIPAAM-VP-AA copolymeric micelles with and without ketorolac. The powder X-ray diagrams of the nanoparticles are shown in Fig. 3. The polymer is an amorphous material with a broad melting point of about 120–140°C. Sharp crystalline peaks of ketorolac in the range of $2\theta = 0–30°$ disappeared in the entrapped system while the characteristic peaks at $2\theta = 45°$ remains intact in the polymeric micelles. It can be suggested that ketorolac entrapped into the nanoparticles remain in the semicrystalline form (Harmia et al., 1986a).

3.2. Size and morphology of NIPAAM-VP-AA copolymeric micelles

The size and size distribution of the nanoparticles of copolymeric micelles were measured by means of dynamic light scattering method. Fig. 4 shows the typical size distribution of the nanoparticles. From DLS measurements the average size was found to be less than 50 nm diameter at 25°C with narrow size distribution and unimodal pattern. Since NIPAAM forms a thermo sensitive polymer, the size of the nanoparticles was found to be temperature dependent as shown in Fig. 5. A quasi-sudden increase of size at a particular temperature depends on the composition of the polymer particularly on the amount of NIPAAM present in the copolymer. Copolymeric micelles formed by 90 mg NIPAAM,10 mg VP and 5 mg AA monomers have shown a rapid increase of size from 35 nm diameter at 25°C to more than 300 nm diameter at 36°C. At and beyond this critical temperature the micellar particles dispersed in aqueous buffer become turbid due to structural changes and consequent enhanced surface hydrophobicity of the particles. Interestingly, the particle size of the copolymeric micelles (at 25°C) does not change significantly when the micelles are loaded with ketorolac.

TEM picture of the stained samples of polymeric micelles loaded with ketorolac is shown in Fig. 6. The samples were dried over the grids at room temperature. The picture shows that the particles are spherical and nearly monodispersed with an approximate size of around 40 nm diameter, which is comparable to the size obtained from DLS measurements.

3.3. Shelf life of the ketorolac loaded copolymeric micelles

The polymeric micelles (lyophilised powder) was dispersed in water and ethanolic solution of ketorolac was dissolved. The clear solution was kept at room temperature till tubidity appears due

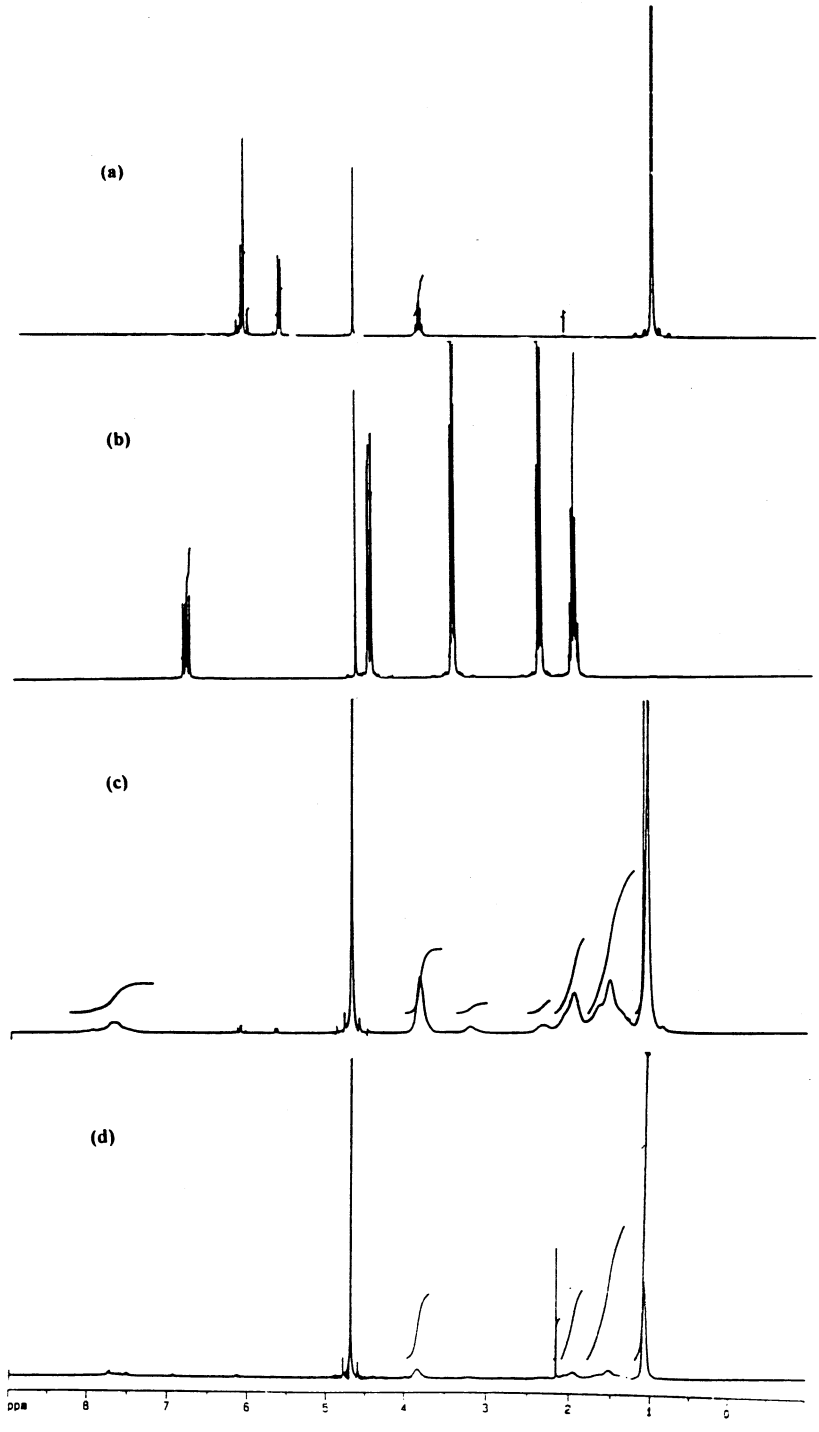

Fig. 2. Typical ^{1}H-NMR spectra of (a) *N*-isopropylacrylamide, (b) *N*-vinyl pyrrolidone, (c) NIPAAM-VP-AA copolymeric micelles, (d) ketorolac loaded polymeric micelles.

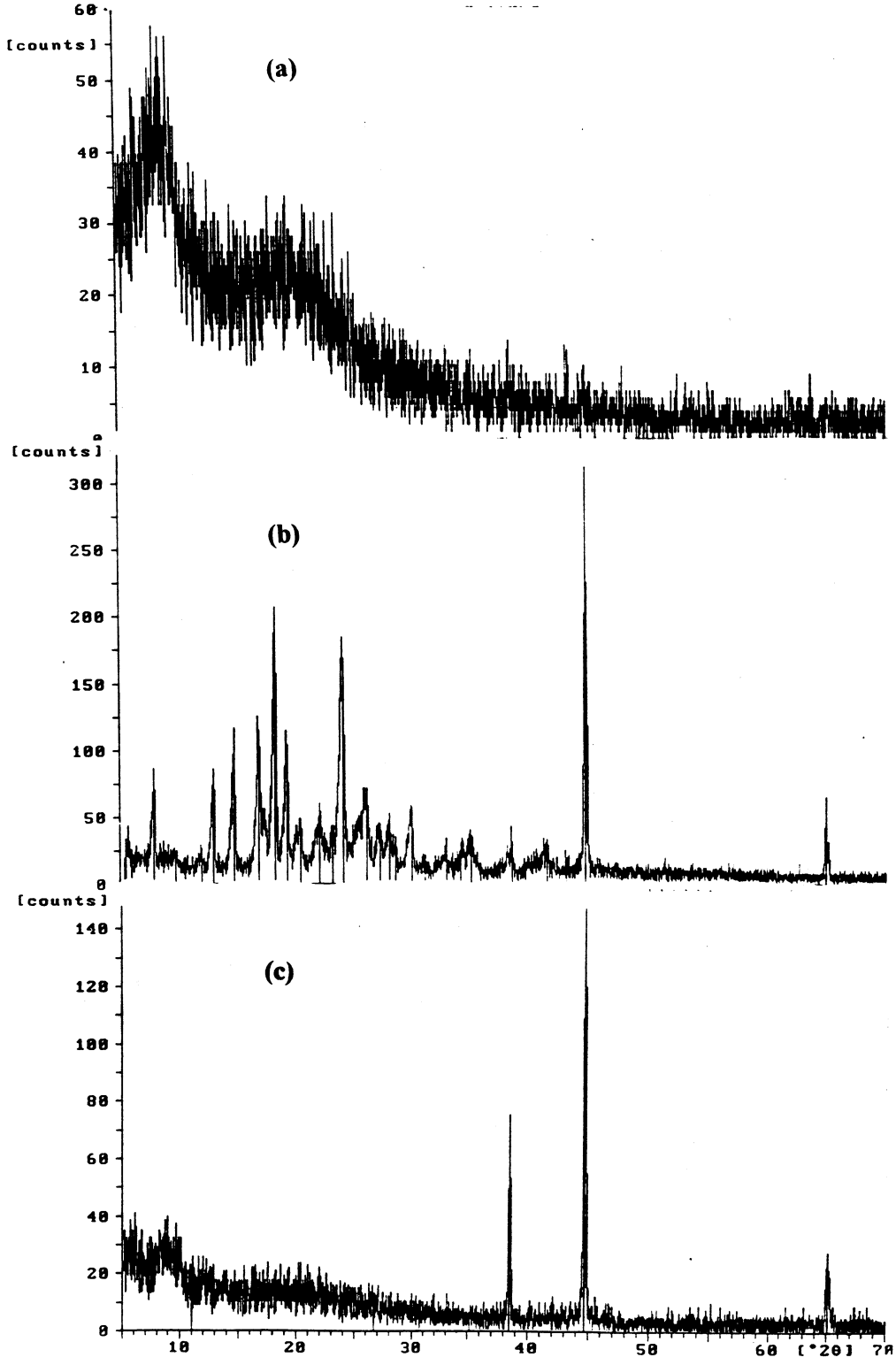

Fig. 3. Powder X-ray diffraction patterns of (a) NIPAAM-VP-AA co-polymeric micelles, (b) ketorolac (free acid) drug, (c) ketorolac loaded polymeric micelles.

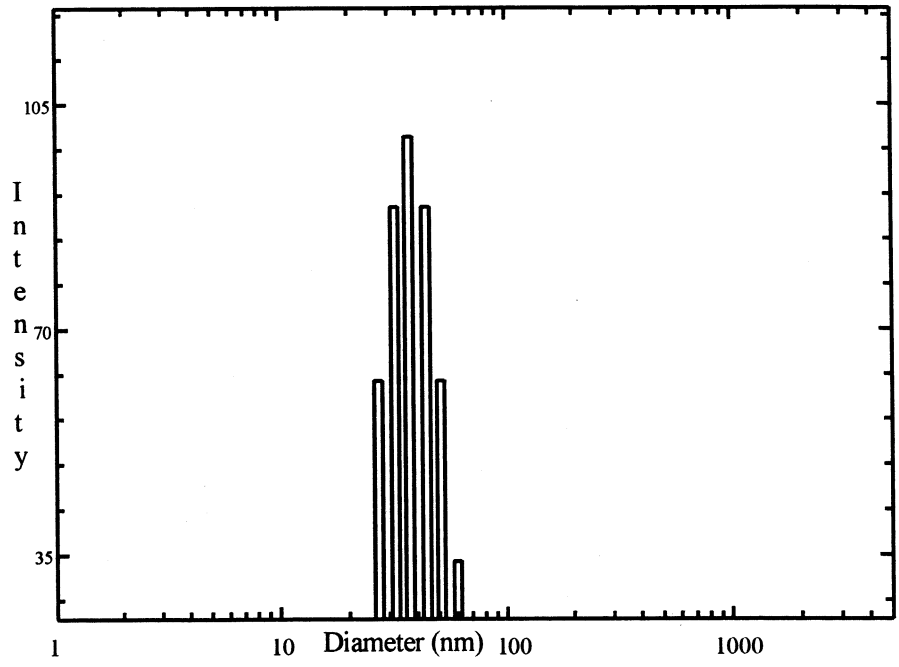

Fig. 4. Typical size distribution of NIPAAM-VP-AA copolymeric micelles by dynamic light scattering measurement.

to sufficient amount of drug releasing out of the particles. This shelf-life for different amounts of ketorolac loaded nanoparticles was noted and the results are shown in Table 1. The shelf life was studied with up to 30% of ketorolac loaded into the nanoparticles. The table shows that while 5% w/w loading keeps the solution clear for more than 15 days, a solution containing nanoparticles of 30% loading gives turbidity within 24 h. This shows that ketorolac entrapped into the nanoparticles can not be used as aqueous solution for a very long time and has to be kept as a lyophilised powder.

3.4. In vitro release kinetic studies

3.4.1. Effect of pH

The in vitro release profiles of the loaded ketorolac from poly(NIPAAM-VP-AA) micelles at pH 5.0, 7.2 and 10.0 were determined at 25°C and the results obtained are shown in Fig. 7. It is evident from the figure that the release rate was slow at acidic pH as compared to that in the alkaline pH and the release rate was found to increase exponentially with time in alkaline pH. About 40% of drug was released within initial 2 h in pH 10.0 followed by gradually slow release for the next 4 h, giving rise to about 75% release of

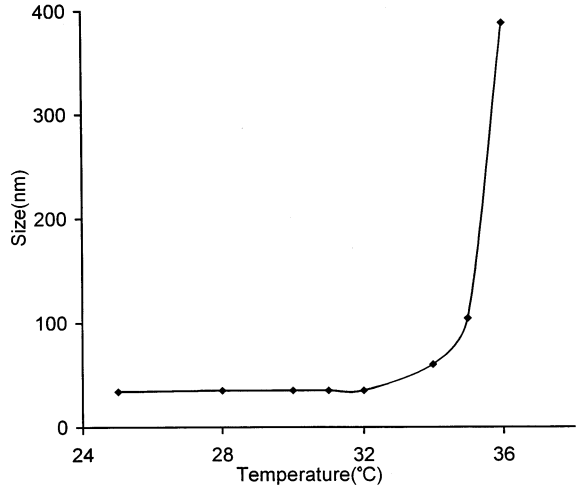

Fig. 5. LCST plot of particle size (nm) vs. temperature (°C) showing diameter change of NIPAAM-VP-AA copolymeric micelles as a function of temperature.

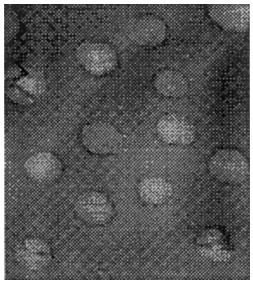

Fig. 6. Transmission electron microscope picture of ketorolac loaded NIPAAM-VP-AA copolymeric micelles (scale: 1 cm = 50 nm).

total drug beyond which the trend falls perhaps due to insolubility of drug. At pH 7.2, only 10% of the drug is released in 1 h and it takes about 6 h to release about 50% of the drug. The release is extremely slow at pH 5.0. Ketorolac free acid is insoluble in water, but as the pH of the solution is increased, the free acid is turned into salt and its solubility is increased. As a result, the release of ketorolac from the polymeric micelles becomes faster. Thus, in a given time, the release of ketorolac from the micelles is faster in alkaline medium than that in the acidic medium. Another important parameter, which synergies the release kinetics in different pH solutions, is the swelling behaviour of the polymer. One of the most important characteristics of the present copoly(NIPAAM-VP-AA) micelles is their ability to ionise/deionise at different pH because of the acrylic acid moiety in the polymer backbone that makes it a pH-sensitive polymer (Dong et al., 1992). The key to the controlling of the pores of the particles include both overall -COOH content and whether the -COOH groups are ionised. At acidic pH, the carboxylic groups are not ionised and the particles are less solvated. The -COOH groups exhibit inter-chain hydrogen bonding creating a tighter overall networks and hence the pore size becomes smaller. Consequently, due to small pore size, the release of drug from the micelles is quite slow. At alkaline pH, -COOH groups becomes ionised, and due to repulsion among the -COOH groups swelling takes place. As a result of swelling, pore size of the polymer increases and the drug release becomes faster.

3.4.2. Effect of temperature

The release of ketorolac was studied at the temperatures below and above the lower critical solution temperature (LCST) of the polymer (Fig. 8). At 25°C ($T < LCST$), the release of the drug was much slower and only 60% of drug is released in 8 h, while nearly 75% w/w of the drug is released from the polymeric micelles in 1 h at

Table 1
Shelf-life studies of drug loaded polymeric micelles with varying amount of ketorolac

Time (h)	w/w % of ketorolac dissolved in aqueous solution of polymer					
	5	10	15	20	25	30
1	Clear	Clear	Clear	Clear	Clear	Clear
2	Clear	Clear	Clear	Clear	Clear	Clear
3	Clear	Clear	Clear	Clear	Clear	Clear
4	Clear	Clear	Clear	Clear	Clear	Clear
5	Clear	Clear	Clear	Clear	Clear	Clear
6	Clear	Clear	Clear	Clear	Clear	Clear
24	Clear	Clear	Clear	Clear	Clear	Turbidity
48	Clear	Clear	Clear	Clear	Clear	–
72	Clear	Clear	Clear	Clear	Clear	–
96	Clear	Clear	Clear	Clear	Clear	–
120	Clear	Clear	Clear	Clear	Clear	–
144	Clear	Clear	Clear	Clear	Clear	–
168	Clear	Clear	Clear	Clear	Clear	–
192	Clear	Clear	Clear	Clear	Clear	–

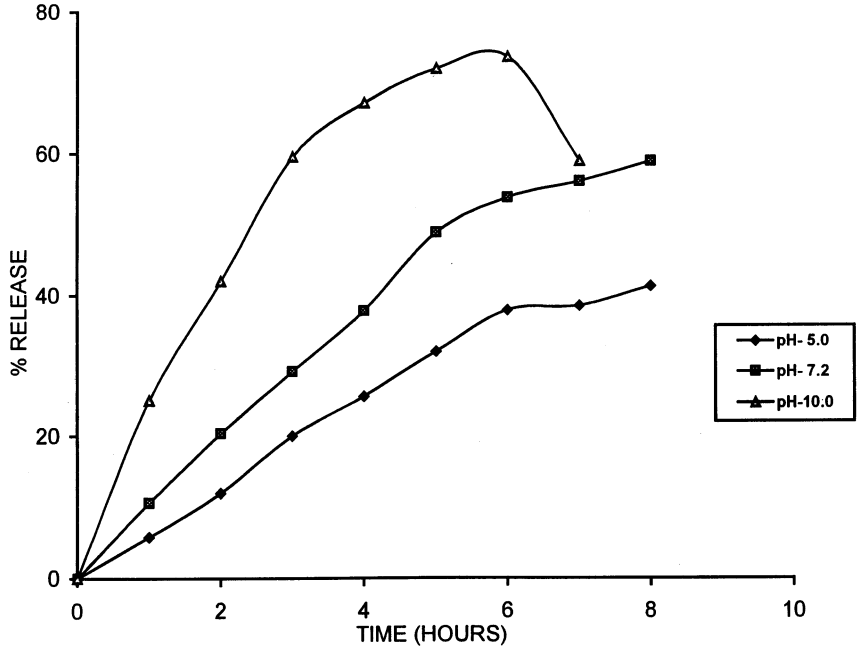

Fig. 7. pH dependent release profile of ketorolac from NIPAAM-VP-AA copolymeric micelles at 25°C.

37°C (T > LCST). The release of drug beyond this time is erratic and the trend is downward probably due to insolubility of the drug in aqueous solution. Therefore, the drug released from the micelles shows dramatical on/off switch upon heating through LCST.

It has already been demonstrated (Chung et al., 1998) that for thermosensitive polymers, strong structural changes takes place when the temperature of the polymer solution is increased through LCST. This structural change above LCST of the present copolymeric micelles accelerate the release of ketorolac at 37°C. At lower temperature particularly below LCST, the inner core of the micelles is hydrophobic while the outer shell is more hydrophilic resulting a decrease of release rate of ketorolac at 25°C from the polymeric micelles.

3.5. Transcorneal permeation studies

Transcorneal permeation studies through excised rabbit cornea indicated about 2-fold increase in drug permeation from nanoparticles formulation compared to that of an aqueous suspension of ketorolac of same concentration (Table 2). This interesting behaviour is thought to be due to slow dissolution of ketorolac from the suspended crystalline material in aqueous buffer and subsequent

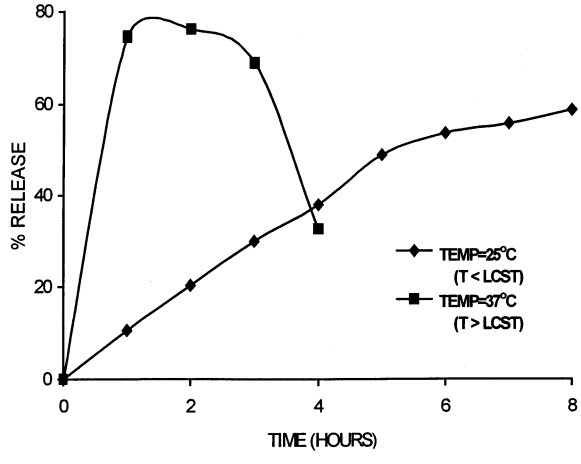

Fig. 8. Temperature dependent release profile of ketorolac from NIPAAM-VP-AA copolymeric micelles in pH 7.2 buffer solution.

Table 2
Comparison of permeation of keterolac from NIPAAM-VP-AA copolymeric micelle formulation with aqueous suspension of drug of same concentration (0.7 mg/ml) through rabbit cornea

Formulation	Cumulative amount of keterolac permeated (mg ± S.E.) values in parentheses indicate % permeation		Corneal hydration (%)
	Time (min) 60 min	120 min	
Polymeric micellar formulation	0.04896 ± 0.00094 (6.9942%)	0.06576 ± 0.00089 (9.3942%)	80.19 ± 0.199
Aqueous suspension of keterolac	0.02736 ± 0.00087 (3.9085%)	0.03624 ± 0.00092 (5.1771%)	79.41 ± 0.0899

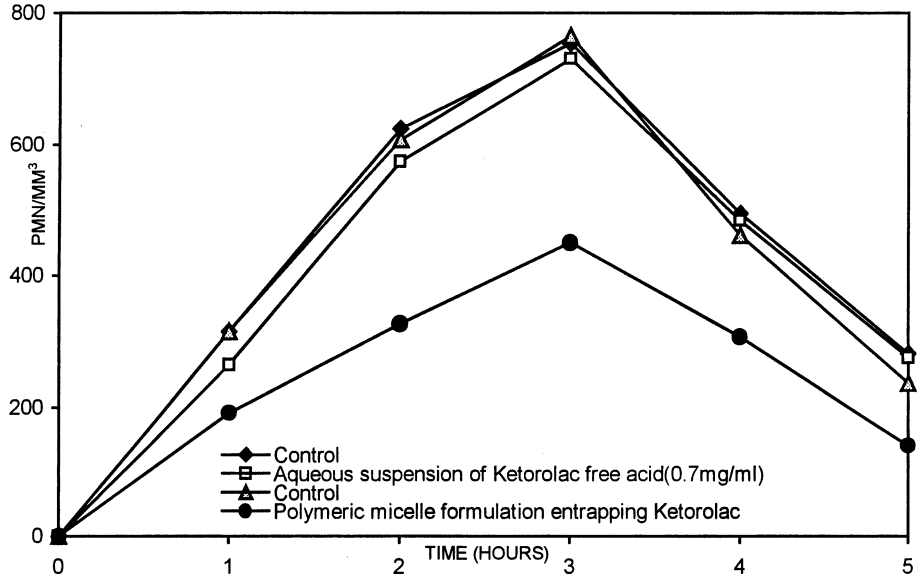

Fig. 9. Comparison of effect of ketorolac loaded NIPAAM-VP-AA copolymeric micelle formulation with aqueous suspension of drug on PGE_2 Induced PMN migration in tears of rabbit.

permeation of dissolved drug through the corneal membrane. In case of nanoparticles, the entrapped ketorolac is hardly in the crystalline form as revealed from the XRD spectrum. Therefore, the release of ketorolac from the nanoparticles and subsequent dissolution in aqueous buffer is much faster. Moreover, the membrane uptake of the ultra low size ketorolac-entrapped nanoparticles can not be ruled out. Higher concentration of drug in aqueous solution would saturate the corneal epithelium quickly followed by fast diffusion of drug to stroma and endothelium. Corneal hydration level was found to be between 79 and 80% indicating no corneal damage has taken place due to addition of ketorolac formulation (Maurice and Riley, 1970).

3.6. In vivo ocular anti-inflammatory activity

Topical instillation prostaglandin (PG) induces ocular inflammation and polymorpho-nuclear leukocytes (PMN) migration in tear fluid (Srinivasan and Kulkarni, 1980). Hence PGE_2 induced lid closure and PMN migration in rabbit were

Table 3
Comparison of effect of ketorolac loaded NIPAAM-VP-AA copolymeric micelle formulation with aqueous suspension of drug of the same concentration on PGE_2 induced lid closure in the rabbit eye

Formulation	30 min	1 h	2 h	3 h	4 h	5 h
Control	1.75 ± 0.25	1.75 ± 0.25	1.66 ± 0.2974	0.5 ± 0.25	0.5 ± 0.2165	0
Aqueous suspension of ketorolac free acid (0.7 mg/ml), $N = 4$	1.66 ± 0.202	1.66 ± 0.202	1.66 ± 0.2974	0.5 ± 0.25	0.5 ± 0.2165	0
Control	1.75 ± 0.25	1.75 ± 0.25	1.75 ± 0.25	1.5 ± 0.25	1.75 ± 0.25	0
Polymeric micellar formulation, $N = 4$	0.5 ± 0.25	0.5 ± 0.25	0.5 ± 0.25	0.5 ± 0.25	0	0

used to evaluate anti-inflammatory effect of nanoparticle formulation of ketorolac. The results as shown in Fig. 9 indicated that the lid closure was prominent up to 3 h after which it subsided. The lid closure score was found to be more in all control eyes as compared to the eyes treated with nanoparticles formulations. Also the lid closure in case of eyes treated with nanoparticle formulations was very less compared to that observed with aqueous suspension of ketorolac of same concentration (Table 3). PMN counts in the tears of rabbit increased up to 3 h and afterward it decreased. In case of nanoparticle formulations, the PMN counts were observed to be less than the control throughout the 5-h study. The percent inhibition of PMN migration with nanoparticle formulations was found to be much higher and longer lasting than that observed with aqueous suspension containing equivalent amount of ketorolac. The results suggest enhanced residence time of the drug on the ocular surface as well as sustained release of drug from nanoparticles.

4. Conclusions

These copolymeric nanoparticles composed of NIPAAM, VP and AA are biocompatible and do not cause any corneal damage. Corneal penetration of ketorolac from nanoparticles was much higher compared to aqueous suspension of drug of equivalent concentration. The formulation also shows much higher anti-inflammatory activity for longer duration compared to that of aqueous suspension of drug. This could be attributed to the ultra small size (< 50 nm diameter) of the polymeric micelles as well as their mucoadhesiveness.

Acknowledgements

The authors (AKG and ANM) are grateful to the Department of Biotechnology, Government of India, New Delhi, for financial assistance in the form of a research project.

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