Thermo-responsive Poly (Vinylcaprolactam-co-Vinylacetate) Nanospheres for Controlled Release of Atenolol

C.S.Rajesh[1], J.N.Suntiha[2], K.S.Rai[3]

Department of Studies in Polymer Science, University of Mysore, Mysore, Karnataka, India

Abstract-Thermo-responsive nanospheres of poly(N-vinylcaprolactam-co-Vinyl acetate) (Poly(NVC-co-VAc)) have been prepared by free radical emulsion polymerization. These nanospheres have been characterized by differential scanning calorimetry (DSC), x-ray diffraction (x-RD), Particle size analyzer, Scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Atenolol, an anti-hypertensive drug, was successfully loaded into these nanospheres during in situ polymerization. DSC and x-RD analyses of the drug-loaded and plain nanospheres have confirmed the uniform molecular dispersion of Atenolol in the nanospheres. Particle size analyzer, SEM and TEM of the particles have shown the formation of spherical particles with a size of 200 nm. In vitro drug dissolution studies have been performed at 250C and 370C to investigate the temperature-dependent drug release characteristics from Poly(NVC-co-VAc) nanospheres. Nanospheres of this study have exhibited a prolonged release of Atenolol for more than 16 h. Drug release profiles of Poly(NVC-co-VAc) nanospheres at different temperatures confirmed that the nanospheres formed are thermo-sensitive, resulting in a pulsatile release of the drug during in vitro dissolution experiments. PNVC domains of the nanospheres seemed responsible to control the release of Atenolol.

Keywords-N-Vinyl caprolactam, Vinyl acetate, Atenolol, Controlled Drug Delivery.

I. INTRODUCTION

In recent years, pharmaceutical research has led to the development of novel drug delivery systems that possess several inherent advantages compared to conventional dosage formulations. This has prompted renewed experimental activities on the development of novel polymeric devices that can release drugs in a controlled manner under different environmental conditions. Particularly, intense research concerning the application of stimuli-responsive polymers as novel drug delivery systems has taken place over the past decades [1, 2]. This is due to their ability to swell or collapse in response to external factors such as pH, temperature, ionic strength, electric field and presence of chemical compounds, during which time, drug release occurs at a specific site over an extended or fixed time period in a controlled manner [3-9]. Such systems include the temperature-sensitive coatings, [10, 11] smart catalysts, [12, 13] pervasive membranes [14], etc. Majority of thermo-responsive polymers dissolve in cold water, but collapse and precipitate upon heating in aqueous solutions above their lower critical solution temperature, LCST. Upon crosslinking, they can reversibly shrink, particularly when heated above the critical temperature.

Over the past few decades, there has been a considerable interest in developing biodegradable nanoparticles (NPs) as the effective drug delivery devices. Various polymers have been used in drug delivery research, since these can effectively deliver the drug to a target site and thereby, increase the therapeutic benefit, while minimizing the side effects [15]. In view of their importance, pharmacists have produced a wide spectrum of polymer network structures that are suitable for drug encapsulation and to deliver drugs in a controlled manner. Nanoparticles vary in the size range from 10 to 1000 nm. Drug is dissolved, entrapped, encapsulated or attached to a nanoparticulate matrix, depending upon the method of their preparations and the method of drug encapsulation employed. Nanocapsules, on the other hand, are vesicular systems in which drug is confined to a cavity surrounded by a unique type of polymer membrane, while nanospheres are the matrix systems in which drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles have attracted a considerable attention as the potential drug delivery devices in view of their applications in the controlled release (CR) of drugs. Ability to target particular organs/tissues, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes through a peroral route of administration [16, 17]. In the earlier literature, thermo-responsive core-shell nanoparticles were prepared with N-isopropylacrylamide and methacrylic acid, methyl methacrylate, styrene, poly(D,L-lactide) for the delivery of short-lived drugs [18,19].

N-vinyl caprolactam (NVCL) has been known to possess noteworthy properties for biomedical applications, e.g., solubility in water and organic solvents, high absorption ability, and a transition temperature within the setting of these applications (330C) [20]. Even though the LCST of PNVCL solutions are close to PNIPAM, however, there

are significant differences in the thermodynamic and molecular mechanisms underlying the phase transition. In contrast to PNIPAM, PNVCL possesses a classical Flory–Huggins thermoresponsive phase diagram. The phase transition behavior depends on the molecular weight of PVCL and the solution concentration [21,22]. This unique feature allows for controlling the temperature sensitivity of the polymer by varying its molecular weights. There are two stages in the phase transition of PNVCL in aqueous solution. The hydrogen bonding transformation is predominant at the first transition stage below the LCST and hydrophobic interaction is predominant at the second stage above the LCST [23]. A "sponge-like" structure may be formed for PVCL mesoglobules due to the absence of topological constrains as well as self-associated hydrogen bonds that could further continuously expel water molecules upon increasing temperature [21]. When the polymer chain length or polymer concentration is increased, the LCST of PNIPAM and PNVCL decreases [24, 25]. In general, hydrophobic compounds decrease the LCST, whereas hydrophilic and charged compounds increase LCST of polymers due to the strong interactions between water and hydrophilic or charged groups [26]. LCST disappears when those polymers

contain too many hydrophilic comonomers [25]. Salts are known to lower the LCST of PNIPAM and PNVCL [24,26–28]. Moreover LCST of PNVCL is known to decrease by addition of a small amount of alcohol [25]. Anionic surfactants usually prevent phase separation of PNVCL solutions when heating, while the hydrophobic interactions of anionic and cationic surfactants would lead them to bind to PNVCL. The behavior of PNVCL looks like a polyelectrolyte upon binding of the surfactant which means the polymer coil swells. As the surfactant concentration increases, the transition temperature increases [29]. Lau and Wu reported that the phase transition temperature of PNVCL depends on the molecular weight of the polymer [30]. Tager et al. showed for PNVCL with Mw =5*105 g/mol the change of the phase transition temperature from 320 C to 340 C depending on the concentration of the polymer in solution [31]. Meeussen et al. estimated the phase diagrams for polymers with different molecular weights. Moreover, cloud point measurements and theoretical calculations of PNVCL were carried out [20]. On the other hand, the lack of popularity among researchers for PNVCL compared to PNIPAM in previous years has likely been due to the affinity of polymerization of NVCL in a controlled manner because the polymerization kinetics is very difficult to measure. Unlike PNIPAM, the hydrolysis of PNVCL would not produce small amide compounds that are unwanted in biomedical applications [24].

Despite this, PNVCL have attracted attention for use in the biomedical field by considering the excellent properties such as biocompatibility [32]. This unique feature together with its overall low toxicity, high complexing ability and film forming properties enables its use in many industrial and medical applications, in particular in the biomedical field [23,24]. So far, various types of PNVCL based temperature responsive carriers such as micelles and vesicles have been reported and used for drug delivery applications [33–36].

Atenolol, a model drug used in this study, is a polar cardio selective □-blocker, widely used alone or in combination, to treat angina or hypertension. Administration of Atenolol conventional tablets in 100 mg/day doses are known to cause fluctuations in plasma concentrations, resulting in side effects or reduction in drug concentration at the receptor sites. Atenolol is a □-adrenergic receptor blocking agent without the membrane stabilizing or intrinsic sympathomimetic activities. It has been used for the treatment of hypertension, either alone or with other antihypertensive drugs, such as thiazide diuretics. It has been reported that during its oral administration, it can induce side effects such as diarrhea, nausea, ischemic colitis, and mesenteric arterial thrombosis, since it has been used in other CR applications [37, 38].

The principal objective of the present paper is to develop Poly(N-Vinylcaprolactam-co-vinyl acetate) copolymeric nanospheres with improved temperature sensitivity over small temperature cycles between 250 to 370C. Nanospheres were prepared by emulsion polymerization using sodium lauryl sulfate (SLS) as a surfactant by varying the amount of Atenolol, N, N'-methylene bisacrylamide (NNMBA) as well as N-Vinylcaprolactam (NVC) content of the copolymer. The prepared formulations have been examined for the CR of Atenolol. In order to optimize the performance of stimuli-sensitive systems, a thorough understanding of the phase transitions is essential. In general, the hydrogel phase transitions are characterized through the pulsatile swelling trends. In this study, parameters like % encapsulation efficiency, drug loading, effect of crosslinking agent, etc., have been studied in vitro in terms of drug release profiles to understand the pulsatile release characteristics of the nanospheres developed.

II. MATERIAL AND METHODS

2.1. Materials

Vinylcaprolactam (NVC) was purchased from Aldrich Chemicals, Milwaukee, WI USA. Vinyl acetate (VAc), N, N□-methylene bisacrylamide (NNMBA), sodium laurylsulfate, potassium persulfate and calcium chloride were all purchased from s.d. fine chemicals, Mumbai, India. Atenolol was purchased from MP Biochemicals, Eschwege, Germany.

2.2. Preparation of Poly (N'-Vinylcaprolactam-co-Vinyl acetate) P(NVC-co-VAc) Nanospheres

Sodium lauryl sulfate (SLS) (1 g) and potassium persulfate (100 mg) were dissolved in 100 ml of water taken in a 250 ml round bottom flask equipped with a reflux condenser and a nitrogen inlet. Different amounts of vinyl acetate (i.e., 40, 60 and 80 wt. %), N-Vinylcaprolactam (i.e., 20, 40 and 60 wt. %), N,N□-methylene bisacrylamide (2, 4 and 6 wt.%) and Atenolol (i.e., 5, 10 and 15 wt. %) were added to SLS solution. The mixture was bubbled with nitrogen gas for 45 min, heated to 700C and stirred well at the rotation speed of 800 rpm for 10 h. At the end of the reaction, the mixture was taken out, cooled to room temperature and transferred to a beaker containing 5 wt. % of calcium chloride solution to break the emulsion. Polymeric nanospheres obtained were isolated by centrifuging the broken emulsion at 14,000 rpm speed for about 15 min and the nanospheres were dried under vacuum at 400C. The prepared nanospheres were purified by washing with water and shaken thoroughly with a shaker for 1 h to remove any excess of SLS and the crosslinking agent. The unreacted monomer present in the nanospheres, dissolved in calcium chloride solution and the polymeric nanospheres were isolated by centrifuging the mixture. The product was again washed with several times with water to remove the unreacted monomer, crosslinking agent, SLS, and isolated nanospheres were dried under vacuum.

2.3. Conversion of Copolymer

The yield of copolymeric nanospheres was determined gravimetrically. After copolymerization, the latex solution was added to 1 % calcium chloride solution and centrifuged to isolate the particles from the mixture. The copolymeric nanospheres were washed several times successively with water and methanol solvents to remove the remaining monomer and initiator, and then dried in a vacuum oven at 500C until attainment of constant weight. The % conversion of monomers was calculated as:

$$\% \text{ Conversion} = (W/M) \times 100 \quad (1)$$

Where W is weight of the dry copolymer obtained from the latex sample and M is weight of the monomers taken. The yield of copolymeric nanoospheres varied between 82 and 87 % for various formulations prepared in this study.

2.4. Differential Scanning Calorimetry (DSC) Studies

Differential scanning calorimetric (DSC) curves were recorded on a Rheometric scientific differential scanning calorimeter (Model-DSC SP, UK). The instrument was calibrated using indium as the standard. Samples were heated in sealed aluminum pans between 300 and 400oC at the heating rate of 10oC/min under inert nitrogen purge gas at the rate of 20 ml/min.

2.5. Scanning Electron Microscopic (SEM) Studies

Morphology of the nanospheres was confirmed by scanning electron microscopy (SEM). Micrographs of the dry nanospheres in powder form, dispersed in acetone, were all recorded using Leica 400, Cambridge, UK instrument.

2.6. Transmission Electron Microscopy

TEM images of Poly(NVC-co-VAc) emulsion and dry particles were recorded using a Technai-12 transmission electron microscope at acceleration voltage of 100 kV. Particles were dispersed in water by sonication. The dispersion was placed on the copper grid and allowed to dry at room temperature.

2.7 Particle Size Analysis

Particle size of the nanospheres was measured by using a particle size analyzer (Mastersizer 2000, Malvern Instruments, UK). About 500 mg of nanospheres were transferred to the dry sample holder and stirred vigorously to avoid the agglomeration of particles during measurements. For measurement of sizes of different formulations/batches, the sample holder was cleaned by vacuum. The particle size was also measured using an optical microscopy as shown in Fig.5.

2.8. Estimation of Drug Loading and Encapsulation Efficiency

Loading efficiency of Atenolol in the nanospheres was determined spectrophotometrically. About 10 mg of the drug-loaded nanospheres were placed in 10 ml of buffer solution and stirred vigorously for 48 h to extract the drug from the nanospheres. The solution was filtered and assayed by UV spectrophotometer (model Anthelie, Secomam, Dumont, France) at the fixed λ_{max} value of 224.20 nm. The results of % drug loading and encapsulation efficiency were calculated, respectively using Equations. (1) and (2). These data are compiled in Tables 1.

$$\% \text{ Drug loading} = \left(\frac{\text{Amount of drug in beads}}{\text{Amount of beads}}\right) x100 \quad (3)$$

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) x100 \quad (4)$$

2.9. In-vitro Release Study

Dissolution was carried out using Tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 370C under 100 rpm speed. Drug release from the nanospheres was studied in 7.4 pH phosphate buffer solution. Aliquot samples were withdrawn at regular time intervals and analyzed by UV spectrophotometer as explained before.

III. RESULTS AND DISCUSSION

4.1. Differential Scanning Calorimetry (DSC)

DSC thermograms of the plain ATNL, ATNL-loaded Poly(NVC-co-VAc) nanospheres and plain Poly(NVC-co-VAc) nanospheres are displayed in Fig.1. Melting point of ATNL was observed at 1570C, but its peak was not observed in the ATNL-loaded nanospheres and plain nanospheres, suggesting that ATNL was molecularly dispersed in the polymeric matrix.

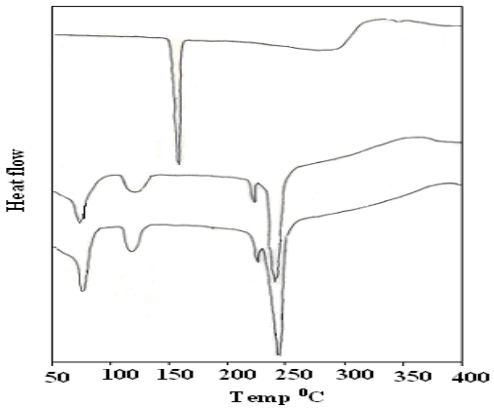

Fig.1. DSC Thermograms of (a) Atenolol, (b) plain poly(NVC-co-VAc) Nanospheres and (c) Atenolol-loaded-poly(NVC-co-VAc) Nanospheres.

4.2. X-ray Diffraction Studies (X-RD)

Figure.2 shows the X-ray diffractograms of (a) placebo ATNL, (b) ATNL-loaded Poly(NVC-co-VAc) nanospheres and (c) pristine Poly(NVC-co-VAc) nanospheres. ATNL has shown characteristic intense peaks at 2θ of 3^0, 6^0, 10^0, 21^0 and 23^0, suggesting its crystallinity, but only peaks observed in the placebo matrices are observed in the ATNL-loaded Poly(NVC-co-VAc) nanospheres. This suggests that ATNL is dispersed at a molecular level within Poly(NVC-co-VAc) nanospheres, since no indication of the crystalline nature of ATNL was observed in the ATNL-loaded Poly(NVC-co-VAc) nanospheres.

Fig.2. X-ray diffraction curves of plain (a) Atenolol, (b) Atenolol loaded- poly(NVC-co-VAc) and (c) plain poly(NVC-co-VAc) nanospheres.

4.3. Scanning Electron Microscopic (SEM) Studies

Morphology of the nanospheres was confirmed by scanning electron microscopy (SEM) in Figure.3. The formed copolymer particles are spherical with the diameters of around 200 nm.

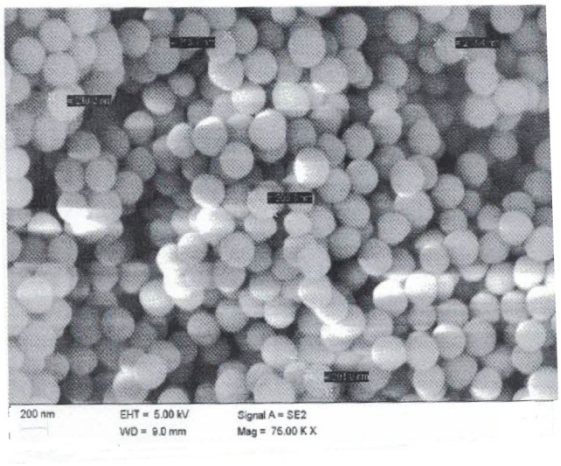

Fig.3. Scanning electron micrographs of poly(NVC-co-VAc) nanospheres

4.4. Transmission Electron Microscope (TEM)

Figure.4 shows the transmission electron micrographs of Poly (NVC-co-VAc) nanospheres. The Poly (NVC-co-VAc) particles are spherical in shape with sizes ranging from 170-200 nm.

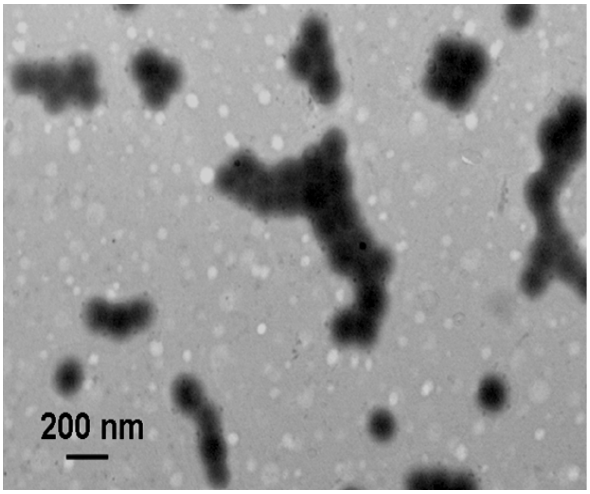

Fig.4. Transmission electron micrographs of poly(NVC-co-VAc) nanospheres

4.5 Particle Size Analysis

Results of the mean particle size with standard errors are presented in Table 1, while the size distribution curve for a typical formulation containing NVCVAc-6 is displayed in Figure 5. It is found that size distribution is broad and volume mean diameter of the particle is around 200 nm.

Particle size of different formulations containing different The particle size decreased with increasing amount of crosslinking due to formation of a rigid structure due to a reduction in chain length of the polymer formed.

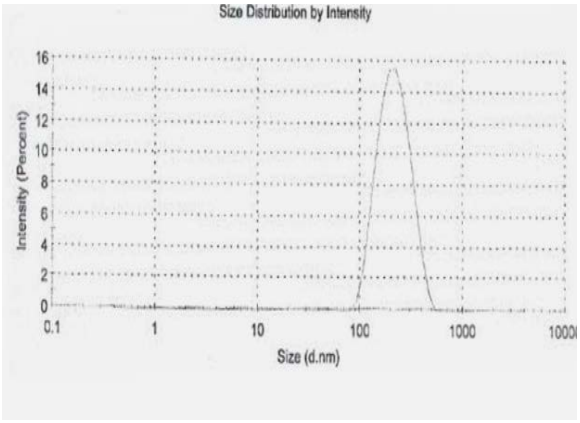

Fig.5. Particle size distribution curve of Poly (NVC-co-VAc) nanospheres.

4.6. Encapsulation Efficiency

Three different concentrations of ATNL (i.e., 5, 10 and 15 wt. %) were loaded during in situ polymerization. Results of % encapsulation efficiency with the standard deviation are included in Table .1. For nanospheres containing 30 wt. % of NVC and 8 wt. % of NNMBA, encapsulation efficiencies were 55, 59 and 64 % for formulations containing 5, 10 and 15 wt. %, of ATNL. The highest % encapsulation efficiency of 70 % was observed for NVCAc-4 formulation, which contained 3 % NNMBA, 30% NVC and 5 % ATNL. For nanospheres containing 30 % NVC and 5 % ATNL, the effect of extent of crosslinking on entrapment efficiency of the nanospheres was studied (e.g., formulations NVCAc-5, NVCAc-6 and NVCAc-7), wherein it was found that with increasing crosslinking, % encapsulation efficiency decreased. For nanospheres crosslinked with 3, 6 and 8 wt. % of NNMBA, the entrapment efficiencies were 70, 58 and 54 %, respectively. This type of decrease in entrapment efficiency is due increased crosslinking density, at which the nanospheres would be rigid, thereby reducing the free volume spaces within the polymer matrix; this might have resulted in a reduction of encapsulation efficiencies. The % encapsulation efficiencies of the nanospheres containing 30, 50 and 70 wt. % of NVC with 5 wt. % of ATNL when crosslinked with 8 wt. % NNMBA were 58, 54 and 51, respectively. Such a decrease in encapsulation efficiency with an increase in NVC content of the nanospheres might due to the lesser interaction of the hydrophobic Atenolol drug molecules with N-Vinylcaprolactam.

Table.1. Formulation Details, particle size and % Encapsulation Efficiency Data of Poly (NVC-co-VAc) Nanospheres

Formulation code	Amount of NVC (wt. %)	Amount of NNMBA (wt. %)	Amount of ATNL (wt%)	% Encapsulation efficiency ± SD	diameter (nm) ± SD
NVCVAc-1	30	8	5	51 ± 1	202 ± 6
NVCVAc-2	30	8	10	55± 2	205 ± 8
NVCVAc-3	30	8	15	61 ± 1	210 ± 6
NVCVAc-4	30	3	5	67 ± 1	235 ± 4
NVCVAc-5	30	6	5	56± 1	225 ± 6
NVCVAc-6	50	8	5	52 ± 2	204 ± 4
NVCVAc-7	70	8	5	50 ± 2	210 ± 1

4.7. In-vitro Release Study

4.7.1. Effect of Drug Concentration

Figures 6a and 6b display the release profiles of P(NVC-co-VAc) microspheres that are loaded with different amounts of ATNL at 250 and 370C, respectively. Notice that initially, during the first hour, the release is quite fast in all the formulations, but later it slowed down. Release data suggest that those formulations containing a highest amount of drug (i.e., 15 wt. %) displayed the higher release rates than those containing smaller amounts of ATNL (i.e., 10 and 5 wt. %). A prolonged and slow release was observed for formulation containing a lower amount of ATNL (i.e., 5 wt. %) at 370C; this is due to the within of large free volume spaces available in the matrix through which, a lesser number of ATNL molecules would transport. Notice that for all the ATNL-loaded formulations, the complete release of ATNL was not observed even after 960 min, since the % cumulative release data tend to increase continuously.

Fig.6. % Cumulative release of Atenolol at 250C (a) and 370C (b) through poly(NVC-co-VAc) nanospheres

crosslinked with 8 % NNMBA and 30 % NVC containing (●) 5 %, (■) 10 % and (▲) 15 % of Atenolol.

4.7.2. Effect of Crosslinking Agent

The % cumulative release data vs time plots for the nanospheres prepared with 3, 6 and 8 wt. % of NNMBA with a fixed amount of ATNL (5 wt. %) at 250 and 370C are displayed in Fig.7a and 7b, respectively. The % cumulative release is quite fast and large at lower amount of NNMBA (i.e., 3 wt. %), but the release became slow at higher amount of NNMBA (i.e., 8 wt. %). The cumulative release is somewhat smaller when a higher amount of NNMBA was present in the matrix because at the higher concentration of NNMBA, polymeric chains became more rigid due to the contraction of microvoids, thereby giving a decrease in % cumulative release of ATNL. The crosslinking agent could help to form a bridge between the copolymeric chains. Therefore, as expected, the drug release becomes slower at the higher amount of NNMBA, but it will be faster when a lower amount of NNMBA is present in the matrix at both 250C and 370C.

Fig.7. % Cumulative release of atenolol at 250C (a) and 370C (b) through poly(NVC-co-VAc) nanospheres loaded with 5% atenolol and 20% NVC containing (●) 3 %, (■) 6 % and (▲) 8 % of NNMBA.

4.7.3. Effect of PNVC

Drug release profiles from the nanospheres containing different amounts of NVC at 250 and 370C are displayed in Fig.8a and 8b, respectively. A systematic increase in % cumulative release is observed with increasing amount of PNVC of the nanospheres, but the time, required in releasing ATNL remained almost the same for all the compositions of PNVC.

Fig.8. % Cumulative release of atenolol at 250C (a) and 370C (b) through poly(NVC-co-VAc) nanospheres loaded with 5 % atenolol and crosslinked with 8 % NNMBA containing (■) 70 %, (●) 50 % and (▲) 30 % of NIPA.

The reason for this effect could be that, during the process of dissolution, a general trend is observed in all formulations i.e., nanospheres have shown a systematic increase in swelling with increasing amount of NVC, due to the loosely crosslinked hydrophilic chains of PNVC. Microscopically speaking, there is a relaxation response of the polymer chains due to stresses that are induced during the drug dissolution stage through the nanospheres, resulting in an increased dimension (radius of gyration) of the polymer coil and subsequently, in a significant increase of molecular volume of the overall hydrated polymer matrix due to an increased swelling of PNVC component of the copolymer. This will further reduce the free volume of the matrices. Notice that the nature of release profiles remained almost identical for all the matrices containing different amounts of PNVC, indicating that swelling of PNVC has a linear relationship with their release profiles.

4.5.4. Effect Temperature

Release profiles of ATNL from P(NVC-co-VAc) nanospheres prepared with different amounts of the crosslinking agent and drug loadings have been studied at two temperatures in the chosen dissolution medium, alternatively from 250C to 370C and vice versa. Drug release profiles exhibited drastic variations due to changes in temperature from 250 to 370C. It may be noted that

drug was released slowly at 370C i.e., above LCST, but release was much faster at 250C (i.e., below LCST) than at 370C. This is due to the fact that at higher temperature, the surface of nanospheres will shrink, thereby causing the drug to migrate towards the surface of the nanospheres as seen by the initial burst effect during the dissolution experiments (Fig.9 and Fig.10). However, dense surfaces of the nanospheres will prohibit the release of more amount of the drug. At lower temperatures, the already collapsed surface layer will start to re-swell, which will allow the drug to be released after a certain period of time, depending upon the minimum time required for re-swelling of the surface. Thus, the time required for drug release was accelerated as a result of cooling below LCST, which further slowed down upon reheating. Nanospheres of this study were proved to be sensitive to changes in temperature. At 250C (in the swollen state), release rate and total amount of the drug were considerably higher than those found at 370C (in a collapsed state). Drug molecules entrapped inside the polymer network will diffuse out of the nanospheres, since they quickly got hydrated in the swollen state. In contrast, at 370C, the network structure is collapsed and exhibits a lesser tendency to uptake water or buffer solution, leading to a decrease in drug diffusion rate.

Figures.9 and 10 displays the pulsatile cumulative release behaviors of ATNL from poly(N-Vinylcaprolactam-co-vinyl acetate) nanospheres under variable temperature conditions. Pulsatile "on-off" release profile is observed. The error bars are indicated to show a close proximity to the x-axis, suggesting that drug release has effectively stopped at that particular temperature. The pulsatile "on-off" release of the drug is due to the presence of poly(N-Vinylcaprolactam) in the nanospheres, which have the propensity to show a transition from the swollen state to the collapsed state at temperature above 330C. This type of swelling/collapse transition alters the permeability of the network and therefore, these properties of the matrices can be used to switch alternatively the release of the drug in a "on-off" pulsatile manner. Similar findings were observed in the earlier literature [39].

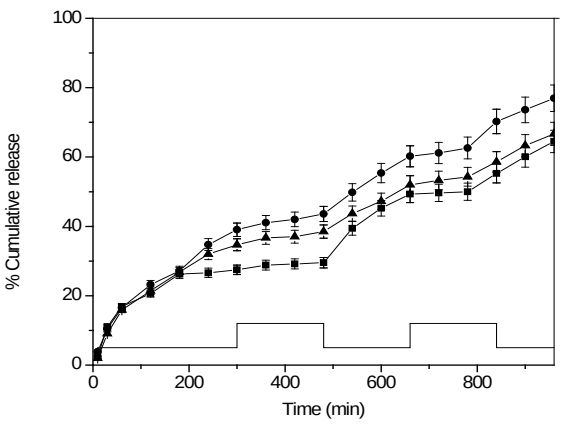

Fig.9. Pulsatile "on-off" cumulative release of Atenolol through poly(NVC-co-VAc) nanospheres crosslinked with 8 % NNMBA and 30 % NIPA containing (■) 5 %, (▲) 10 % and (●) 15 % of Atenolol.

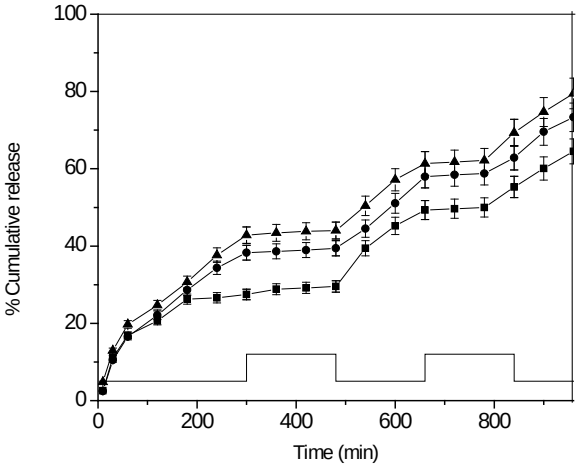

Fig.10. Pulsatile "on-off" cumulative release of Atenolol through poly(NVC-co-VAc) nanospheres loaded with 5 % Atenolol and 30 % NVC containing (▲) 3 %, (●) 6 % and (■) 8 % of NNMBA.

4.8. Drug Release Kinetics

In the area of pharmaceutics, it has been the usual practice to understand the release kinetics of a drug through a polymeric matrix using the empirical relationship proposed by Ritger and Peppas [40]. Following this practice, in the present study, we have analyzed the cumulative release data using the following equation:

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \qquad (1)$$

Here, the ratio, Mt/M∞ represents the fractional drug release at time, t; k is a constant that is characteristic of the drug-polymer system, and n is an empirical parameter characterizing the release mechanism. Using the least-squares procedure, we have estimated the values of n and k for all the nine formulations developed at 95% confidence limit; these data are given in Table.2 at both 250 and 370C. If the values of n = 0.5, then drug diffuses and releases out of the nanosphere matrix following a Fickian diffusion. If n > 0.5, anomalous or non-Fickian transport occurs. For n = 1, non-Fickian or more commonly called Case II release kinetics is operative. The values of n ranging between 0.5 and 1 indicate the anomalous type transport [41].

In the present investigation, the values of k and n have shown a dependence on the extent of crosslinking, % drug loading as well as NVC content of the nanospheres. The values of n for nanospheres, prepared with varying amounts of NVC (i.e., 30, 50 and 70 wt. %) by keeping

ATNL (5 %) and NNMBA (8 %) as constant, ranged from 0.42 to 0.47 and 0.36 to 0.56, respectively at 250 and 370C, suggesting a slight deviation from the Fickian mode of diffusion. The ATNL-loaded nanospheres exhibited the n values ranging from 0.41 to 0.63 and 0.26 to 0.47, respectively at 250 and 370C (see Table.2.), indicating a shift from the erosion type release trend to a swelling-controlled non-Fickian trend. Values of the correlation coefficient, 'r' falls in the range of 0.912 to 0.995 and 0.952 to 0.995 for 250 and 370C, respectively, indicating a good fit of the experimental data. This is due to reduction in the regions of low micro viscosity of the medium and closure of the micro cavities in the swollen nanospheres.

Table .2.Release Kinetics Parameters of Different Formulations at 250 and 370C

Formulation code	*K*	*n*	Correlation coefficient, *r*
25^0C			
NVCAc-1	0.033	0.47	0.944
NVCAc-2	0.013	0.63	0.912
NVCAc-3	0.058	0.41	0.98
NVCAc-4	0.011	0.66	0.945
NVCAc-5	0.037	0.48	0.987
NVCAc-6	0.037	0.47	0.987
NVCAc-7	0.047	0.42	0.995
37^0C			
NVCAc-1	0.021	0.47	0.983
NVCAc-2	0.108	0.26	0.967
NVCAc-3	0.045	0.41	0.992
NVCAc-4	0.109	0.25	0.989
NVCAc-5	0.049	0.38	0.995
NVCAc-6	0.054	0.36	0.974
NVCAc-7	0.017	0.56	0.952

IV. CONCLUSIONS

Novel types of thermo-responsive Atenolol-loaded poly(N-Vinylcaprolactam-co-vinyl acetate) nanospheres were prepared by emulsion polymerization using sodium dodecylsulfate as a surfactant. Atenolol, a hypertensive drug, was chosen as a model drug to investigate the pulsatile "on-off" release pattern using the developed matrices. The nanospheres prepared were characterized by differential scanning calorimetry, x-ray diffractometry, SEM, Particle size analyzer and transmission electron microscopy. DSC indicated that Atenolol is molecularly distributed in the nanospheres, which exhibited a prolonged release of Atenolol over an extended period of time. In the dry state, the size of nanospheres shown by TEM and Particle size analyzer is much smaller (i.e., 170-200 nm). The surface morphology and size of nanospheres by SEM analysis. The nanospheres exhibited a switch "on-off" pulsatile drug release pattern by varying the temperature from 250 to 370C. The prepared nanospheres have thus shown thermo-responsive trends during in vitro drug release studies of Atenolol when dissolution experiments were performed at 250 and 370C.

REFERENCES

[1]. J. Kost, R. Langer, Adv. Drug. Deliv. Rev. 2001, 46,125.
[2]. A.S. Hoffman, Adv. Drug. Deliv. Rev. 2002, 54, 3.
[3]. V.Ramesh Babu, K.S.V. Krishna Rao, M. Sairam, B.V.K. Naidu, K.M. Hosamani, Aminabhavi TM, J. Appl. Polym. Sci. 2006, 99, 2671.
[4]. K. Park, J.R. Robinson, J Control. Rel. 1985, 2, 47.
[5]. Y.H. Bae, T.Okano, S.W. Kim, J. Control. Rel. 1989, 9, 271.
[6]. T. Tanaka, Polymer 1979, 20, 1404.
[7]. D. Jones, L.A. Lyon, Macromolecules 2000, 33, 8301.
[8]. A.Gallardo, G.R. Guez, M.R. Aguilar, M. Fernandez, J.S. Roman, Macromolecules 2003, 36, 8876.
[9]. S.Y. Kim, Y.M. Lee, J Appl. Polym. Sci. 1999, 74, 1752.
[10]. X.S. Wu, A.S. Hoffmann, P. Yager, Polymer 1992, 33, 4659.
[11]. H.G. Schild, R.A. Gaudiana, Prog. Polym. Sci. 1992, 17, 163.
[12]. D.E. Bergbreiter, V.M. Mariagnaman, L. Zhang, Adv Mater 1995,7,69.
[13]. D.E. Bergbreiter, L. Zhang, V.M. Mariagnaman, J Am. Chem. Soc. 1993, 115, 9295.
[14]. Y.M Sun, T.L. Huang, J Membr. Sci. 1996, 110, 211.
[15]. J. Kreuter, Nanoparticles, in: J. Kreuter (Ed.), Colloidal Delivery Systems. Marcel Dekker, New York, 1994, pp. 219–342.
[16]. R. Langer, Acc. Chem. Res. 2000, 33, 94.
[17]. R.P. Lanza, R. Langer, W.L. Chick, Principles of Tissue Engineering, in: Academic Press. Austin. TX, 1997, pp. 405–427.
[18]. C.L. Lo, K.M. Lin, Hsiue GH, J Control Rel 2005, 104, 477.
[19]. S. Cammas, K. Suzuki, C. Sone, Y. Sakurai, K. Kataoka, T. Okano, J Control. Rel. 1997, 48,157.
[20]. Meeussen, F.; Nies, E.; Bergmans, H.; Verbrugghe, S.; Goethals, E.; Du Prez, F. Phase behaviour of poly (N-vinyl caprolactam) in water. Polymer 2000, 41, 8597–8602.
[21]. Sun, S.; Wu, P. Infrared Spectroscopic Insight into Hydration Behavior of Poly(N-vinylcaprolactam) in Water.J. Phys. Chem. B 2011, 115, 11609–11618.
[22]. Jirˇí, S. NMR investigations of phase transition in aqueous polymer solutions and gels. Curr. Opin. Colloid Interface Sci. 2009, 14, 184–191.
[23]. Kermagoret, A.; Fustin, C.-A.; Bourguignon, M.; Detrembleur, C.; Jerome, C.; Debuigne, A. One-pot controlled synthesis of double thermoresponsive N-

vinylcaprolactam-based copolymers with tunable LCSTs. Polym. Chem. 2013, 4, 2575–2583.
[24]. Schild, H.G.; Tirrell, D.A. Micro calorimetric determination of lower critical solution temperature in aqueous polymer solutions. J. Phys. Chem. 1990, 94, 4352–4356.
[25]. Kirsh, Y.E. Water Soluble Poly-N-Vinylamides; John Wiley & Sons: Chichester, UK, 1998.
[26]. Chilkoti, A.; Dreher, M.R.; Meyer, D.E.; Raucher, D. Targeted Drug Delivery by Thermally Responsive Polymers. Adv. Drug Deliv. 2002, 54, 613–630.
[27]. Vihola, H. Studies on Thermosensitive Poly(N-vinylcaprolactam) Based Polymers for Pharmaceutical Applications; University of Helsinki: Helsinki, Finland, 2007.
[28]. Shtanko, N.I.; Lequieu, W.; Goethals, E.J.; Prez, F.E. pH- and Thermoresponsive Properties of Poly(N-vinylcaprolactam-co-acrylic acid) Copolymers. Polym. Int. 2003, 52, 1605–1610.
[29]. Makhaeva, E.E.; Tenhu, H.; Khoklov, A.R. Conformational Changes of Poly(vinylcaprolactam) Macromolecules and Their Complexes with Ionic Surfactants in Aqueous Solution. Macromolecules 1998, 31, 6112–6118.
[30]. Lau, A.C.W.; Wu, C. Thermally Sensitive and Biocompatible Poly(Nvinylcaprolactam): Synthesis and Characterization of High Molar Mass Linear Chains. Macromolecules 1999, 32, 581–584.
[31]. Tager, A.A.; Safronov, A.P.; Berezyuk, E.A.; Galaev, I.Yu. Lower Critical Solution Temperature and Hydrophobic Hydration in Aqueous Polymer Solutions. Colloid Polym. Sci. 1994, 272, 1234–1239.
[32]. Vihola, H.; Laukkanen, A.; Valtola, L.; Tenhu, H.; Hirvonen. Cytotoxicity of thermosensitive polymers poly(N-isopropyl acrylamide), poly(N-vinyl caprolactam) and amphiphilically modified poly(N-vinyl caprolactam). J. Biomater. 2005, 26, 3055–3064.
[33]. Liang, X.; Liu, F.; Kozlovskaya, V.; Palchak, Z.; Kharlampieva, E. Thermoresponsive Micelles from Double LCST-Poly(3- methyl-N-vinylcaprolactam) Block Copolymers for Cancer Therapy. ACS Macro Lett. 2015, 4, 308–311.
[34]. Nakayama, M.; Okano, T.; Miyazaki, T.; Kohori, F.; Sakai, K.; Yokoyama, M. Molecular design of biodegradable polymeric micelles for temperature-responsive drug release. J. Controll. Release 2006, 115, 46–56.
[35]. Akimoto, J.; Nakayama, M.; Okano, T. Temperature-responsive polymeric micelles for optimizing drug targeting to solid tumors. J. Controlled Release 2014, 193, 2–8.
[36]. Liu, F.; Kozlovskaya, V.; Medipelli, S.; Xue, B.; Ahmad, F.; Saeed, M.; Cropek, D.; Kharlampieva, E. Temperature-Sensitive Polymersomes for Controlled Delivery of Anticancer Drugs. Chem. Mater. 2015, 27, 7945–7956
[37]. A.F.J. Kairuz, J.M. Llabot, D.A. R.H. Allemandi, Manzo, Int. J Pharm. 2005, 288, 87.
[38]. J. Jacobsen, J. Control. Rel. 2001, 70, 83.
[39]. D.C. Coughlan, F. P. Quilty, O. I.Corrigan, J Control. Rel. 2004, 98, 97.
[40]. P.L. Ritger, N.A. Peppas, J Control. Rel. 1987, 5, 37.
[41]. S.B. Harogoppad, T.M. Aminabhavi, Macromolecules 1991, 24, 2598.