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A NOVEL APPROACH TO PREPARE ETOPOSIDE LOADED POLY(N-VINYLCAPROLACTAM-CO-METHYLMETHACRYLATE) COPOLYMERIC MICROSPHERES AND THEIR CONTROLLED RELEASE STUDIES

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ABSTRACT

Thermoresponsive Poly N-Vinylcaprolactam -co- Methylmethacrylate designated as Poly (NVCL-co-MMA) copolymeric microspheres crosslinked with N, N– methylene *bis*-acrylamide (NNMBA) have been prepared by dispersion polymerization using varying amounts of NVCL, MMA and NNMBA. Etoposide, an anticancer drug, was loaded into the microspheres during *in situ* polymerization and *in vitro* release of Etoposide has been studied. The microspheres were characterized by Fourier Transform Infrared Spectroscopy (FTIR) Differential Scanning Calorimetry (DSC), X-Ray Diffractometry (X-RD) and Scanning Electron Microscopy (SEM). The release of Etoposide drug from these microspheres was studied in pH 7.4 media, at the temperatures 25^0C and 37^0C. The microspheres consisting of NVCLand MMA provide thermo responsive nature to the microspheres. The system developed in this study showed a thermoresponsive for the controlled release of Etoposide. The DSC and XRD techniques indicated that the uniform distribution of drug in the microspheres and the drug was released in a controlled manner up to 12 h.

KEYWORDS: N-Vinylcparolactam(NVCL), Methylmethacrylate (MMA), Drug delivery, Etoposide, Controlled release.

INTRODUCTION

Thermoresponsive polymers belongs to the class of “smart” materials that have the ability to respond to a change in temperature; a property that makes them useful materials in a wide

range of applications and consequently attracts much scientific interest. Thermoresponsive polymers are used for biomedical applications including drug delivery, tissue engineering and gene delivery.[1-5] Thermoresponsive polymers exhibit a volume phase transition at a certain temperature, which causes a sudden change in the solvation state. Polymers, which become insoluble upon heating, have a so-called LCST. Systems, which become soluble upon heating, have an UCST. LCST and UCST systems are not restricted to an aqueous solvent environment, but only the aqueous systems are of interest for biomedical applications. The change in the hydration state, which causes the volume phase transition, reflects competing hydrogen bonding properties, where intra and intermolecular hydrogen bonding of the polymer molecules are favoured compared to a solubilisation by water.[6-12]

Special interest is focused on the microspheres/nanospheres based on polymers, which have a lower critical solution temperature (LCST) near the physiological one, such as PNIPAM and PNVCL. In the case of PNIPAM- or PNVCL-based nanogels the phase transition of the nanoparticles is observed as a volume phase transition temperature (VPTT), since together with the increase in the hydrophobicity of the polymer chains, the dissociated water molecules are expelled from the micro/nanogel interior, thus causing a decrease in volume occurring above the LCST of the polymer. Although both polymers are suitable for several applications, PNVCL is biocompatible and more useful than PNIPAM. Both PNVCL and PNIPAM are water soluble, nonionic polymers and exhibit a LCST at similar temperatures; however, they differ in the mechanisms and thermodynamics of the phase transition. PNIPAM shows an almost complete independence of the critical temperature on the polymer chain length that is a thermoresponsive phase behavior in water Type II,[13] which means that the cloud point of PNIPAM aqueous solutions is only slightly affected by environmental conditions such as pH or polymer concentration. Contrary to PNIPAM, PNVCL exhibits a ‘‘classical’’ Flory–Huggins thermo-responsive behavior in water (Type I) and, consequently, its LCST value decreases with increasing both the polymer chain length and concentration. Thus, one can easily modify the cloud point of a PNVCL-based thermoresponsive system by controlling the polymer molecular weight, with no requirement of using a comonomer. [14]

PNVCL has a carboxylic and a cyclic amide hydrophilic group and a hydrophobic carbon–carbon backbone chain. The amide group is directly connected to the hydrophobic carbon–carbon backbone chain, so the hydrolysis of PNVCL under strongly acidic conditions, if it occurs, will produce a polymeric carboxylic acid and not a small toxic amide compound,

which is unwanted for biomedical applications, as in the case of PNIPAM. Therefore, the use of PNIPAM as a biomaterial may be limited because of its higher cytotoxicity and its lower cell viability compared to PNVCL.[15] thus its application in drug delivery systems and also in any *in vivo* applications may be extremely restricted.[16] In addition, the biocompatibility of PNVCL-based nanogels was also proved by Imaz and Forcada,[17] concluding their suitability to be used in biomedical applications.

Etoposide is a semi synthetic derivative of podophyllotoxin that exhibits antitumor activity. Etoposide inhibits DNA synthesis by forming a complex with topoisomerase II and DNA. This complex induces breaks in double stranded DNA and prevents repair by topoisomerase II binding. Accumulated breaks in DNA prevent entry into the mitotic phase of cell division, and lead to cell death. Etoposide acts primarily in the G2 and S phases of the cell cycle. It does not interfere with microtubular assembly. It is the first line treatment in patients with small cell lung cancer and indicated for use in combination with other chemotherapeutic agents in the treatment of refractory testicular tumors and to treat other malignancies such as lymphoma, non-lymphocytic leukemia, and glioblastoma multiform.The chemotherapy regimens that utilize etoposide are more effective when the drug is given over an extended period of time.[18, 19]

The main objectives of this paper were the preparation of stable thermoresponsive microspheres from preformed polymers and the study of the influence of physico – chemical characteristics of drug on their release profile. Here the Poly(NVCL-co-MMA) microspheres were prepared as a thermoresponsive polymer with its LCST tailored towards the body temperature. This copolymer was transformed into thermoresponsive stable microspheres by an original approach that assumes the crosslinking of two monomers with methylene bis acrylamide (NNMBA). In this thermoresponsive copolymer Etoposide is an anti cancer drug, was incorporated. Then the *in- vitro* study was carried out in 7.4 pH buffer solution at two different predetermined temperatures (25^0C & 37^0C), the concentration of Etoposide drug release was monitored at 285 nm on an UV spectrophotometer. It was found that Etoposide was released more rapidly at 25^0C than at 37^0C. The plasma a half-life time of Etoposide is about 1–1.2 h, but in the present study, it was possible to extend its release up to 12 h after encapsulation. The microspheres have been characterized by scanning electron microscopy (SEM) to understand their size and surface morphology, respectively. This thermoresponsive

Poly(NVCL-co-MMA) polymeric microspheres may be more useful in the cases where controlled drug delivery system is needed.

EXPERIMENTAL

MATERIALS AND METHODS

Vinylcaprolactam (NVCL) was purchased from Aldrich (Milwaukee, WI, USA). Methylmethacrylate (MMA), *N,-N'*- methylenebisacrylamide (NNMBA), sodium lauryl sulfate, potassium per sulfate and calcium chloride were all purchased from s.d. fine chemicals (Mumbai, India). Etoposide was a generous gift sample from Biological E. Limited, Hyderabad.

Synthesis of thermoresponsive NVCL-co-MMA microspheres

Sodium lauryl sulfate (1g) was dissolved in 75mL of water taken in a three necked round bottom flask equipped with a mechanical stirrer, a condenser and a gas inlet to maintain the inert nitrogen atmosphere. The flask was immersed in an oil bath with a thermostatic control to maintain the desired temperature accurate to ± 0.1^0C. The solution was stirred at 800 rpm speed until it became clear and 100 mg of potassium per sulfate was added. Required amount of NVCL, MMA the crosslinking agent NNMBA and Etoposide were dissolved separately in 25mL of water. This mixture was added to the reaction mixture drop wise using a dropping funnel and the reaction was continued for 8 h at 70^0C to obtain the maximum yield. The reaction mixture was taken out after 8 h and added to 1% calcium chloride solution drop wise to break the emulsion. Particles were then isolated by centrifuging the product at the rotor speed of 12,000 rpm, washed with water and dried under vacuum at 40^0C for 24 h. Eight different batches of formulations were prepared by varying the amounts of monomer, drug and the crosslinking agent. The compositions of all the formulations, *viz.*, NVCL-1 to NVCL-8, prepared in this work are given in Table 1.

CHARACTERIZATION TECHNIQUES

Fourier Transform Infrared (FTIR) Studies

Fourier transform infrared spectroscopy (FTIR) measurements were performed using Perkin Elmer (model Impact 410, Wisconsin, MI,USA) spectrophotometer to confirm the formation of copolymer NVCL-MMA. The copolymer particles are finely grounded with KBr to prepare the pellets under a hydraulic pressure of 600 dynes/m2 and spectra were scanned between 4000 to 400 cm^{-1}.

Differential Scanning Calorimetric (DSC) Studies

Differential Scanning Calorimetry (DSC) curves of the placebo copolymer, plain Etoposide drug and drug loaded copolymer microspheres were recorded using a Rheometric Scientific differential scanning calorimeter (Model-DSC SP, UK).The analysis was performed by heating the samples at the rate of 10^0C/min under inert atmosphere.

X-Ray Diffraction (X-RD) Studies

The X-Ray diffraction (X-RD) patterns of plain drug, plain microspheres and drug loaded microspheres were recorded using a Rigaku Geigerflex diffractometer (Tokyo, Japan) equipped with Ni-filtered CuKα radiation (λ=1.5418A^0). The dried microspheres of uniform size were mounted on a sample holder and the patterns were recorded in the range 0 to 50^0 at the speed of 5^0C/min to know the crystallinity.

Scanning Electron Microscopic (SEM) studies

SEM micrographs of microspheres were obtained under high resolution (Mag.300X 5kV) using JOEL MODEL JSM 840A, scanning electron microscope (SEM), equipped with phoenix energy dispersive analysis of X-rays (EDAX) and Leica 400, Cambridge, UK instrument.

Estimation of drug loading and encapsulation efficiency

Loading efficiency of Etoposide in the microspheres was determined spectrophotometrically. About 10 mg of the drug loaded microspheres were placed in 10 mL buffer solution and stirred vigorously for 24 h to extract the drug from the microspheres. The solution was filtered and assayed by UVspectrophotometer (model Labindia-3000$^+$, Mumbai, India) at the fixed λ_{max} value of 285nm. The results of % drug loading and encapsulation efficiency were calculated using Eqs**.** (1) and (2) respectively, and the results are compiled in Tables 1 and 2:

$$\% \text{ Drug loading} = \left(\frac{Amount\ of\ drug\ in\ Microspheres}{Amount\ of\ Microspheres}\right) X100 \text{ -- (1)}$$

$$\%\text{Encapsulation efficiency} = \left(\frac{Actual\ lodong}{Theoretical\ loading}\right) X100 \text{ -- (2)}$$

Conversion of copolymer

The yield of the copolymeric microspheres 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 microspheres were

washed several times successively with distilled water and methanol solvents to remove the remaining monomer and initiator, and then dried in a vacuum oven at 50^0C till constant weight is attained. The conversion of monomers was calculated as:

Conversion = (W/M) X 100 ------- (3)

***In-vitro* release studies**

Dissolution was carried out using the Tablet dissolution tester (LabIndia, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at two temperatures i.e 25^0C and 37^0C under 100 rpm speed. Drug release from the microspheres was studied in intestinal (7.4 pH phosphate buffer media) fluids. At regular intervals of time, aliquot sample were withdrawn and analyzed by using UV spectrophotometer (Model LabIndia-3000 +,Mumbai, India) at the fixed λmax value of 285 nm.

RESULTS AND DISCUSSIONS

FTIR Analysis

FTIR spectra of copolymeric microspheres, pure Etoposide, and Etoposide loaded copolymeric microspheres were recorded and are given in **Fig: 1**. The carbonyl stretching vibrations of the MMA and NVCL units of the copolymer **Fig:1**(A) appear as a very strong absorption bands at 1732 and 1640 cm^{-1}, respectively. The copolymer being hydrogel in nature, the appearance of a strong but broadband at 3436 cm^{-1} can be attributed to the presence of water of hydration in copolymer. *Krish et al.* [20] studied detailed structural transformations and interactions of NVCL and water using various methods such as IR-spectroscopy, quantum chemical calculations, DSC, and optical microscopy and have reported that NVCL macromolecules in aqueous solution are the highly modified water associated structures, being affected by the polarization action of highly polar amide groups due to specific configurational and conformational structures of the polymer. The bands appearing at 2922, 2852, 1447, and 1387 cm^{-1} are attributed to stretching and bending vibrations of $>CH_2$, >CH- groups respectively. Bands appearing at 1045 cm^{-1}, 1245 cm^{-1} correspond to ester stretching vibration of acrylate polymer and bands appearing at 719 cm^{-1} correspond to the presence of more than three $>CH_2$ groups in a cyclic structure, providing evidence of copolymerization. IR spectrum of Etoposide **Fig: 1**(B) exhibits, strong absorption peaks at 3392, 2912, 1763 - 1610, 1500 –1300, 1250–1050, and 950–850 cm^{-1} corresponding to -OH, $-CH_2$ stretching, lactone group, ring stretching, C-O-C linkages and substituted ring stretching, respectively, as reported earlier. Although in the case of Etoposide loaded

copolymeric microspheres **Fig: 1**(C), presence of characteristics bands of drug and copolymer at 3401, 2922, 2852, 1763–1610, 1520–1300, 1250–1050, and 900–750 cm^{-1} correspond to -OH, $>CH_2$ stretching, lactone group, ring stretching, C-O-C linkages and the presence of more than three $-CH_2$ groups in cyclic structure supporting the existence of Etoposide in microspheres. Changes observed in FTIR spectrum of the Etoposide loaded copolymer might be due to the interaction between drug and polymer or difference in the polymer morphology due to the presence of small quantity of Etoposide. [21]

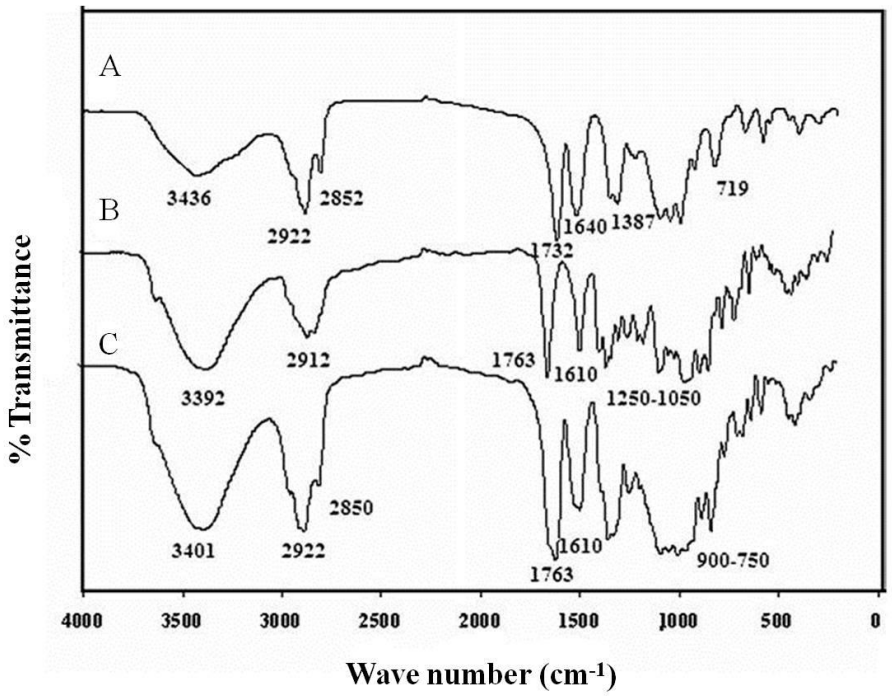


Fig 1. FTIR spectra of (A) poly (NVCL-co-MMA) copolymer, (B) Etoposide and (C) Etoposide loaded copolymeric microspheres.

Differential scanning calorimetry (DSC) studies

Fig.2 displays the DSC thermograms of (A) Etoposide, (B) copolymeric microspheres, (C) physical mixture of Etoposide and copolymeric microspheres, and (D) Etoposide loaded copolymeric microspheres are given in Figure 2. DSC thermogram of pure Etoposide evidenced an endotherm over the range of 80–120^{0}C due to dehydration of water molecules and endothermic peak at 269^{0}C for melting followed by decomposition observed above 290^{0}C. A similar observation is reported by *Jasi et al.* [22] Although endothermic peaks at 84 and 223^{0}C are observed for lyophilized 80:20 poly (NVCL-co-MMA) copolymeric microspheres. However, no characteristic endothermic peaks at 84^{0}C and 269^{0}C corresponding to copolymer and etoposide were observed in DSC plot of the etoposide-

loaded copolymeric microspheres (D) might be due to low loading of drug in copolymer structure along with strong interaction of drug with copolymer, but two separate peaks at 84^0C and 282^0C corresponds to copolymeric microspheres and etoposide were observed in a physical mixture of copolymeric microspheres and etoposide (C). From these results, we can also say the presence of possible interaction takes place between drug and polymers.

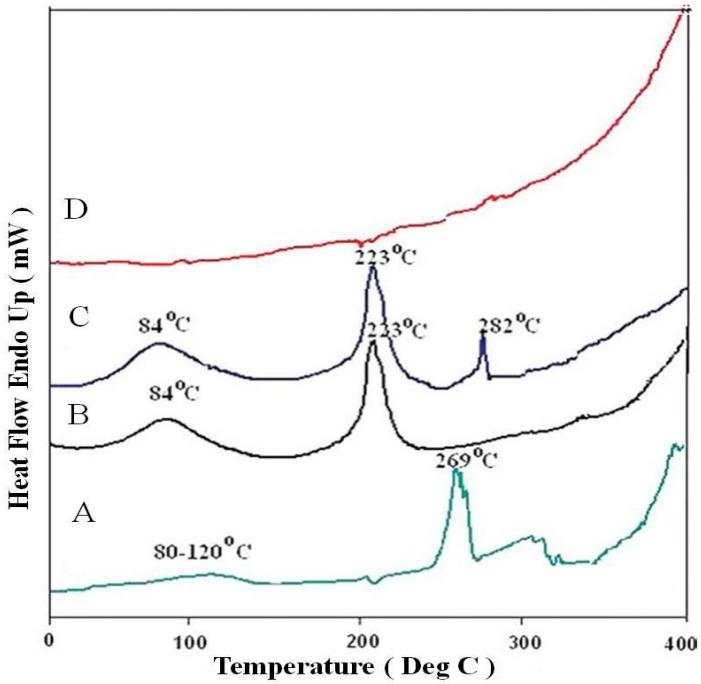


Fig.2. DSC thermo grams of (a) Etoposide, (b) plain (NVCL-co-MMA) microspheres, and (C) physical mixture of Etoposide and copolymeric microspheres (D) Etoposide loaded copolymeric microspheres.

X-ray diffraction studies

X-ray Diffraction Powder X-ray diffraction (XRD) patterns for the (A) Etoposide, (B) physical mixture of Etoposide and copolymeric microspheres (1: 1 w/w) (C) copolymeric microspheres, and (D) Etoposide - loaded copolymeric microspheres are given in Figure 3. The peaks of interest for pure drug observed at 4.2, 9.46, 10.22, 13.18, 16.15, 17.08, 17.67, 19.26, 19.89, 22.14, 23.03, 23.67, 24.17, and 26.78; indicate highly crystalline nature of the drug. Although for Etoposide -loaded copolymeric microspheres, all the crystalline peaks of drug disappear, as only 1% of drug is present in the sample that contains Etoposide in the polymer matrix.

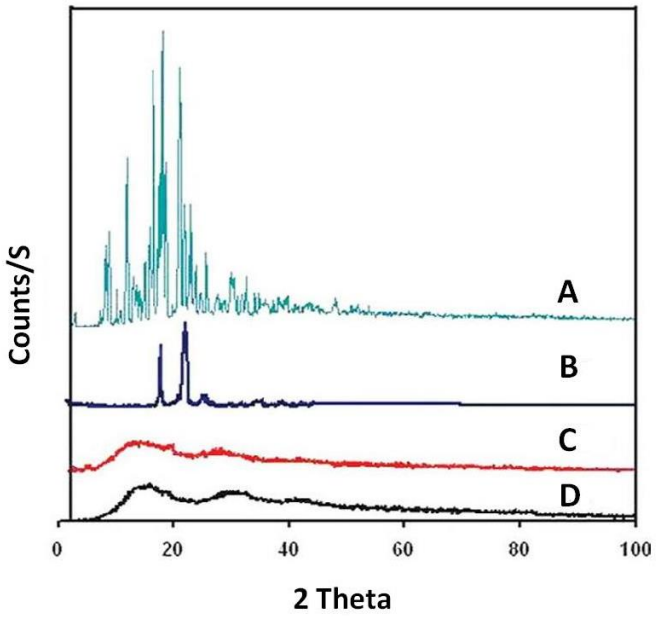


Fig:3. XRD spectra of (D) Etoposide, (C) Physical Mixture of Etoposide and copolymeric microspheres (B) poly (MMA-co-NVCL) copolymeric microspheres and (A) Etoposide loaded copolymeric microspheres

Scanning Electron Microscopy (SEM)

Fig: 4 displays the SEM photograph, where in the morphology of NVCL-co-MMA microspheres can be observed. The formed copolymer particles are spherical in nature with diameters around 10µm and have microporous surface structures.

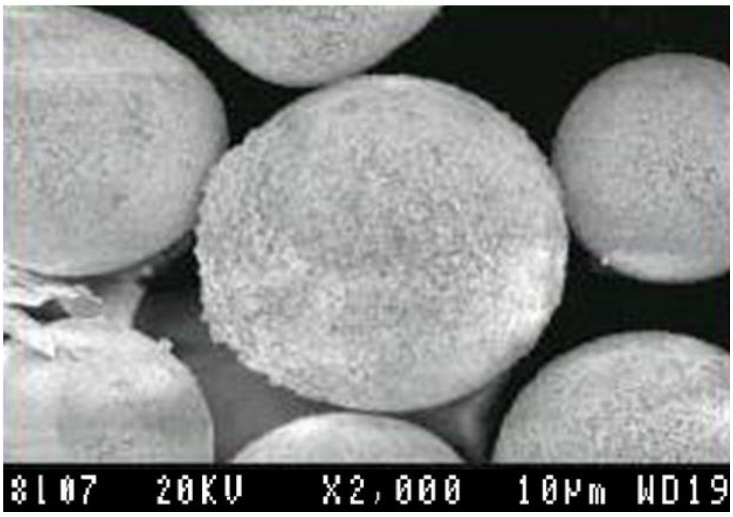


Figure .4. Scanning electron micrograph of NVCL-co-MMA microspheres

Encapsulation Efficiency

In order to investigate the effect of process variables on encapsulation efficiency, we used the same parameters as described in Table 1. Notice that formulations NVCL- 1 to NVCL-8 are

prepared by taking 20 wt% MMA and 80 wt% NVCL along with the variations of drug and cross-linking agent. For formulations NVCL- 6 and NVCL-7, the amount of NVCL and MAA polymers were varied, keeping the cross-linking agent and Etoposide constant. However, in the case of NVCL- 8, 100% MMA was polymerized by adding 1 wt% NNMBA and 5 wt% drug. Encapsulation efficiency data given in Table 1 of all the formulations varied from 69 to 79%, depending on the nature of the matrix material. Notice that for a matrix that has the highest amount (15 wt %) of Etoposide, the highest encapsulation efficiency of 79% was seen. On the other hand, the matrix containing 90 wt% NVCL and 10 wt% MMA with 5wt% Etoposide gave the lowest encapsulation efficiency of 69%. This clearly suggests that the nature of the polymer and the amount of drug affects the encapsulation efficiency of the matrices. In case of formulations NVCL-1 to NVCL-3, the encapsulation efficiency increased systematically with increasing drug content of the matrices. At higher concentration of cross-linking agent (i.e.,10 or 15wt% of NNMBA), the encapsulation efficiency was reduced from 75 to 79% due to the inward wrapping of the monomeric chains in the co-polymer.

Table: 1

Formulation	VCL (%)	MMA (%)	NNMBA (%)	Etoposide (%)	Encapsulation efficiency ± SD (%)
NVCL-1	80	20	1	5	71 ±1
NVCL-2	80	20	1	10	75 ±2
NVCL-3	80	20	1	15	79 ±2
NVCL-4	80	20	2	5	76 ±9
NVCL-5	80	20	3	5	72 ±8
NVCL-6	90	10	1	5	69 ±6
NVCL-7	70	30	1	5	72±5
NVCL-8	00	100	1	5	73 ±1

***IN VITRO* RELEASE STUDIES**

Effect of Temperature

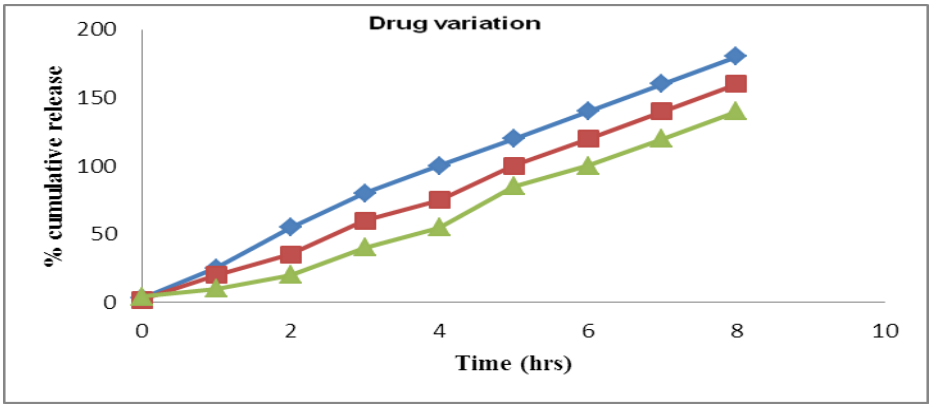
Release profiles of Etoposide from NVCL-co-MMA microspheres prepared with different amounts of the crosslinking agent and drug loadings have been studied at two temperatures 25^0C & 37^0C in the chosen dissolution medium. Drug release profiles exhibited drastic variations due to changes in temperature. It may be noted that drug was released slowly at 37^0C (i.e., above LCST), but release was much faster at 25^0C (i.e., below LCST) than at 37^0C. This is due to the fact that at higher temperature, the surface of microspheres will shrink, thereby causing the drug migrate towards the surface of the microspheres as seen by the initial burst effect during the dissolution experiments. However, dense surfaces of the

microspheres will prohibit the release of more amount of the drug. At lower temperatures, 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 reswelling 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. Microspheres of this study were proved to be sensitive to changes in temperature. At 25^0C (in the swollen state), release rate and total amount of the drug were considerably higher than those found at 37^0C (in a collapsed state). Drug molecules entrapped inside the polymer network will diffuse out of the microspheres, since they quickly got hydrated in the swollen state. In contrast, at 37^0C, the network structure is collapsed and exhibits a lesser tendency to uptake water or buffer solution, leading to decrease in drug diffusion rate.

Effect of Drug Concentration

Fig.5.a and b displayed the release profiles of NVCL-co-MMA microspheres that are loaded with different amounts of Etoposide at 25^0C &37^0C, respectively. From the Fig. 5.a and b, it is explained initially that during the first hour the release is quiet fast in all formulations, but later it is slowed down. Release data suggest that those formulations containing the highest amount of drug (i.e., 15 wt %) shows the higher release rates than those formulations containing smaller amounts of Etoposide (i.e., 10 and 5 wt %). A prolonged and slow release was observed for formulation containing a lower amount of Etoposide (i.e., 5 wt %) at 37^0C. This may be due to the free volume spaces are available in the matrix through which, a lesser number of Etoposide molecules would transport and it is further explained from the Fig. 5.a and Fig. 5.b that for all the Etoposide loaded formulations, the complete release of Etoposode was not observed even after 720 min, since the % cumulative release data tend to increase continuously.

(a) At 25^0C



(b) At 37^0C

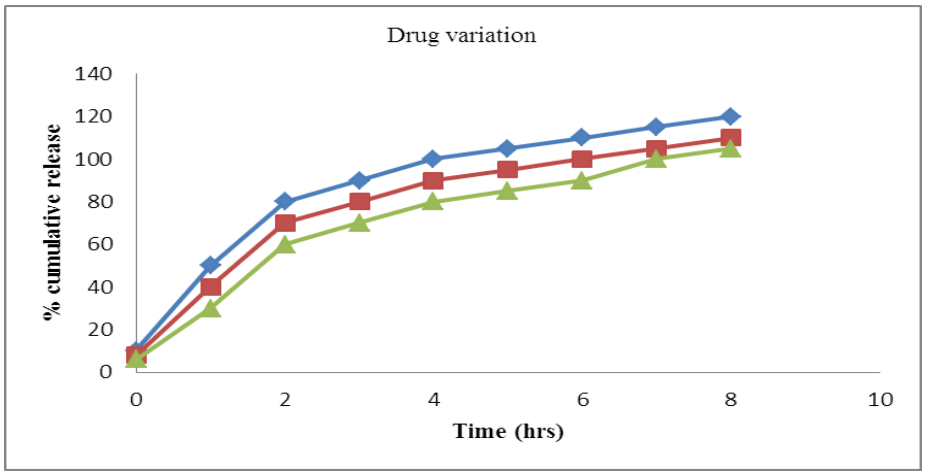
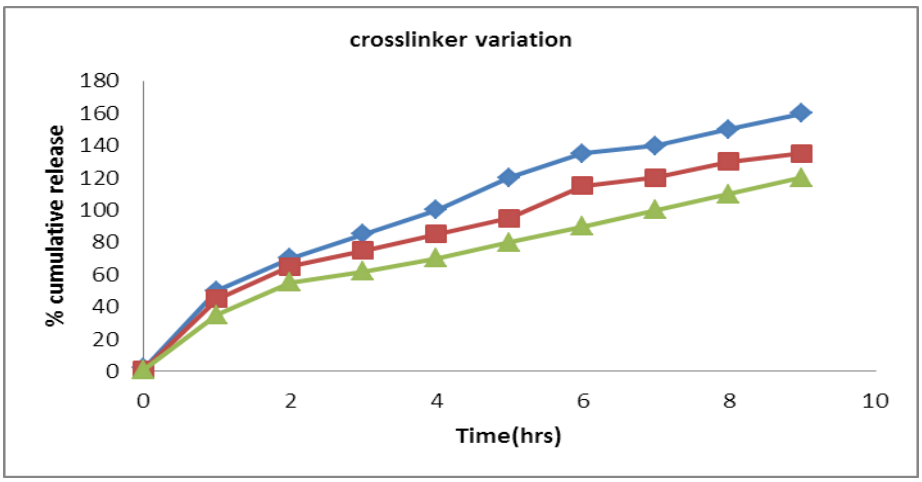


Fig.5 (a, b). % of cumulative release with drug variation at 25^0C & 37^0C.

Effect of cross-linking agent

The % cumulative release data *versus* time plots for the microspheres prepared with varying amounts of NNMBA, i.e., 1, 2 and 3wt% at the fixed amount of the drug (5 wt %) at 25^0C &37^0C are displayed in **Fig.6**.a and **6**.b respectively. The % cumulative release is quite fast and large at the lower amount of crosslinker, (i.e., 1% of MBA), whereas the release is quite slower at higher amount of crosslinker, (i.e., 3 wt% MBA). The cumulative release is also higher at the lower amount of NNMBA, because at higher concentration of NNMBA, the polymeric chains will become rigid due to contraction of microvoids thereby, giving a decrease in % cumulative release of the drug. The crosslinking agent could help to form a bridge between the copolymeric chains. Therefore, as expected, the drug release becomes slower at higher amount of NNMBA, but it will be faster when a lower amount of MBA present in the polymer matrix at both 25^0 &37^0C

(A) At 25^0C



(B) At 37^{0}C

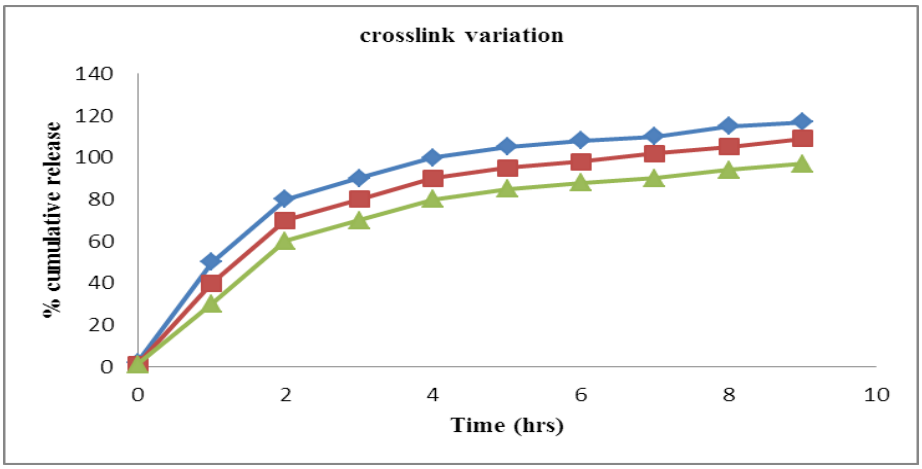
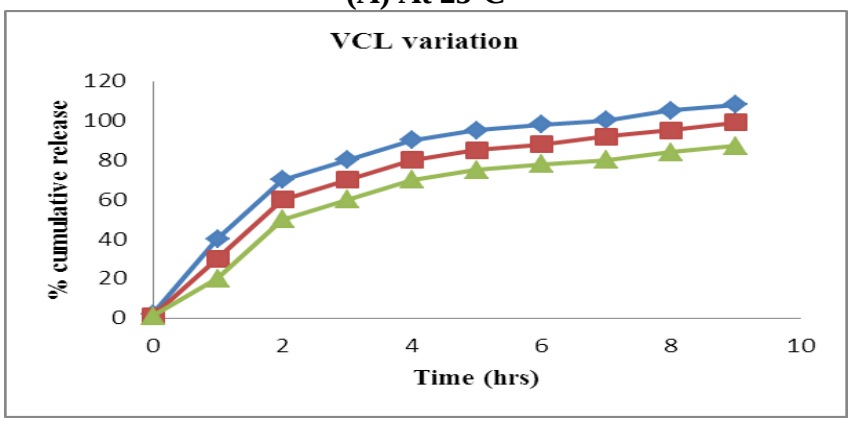


Fig.6 (a, b). % of cumulative release with drug variation at 25^{0}C & 37^{0}C.

Effect of vinylcaprolactam

Drug release profiles from the microspheres containing different amounts of NVCL at 25^{0}C & 37^{0}C are displayed in **Fig.7**.a and **7**.b respectively. Effect of NVCL content on encapsulation efficiency and *in vitro* release of Etoposide was investigated and the data given in Table: 1. *In vitro* release profiles of Etoposide for formulations prepared by taking different amounts of NVCL with 15% of Etoposide and 1% NNMBA are shown in Figure 7. Higher release rates were observed for the formulations prepared with higher amounts of NVCL (i.e., 90%) than those formulations prepared using lower amounts of NVCL. Slower drug release is observed from formulations prepared with lower amount of NVCL, which is due to the hydrophilic nature of both the drug and NVCL in the copolymer. Similar observation was reported by *Prabaharan et al*. [23] in case of Chitosan-g-Poly (N-Vinyl caprolactam).

(A) At 25^{0}C



(B) At 37^{0}C

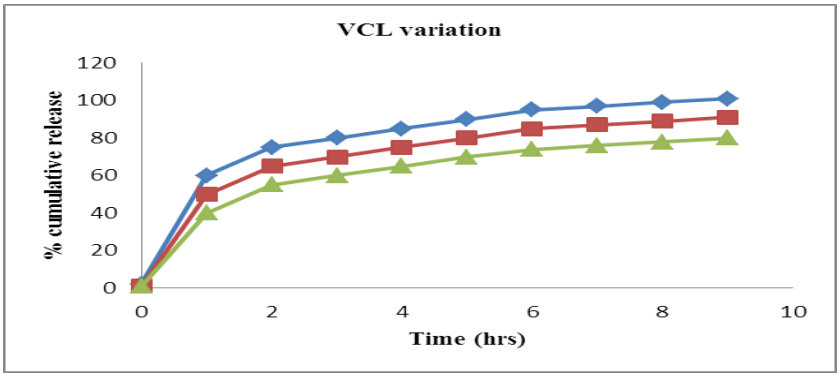


Fig.7 (a, b). % of cumulative release with drug variation at 25^{0}C & 37^{0}C.

Drug release kinetics

Drug release kinetics was analyzed by plotting the cumulative release data *versus* time and by fitting these data to the exponential equation of the type (Ritger and Peppas, 1987).

$$M_t/M_\infty = kt^n \text{------ (4)}$$

Here, M_t/M_∞ represents the fractional drug release at time t; k is a constant 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 seven formulations and these values are given in Table.2.

Table.2 Release kinetic parameters for different formulation at-25^{0}C and 37^{0}C.

At 25^{0}C

Formulation code	*k*	*n*	Correlation coefficient, *r*
NVCL-1	0.6569	0.3841	0.9654
NVCL-2	0.5581	0.3657	0.9115
NVCL-3	0.3184	0.3429	0.9545
NVCL -2	0.5581	0.3657	0.9115
NVCL -4	0.6396	0.4593	0.9381
NVCL -5	0.6883	0.4961	0.9361
NVCL -2	0.5581	0.3657	0.9115
NVCL -6	0.6312	0.3594	0.9918
NVCL -7	0.7414	0.4961	0.8582

At 37^{0}C

Formulation code	*k*	*n*	Correlation coefficient, *r*
NVCL-1	0.6296	0.5124	0.9723
NVCL-2	0.4955	0.5365	0.9255
NVCL-3	0.3564	0.7337	0.9272
NVCL -2	0.4955	0.5365	0.9255
NVCL -4	0.5773	0.6754	0.9648
NVCL -5	0.7305	0.5372	0.9457
NVCL -2	0.4955	0.5365	0.9255

NVCL -6	0.5828	0.5658	0.9213
NVCL -7	0.6067	0.8354	0.9825

If *n* = 0.5, then drug diffuses and releases from the polymer matrix following a Fickian diffusion. For *n* > 0.5, anomalous or non-Fickian type drug diffusion occurs. If *n*=1, a completely non-Fickian is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to the anomalous type transport (Ritger and Peppas, 1987).

In the present investigation, the values of *k* and n have shown a dependence on the extent of crosslinking, % drug loading as well as NVCL content of the microspheres. The values of n for microspheres, prepared with varying amounts of NVCL (i.e., 70, 80 and 90 wt %) by keeping Etoposide (5 %) and MBA (1%) as constant, ranged from 0.3429 to 0.4961 and 0. 5124 to 0.8354, respectively at 25^0C &37^0C, suggesting a slight deviation from the Fickian mode of diffusion.

The Etoposide loaded microspheres exhibited the n values ranging from 0.8582 to 0.9918 and 0.9213 to 0.9825, respectively at 25^0C &37^0C (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 0.8582 to 0.9918 and 0.9213 to 0.9825 at 25^0C &37^0C, respectively, indicating a good fit of the experimental data. This is due to reduction in the regions of low microviscosity of the medium and closure of the microcavities in the swollen microspheres.

CONCLUSION

The thermoresponsive Etoposide loaded NVCL-co-MMA microspheres were prepared by dispersion polymerization using sodium laurylsulfate as a surfactant. Etoposide, anti- cancer drug, was chosen as model drug to investigate the percentage of cumulative release using the developed matrices. The developed thermoresponsive microspheres show a prolonged release of Etoposide over an extended period of 12h. The prepared microspheres have thus shown thermoresponsive trends during in vitro drug release studies of Etoposide. The fast release rates were observed at 25^0C whereas slow release rates were observed at 37^0C when dissolution experiments were performed at 25^0C & 37^0C.

REFERENCES

1. Shimizu K, Fujita H, Nagamori E. Oxygen plasma-treated thermoresponsive polymer surfaces for cell sheet engineering. Biotechnol Bioeng., 2010; 106: 303-310.
2. Twaites BR, Alarcon CDH, Lavigne M, et al. Thermoresponsive polymers as gene delivery vectors: cell viability, DNA transport and transfection studies. J Control Release., 2005; 108: 472-483.
3. Doorty KB, Golubeva TA, Gorelov AV, et al. Poly (N-isopropylacrylamide) co-polymer films as potential vehicles for delivery of an antimitotic agent to vascular smooth muscle cells. Cardiovasc Pathol., 2003; 12: 105-110.
4. Stile RA, Healy KE. Thermo-responsive peptide-modified hydrogels for tissue regeneration. Biomacromolecules., 2001; 2: 185-194.
5. Hacker MC, Klouda L, Ma BB, et al. Synthesis and characterization of injectable, thermally and chemically gelable, amphiphilic poly(N-isopropylacrylamide)-based macromers. Biomacromolecules., 2008; 9: 1558-1570.
6. Feil H, Bae YH, Feijen J, et al. Effect of comonomer hydrophilicity and ionization on the lower critical solution temperature of N-isopropylacrylamide copolymers. Macromolecules., 1993; 26: 2496-2500.
7. Vihola H, Laukkanen A, Tenhu H, et al. Drug release characteristics of physically cross-linked thermosensitive poly (N-vinylcaprolactam) hydrogel particles. J Pharm Sci., 2008; 97: 4783-4793.
8. Vihola H, Marttila AK, Pakkanen JS, et al. Cell-polymer interactions of fluorescent polystyrene latex particles coated with thermosensitive poly(N-isopropylacrylamide) and poly(N-vinylcaprolactam) or grafted with poly(ethylene oxide)-macromonomer. Int J Pharm., 2007; 343: 238-246.
9. Klouda L, Mikos AG. Thermoresponsive hydrogels in biomedical applications. Eur J Pharm Biopharm., 2008; 68: 34-45.
10. Lutz JF. Polymerization of oligo(ethylene glycol) (meth) acrylates: toward new generations of smart biocompatible materials. J Polym Sci Part A., 2008; 46: 3459-3470.
11. Pasparakis G, Vamvakaki M. Multiresponsive polymers: nano-sized assemblies, stimuli-sensitive gels and smart surfaces. Polym Chem., 2011; 2: 1234-1248.
12. Liu F, Urban MW. Recent advances and challenges in designing stimuli-responsive polymers. Prog Polym Sci., 2010; 35:3-23.
13. Meeussen F, Nies E, Berghmans H, Verbrugghe S, Goethals E J, Du Prez F. Polymer., 2000; 41: 8597–8600.

14. Beija M, Marty JD, Destarac M. Thermoresponsive Poly (N-Vinyl Caprolactam)-Coated Gold Nanoparticles: Sharp Reversible Response and Easy Tunability. Chem. Commun., 2011; 47: 2826–2828.
15. Vihola H, Laukkanen A, Valtola L, Tenhu H, Hirvonen.Cytotoxicity of thermosensitive polymers poly (N-isopropylacrylamide), poly (N-vinylcaprolactam) and amphiphilically modified poly (N-vinylcaprolactam). J, Biomaterials., 2005; 26: 3055–3064.
16. Jun L, Bochu W, Yazhou W. Thermo-sensitive Polymers for Controlled-release Drug delivery systems. Int. J. Pharmacol., 2006; 2; 513–519.
17. Imaz A, Forcada J. *N*-vinylcaprolactam-based microgels for biomedical applications. J. Polym. Sci., Part A: Polym. Chem., 2010; 48: 1173–1181
18. Hande KR.Etoposide: four decades of development of a topoisomerase II inhibitor. Eur. J. Cancer., 1998; 34: 1514-1521.
19. Wolf SN, Grosh WW, Prater K. *In vitro* pharmacodynamic evaluation of VP-16213 and implications for chemotherapy. Cancer. Chemother. Pharmacol., 1987; 19: 246-249.
20. Kirsh Y E, Yanula NA, Kalninsh KK.structural transformation and water associate interactions in poly N-vinylcaprolactam-water system Eur. Polym. J., 1999; 35: 305.
21. Lamprecht A, Benoit JP. Etoposide nanocarriers suppress glioma cell growth by intracellular drug delivery and simultaneous P-glycoprotein inhibition. J Controlled Release., 2006; 112: 208-13.
22. Jasti BR, Du J, Vasavada RC. Characterization of thermal behavior of etoposide. Int. J. Pharm., 1995; 118: 161-67.
23. Prabaharn M, Grailer JJ, Steeber DA, Gong S. Stimuli responsive chitosan-graft-poly (N-vinyl caprolactam) as promising material for controlled hydrophobic drug delivery. Macromol Biosci., 2008; 8: 843-51.