In the past decade, temperature-induced hydrogels have gained for practical biomedical or pharmaceutical applications since the introduction of microspheres and the drug was released in a controlled manner up to 10 h.

Keywords: Caprolactam, N-isopropylacrylamide, Drug delivery, Acebutolol hydrochloride, Controlled release.

INTRODUCTION

In the past decade, temperature-induced hydrogels have gained increasing attention for many investigators for scientific interest and for practical biomedical or pharmaceutical applications since the administration is convenient. Among the temperature-responsive hydrogels reported to date, Poly(N-isopropylacrylamide) (PNIPAAm) homopolymer and its copolymers have been most extensively investigated. Controlled delivery systems via targeted drug delivery of a predetermined dose over a sustained period have been used to overcome the short comings of conventional dosage forms. This is because the controlled drug delivery system can provide sustained therapeutic level of drug concentration without toxicity. Drug delivery technology also provides convenience for patients. It would be more beneficial and ideal if the drug could be delivered by a device that would respond to physiopathological signals from an underlying disease. The correct amount of drug would be released upon the stimulation of such a physiopathological signal.

There have been many reports on the thermally sensitive PNIPAAm hydrogel in the past 30 years because of its particular property, its volume or weight changes drastically and almost all the inside water is lost when the temperature is raised from room temperature to above the lower critical solution temperature (LCST). This useful property has been used in many biomedical and pharmaceutical applications, such as drug-delivery systems, enzyme immobilization, tissue culture substrates, and gene carriers. However, for some applications, the conventional PNIPAAm hydrogel has some serious limitations, such as weak mechanical strength in the highly swollen state and a slow response rate when the temperature is greater than the LCST.

Recently, there has been increased interest in responsive hydrogels for utilization as the smart drug delivery system (smart-DDS) in the field of controlled drug release, to meet the need for prolonged and better control of drug administration. As we know, in conventional drug delivery, the drug concentration in the blood increases to a toxic level as the drug is taken, and then the drug concentration decreases to an ineffective level and the patients have to take the drug frequently. In order to eliminate or reduce the above disadvantages, drug delivery system (DDS) for control release was designed to maintain the drug release with the predetermined dose and prolong the curing-time in the targeted body compartment. Compared to the conventional DDS, the advantages of the smart-DDS are self-evident because the drug amount can be auto-controlled by external changes, such as temperature, electric fields, pH and photo fields etc.

Thermo-responsive hydrogel is a most extensively studied, responsive and polymeric material, including various polymers, such as the N-substituted polycrylamide, polymethylacrylamide and poly(ethylene oxide) etc. Poly(N-isopropylacrylamide) (PNIPAAm) hydrogel is a typically thermosensitive material, which exhibits a phase transition temperature (Tc) or lower critical solution temperature (LCST) at ~ 33 °C. As the external temperature cycles around this phase transition temperature, the polymer chains undergo a coil-globule transition. Correspondingly, the three-dimensional PNIPAAm hydrogel returns to a shrunken state and displays phase separation, i.e. abrupt collapse in volume as the temperature is increased above LCST. The abrupt shrinking in the volume of the PNIPAAm hydrogel to the increased temperature has produced extensive research interest directed at applications to the controlled release of drugs.

Normally, the selected drug is physically loaded in the swollen thermo-responsive hydrogel and the drug release is controlled by the external temperature changes due to the thermo-reversible properties of the PNIPAAm hydrogel. Generally regarded, the drug exhibits a Fickian release, which depends on the swelling ratio of the hydrogel. As the temperature is increased above the LCST, PNIPAAm hydrogel may shrink and quickly form a dense, thick skin layer, which leads to the burst release initially and then the release of the drug in the network matrix is stopped.

A typical release pattern was reported by Kim’s research group and an on-off release pattern of the model drug, indomethacin, was achieved by regulating the temperature between 20 and 30°C. A series of investigations based on the thermo-responsive hydrogels was carried out and much useful data were obtained. In these cases, the thermo-responsive hydrogels provide a negative temperature-responsibility to the drug release, i.e. slow drug release at increased temperature and rapid drug release at decreased temperature. In some cases, a positive controlled release pattern, i.e. rapid drug release at increased temperature and slow drug release at decreased temperature, is urgently needed when the DDS is
specially designed to respond to an increase in the body
temperature resulting from diseases, such as inflammation or
cancers etc.

In our research, the author prepared a novel thermo-responsive DDS
to give a positive controlled release pattern. Acebutolol hydrochloride is an anti hypertensive drug, used in the treatment of
curing the hypertension, which is easily soluble in water. Here, the author has chosen as model drug which was loaded into the
PNIPAAm and Caprolactam microspheres. Then the drug
incorporated microspheres was carefully enveloped in the dialysis bag to form a novel DDS with double controlled release layers (the polymer network and the dialysis membrane). At different
predetermined temperatures (25°C and 37°C) the concentration of
released ABH was monitored at 240 nm on an UV
spectrophotometer. It was found that ABH was released more
rapidly at 37°C (>25 °C) than at 25 °C. This novel, thermoresponsive
PNIPAAm-co-CL) polymeric matrix may be more useful in cases
where controlled drug delivery system is needed.

EXPERIMENTAL

Materials and methods

N-isopropylacrylamide (NIPAAm), caprolactam were purchased
from Aldrich, Milawakee, WI, USA. N,N'-methylene bis-acrylamide
(NNMBa), potassium persulate (KPS), sodium lauryl sulphate (SLS)
were all of analytical grade purchased from s.dine chemicals,
Mumbai, India and used without further purification. The model
drug acebutolol hydrochloride was obtained as a gift sample from
waksan salesmen pharmaceuticals, Anantapur, A.P. India.

Synthesis of thermo responsive poly (NIPAAm-co-CL)
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°C.

The solution was stirred at 800 rpm speed until it became clear and
100 mg of potassium per sulfate was added. Required amount of
NIPAAm, Caprolactam, the crosslinking agent NNMBa and
acebutolol hydrochloride 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°C 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°C for 24 h. The blank microspheres
without drug incorporation were by prepared by above method. The
model drug acebutolol hydrochloride was loaded into polymeric microspheres by keeping the weighed
amount of drug in the crosslinked microspheres. X-RD patterns recorded
for the plain ABH drug (A) placebo polymeric microparticles (B)
and drug-loaded microspheres (C) are shown in Fig. 2. The
acebutolol hydrochloride peaks are observed at 2θ of 7°, 17°, 20°,
21° and 25° suggesting its crystalline nature. But, these peaks are
not found in acebutolol hydrochloride loaded microspheres,
suggesting that drug is molecularly dispersed in the polymer matrix.

X-ray diffraction (X-RD) studies

Dried microspheres of uniform size were mounted on a sample
holder and X-RD patterns were recorded in the range 0- 60° at the
speed of 5°/min. X-RD analysis provide a clue about crystallinity of
the drug in the crosslinked microspheres. X-RD patterns recorded
for the plain ABH drug (A) placebo polymeric microparticles (B)
and drug-loaded microspheres (C) are shown in Fig. 2. The
acebutolol hydrochloride peaks are observed at 2θ of 7°, 17°, 20°,
21° and 25° suggesting its crystalline nature. But, these peaks are
not found in acebutolol hydrochloride loaded microspheres,
suggesting that drug is dispersed at a molecular level in the
polymer matrix.

Scanning electron microscopic (SEM) studies

Scanning electron microscopy has been used to confirm the
formation of spherical structures of the microspheres. SEM
micrographs of Poly(NIPAAm-co-CL) microspheres are displayed in
Fig. 3. The microspheres were coated with gold colour and subjected
to SEM, which revealed that the formation nearly spherical structure
of the microspheres with porous nature.
Encapsulation efficiency

Three different concentrations of drug (5, 10 and 15 wt %) were loaded in the Poly(NIPAAm-co-CL) microspheres during crosslinking. The % encapsulation efficiency was included in Table 1. From the Table 1, it is noticed that the encapsulation efficiency values increased with increasing drug loading in the polymeric microspheres. In the case of ABH-1, ABH-2 and ABH-3 microspheres, the % encapsulation efficiency increased from 50.3 % to 60.9 % as the drug content increased from 5 to 15 wt %. The % encapsulation efficiency increased with an increasing amount of NIPAAm in the microspheres. For example, to study the effect of NIPAAm in the microspheres [e.g., formulations containing different ratios of NIPAAm and Caprolactam with 10 % of ABH (NIPAAm-1, NIPAAm-2, NIPAAm-3)], encapsulation efficiencies were found to be 68.3 %, 57.6 % and 53.2 %, respectively. The effect of crosslinking on size and entrapment efficiency of the microspheres using percentage of crosslinker 10, 20 and 30 % containing Poly (NIPAAm-co-CL) microspheres are also represented in Table 1. With an increase in degree of crosslinking, the % encapsulation efficiency was decreased, e.g., formulations crosslinked with 10, 20, 30 wt% of NNMB (NNMBA-1, NNMBA-2 and NNMBA-3), entrapment efficiencies were 57.6 %, 51.2 % and 48.6 %, respectively. This may be due to the increasing degree of crosslinking, which leads to microspheres becoming more rigid and thus, reducing the free volume space within the polymeric network to yield a reduction in the percentage of encapsulation efficiency.

Effect of drug concentration

Fig.4.a. and 4.b displayed the release profiles of Poly (NIPAAm-co-Caprolactam) microspheres that are loaded with different amounts of ABH at 25°C and 37°C, respectively. From the figures 4.a & 4.b it is noticed that initially, during the first hour the release is quite 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 %) displayed the higher release rates than those containing smaller amounts of ABH (i.e., 10 and 5 wt %). A prolonged and slow release was observed for formulation containing a lower amount of ABH (i.e., 5 wt %) at 37°C. This may be attributed to the factor that free volume spaces are available in the matrix through which, a lesser number of ABH molecules would transport showing. Generally, drug release through microspheres depend upon the particle size, polymer crystallinity, surface character, molecular weight, polymer...
composition, swelling ratio, degradation rate, drug binding affinity, rate of hydration, etc. In the present study among the factors responsible for this in vitro release of the drug through this system, the binding affinity might be predominant. It is further noticed from the figures 4a and 4b that for all the ABH loaded formulations, the complete release of ABH was not observed even after 600 min, since the % cumulative release data tend to increase continuously.

Effect of crosslinking agent

The % cumulative release data versus time plots for the microspheres prepared with varying amounts of NNMBA, i.e., 1, 2 and 3% at the fixed amount of the drug (5 wt %) at 25°C and 37°C are displayed in Fig.5a and Fig 5b, respectively. The % cumulative release is quite fast and large at the lower amount, i.e., 1% of NNMBA, whereas the release is quite slower at higher amount, i.e., 3% NNMBA. 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 NNMBA is present in the polymer matrix at both 25°C and 37°C.

Table 1: Formulation details and % of encapsulation efficiency data for Poly (NIPAAm-co-CL) microspheres

<table>
<thead>
<tr>
<th>Formulation codes</th>
<th>% NIPAAm</th>
<th>% Caprolactam</th>
<th>% ABH</th>
<th>Amount of NNMBA added (gm)</th>
<th>% Encapsulation efficiency ± S.D.</th>
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<tr>
<td>Drug Variation at constant monomer and crosslinker</td>
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<td></td>
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<td></td>
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<tr>
<td>ABH-1</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>49.5 ± 0.8</td>
</tr>
<tr>
<td>ABH-2</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>56.2 ± 1.4</td>
</tr>
<tr>
<td>ABH-3</td>
<td>4</td>
<td>6</td>
<td>15</td>
<td>1</td>
<td>60.7 ± 0.2</td>
</tr>
<tr>
<td>Crosslinker Variation at constant monomer and drug</td>
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<td></td>
</tr>
<tr>
<td>NNMBA-1</td>
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<td>6</td>
<td>10</td>
<td>1</td>
<td>56.2 ± 1.4</td>
</tr>
<tr>
<td>NNMBA-2</td>
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<td>6</td>
<td>10</td>
<td>2</td>
<td>50.4 ± 0.8</td>
</tr>
<tr>
<td>NNMBA-3</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td>47.6 ± 1.0</td>
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<tr>
<td>Monomer Variation at constant drug and crosslinker</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NIPAAm-1</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>56.2 ± 1.4</td>
</tr>
<tr>
<td>NIPAAm-2</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>56.7 ± 0.6</td>
</tr>
<tr>
<td>NIPAAm-3</td>
<td>2</td>
<td>8</td>
<td>10</td>
<td>1</td>
<td>52.8 ± 0.4</td>
</tr>
</tbody>
</table>

NIPAAm = N-isopropylacrylamide
ABH= Acebutolol hydrochloride
NNMBA = N,N-methylene bis-acrylamide
S.D = standard deviation calculated 95% accurately

Fig. 4: % cumulative release of ABH at 25°C (a) and 37°C (b) through poly (NIPAAm-co-CL) microspheres crosslinked with 1% NNMBA and % NIPAAm containing (●) 5%, (■) 10% and (▲) 15% of ABH.
Effect of temperature

Release profiles of ABH from poly (NIPAam-co-Caprolactam) microspheres prepared with different amounts of the crosslinking agent and drug loadings have been studied at two temperatures in the chosen dissolution medium, alternatively from 25°C to 37°C and vice versa. Drug release profiles exhibited drastic variations due to changes in temperature from 25°C to 37°C. It may be noted that drug was released slowly at 37°C i.e., above LCST, but release was much faster at 25°C (i.e., below LCST) than at 37°C. This is due to the fact that at higher temperature, the surface of microspheres will shrink, thereby causing the drug to 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, 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 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°C (in the swollen state), release rate and total amount of the drug were considerably higher than those found at 37°C (in a collapsed state). Drug molecules entrapped inside the polymer network will diffuse out of the microspheres, since they quickly get hydrated in the swollen state. In contrast, at 37°C, the network structure is collapsed and exhibits a lesser tendency to uptake water or buffer solution, leading to decrease in drug diffusion rate.

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\(^3\)^4.

\[
\frac{M_t}{M_\infty} = k t^n
\]  

Here, \(M_t/M_\infty\) 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. 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

\fig

Fig. 5: % Cumulative release of ABH at 25°C (a) and 37°C (b) through Poly (NIPAam-co-CL) microspheres loaded with 10% ABH and 40% NIPAam containing (●) 1%, (★) 2% and (▲) 3% of NNMB.

Fig. 6: % Cumulative release of ABH at 25°C (a) and 37°C (b) through Poly (NIPAam-co-CL) microspheres loaded with 10% ABH and crosslinked with 2% NNMB containing (●) 60%, (■) 40% and (▲) 20% of NIPAam.
type drug diffusion occurs. If ν = 1, a completely non-Fickian is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to the anomalous type transport.\textsuperscript{14}

In the present investigation, the values of k and n have shown a dependence on the extent of crosslinking, % drug loading as well as NIPAAm content of the microspheres. The values of n for microspheres, prepared with varying amounts of NIPAAm (i.e., 20, 40 and 60 wt %) by keeping ABH (10 %) and NNMBA (10 %) as constant, ranged from 0.201 to 0.342 and 0.561 to 0.991, respectively at 25°C and 37°C, suggesting a slight deviation from the Fickian mode of diffusion. The ABH-loaded microspheres exhibited the n values ranging from 0.201 to 0.486 and 0.561 to 0.991, respectively at 25°C and 37°C (Table 2), indicating a shift from the n values ranging from 0.201 to 0.486 and 0.561 to 0.991, and n values ranging from 0.201 to 0.486 and 0.561 to 0.991, respectively at 25°C and 37°C, indicating a good fit of the experimental data. This is due to reduction in the regions of low microviscosity of the medium and closeup of the microcavities in the swollen microspheres.

Table 2: Release kinetics parameters of k, n and correlation coefficients(\(r^2\)) for different formulations at different temperatures

<table>
<thead>
<tr>
<th>Formulation codes</th>
<th>k</th>
<th>n</th>
<th>Correlation coefficient, (r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABH-1</td>
<td>1.214</td>
<td>0.365</td>
<td>0.970</td>
</tr>
<tr>
<td>ABH-2</td>
<td>1.131</td>
<td>0.342</td>
<td>0.981</td>
</tr>
<tr>
<td>ABH-3</td>
<td>1.068</td>
<td>0.340</td>
<td>0.970</td>
</tr>
<tr>
<td>NNMBA-1</td>
<td>1.131</td>
<td>0.342</td>
<td>0.981</td>
</tr>
<tr>
<td>NNMBA-2</td>
<td>1.502</td>
<td>0.413</td>
<td>0.902</td>
</tr>
<tr>
<td>NNMBA-3</td>
<td>1.732</td>
<td>0.486</td>
<td>0.980</td>
</tr>
<tr>
<td>NIPAM-1</td>
<td>1.131</td>
<td>0.342</td>
<td>0.981</td>
</tr>
<tr>
<td>NIPAM-2</td>
<td>0.757</td>
<td>0.201</td>
<td>0.974</td>
</tr>
<tr>
<td>NIPAM-3</td>
<td>1.251</td>
<td>0.339</td>
<td>0.976</td>
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</table>

**CONCLUSION**

Novel types of thermo-responsive Acebutolol hydrochloride poly(N-isopropylacrylamide-co-caprolactam) microspheres were prepared by dispersion polymerization using sodium dodecylsulphate as a surfactant. Acebutolol hydrochloride, a hypertensive drug, was chosen as model drug to investigate the percentage of cumulative release using the developed matrices. The microspheres prepared were characterized by scanning electron microscopy, X-ray diffractometry and scanning electron microscopy. DSC indicated that ABH is molecularly distributed in the microspheres, which exhibited a prolonged release of ABH over an extended period of time. In the dry state, the size of microspheres exhibited and differentiated the % of cumulative drug release by varying the temperature from 25°C to 37°C. The prepared microspheres have thus shown thermo-responsive trends during in vitro drug release studies of acebutolol hydrochloride when dissolution experiments were performed at 25°C and 37°C.

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