NEW POLY(ACRYLIC ACID)-BASED FUNCTIONAL NANOGELS AS SUPPORTS FOR DOXORUBICIN LOADING AND RELEASE

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ABSTRACT

Nanogels are emerging as excellent supports for site specific drug delivery. Doxorubicin is a chemotherapeutic drug used for treatment of a varied range of cancers. A series of poly(acrylic acid) [poly(AAc)] nanogels using four different crosslinkers(-cl-), divinyl benzene (DVB), N,N-methylenebisacryl amide (N,N-MBAAm), ethyleneglycol dimethacrylate (EGDMA), and tripropylene glycol dimethacrylate (TPGDA) were synthesized. Characterization of these nanogels was carried out by X-Ray diffraction and Fourier transformed infra red spectroscopy to get evidence of the network formation. The swelling studies of the nanogels were also carried out to undermine their therapeutic applications. The well characterized nanogels were used for doxorubicin loading and release studies. It was observed that poly(AAc)-cl- EGDMA showed the highest % uptake of 98.7 % within 5h and a sustained release over 240h.

Key words: Doxorubicin, Nanogel, Release, Swelling, Uptake

1. INTRODUCTION

Nanogels are the most important classes of hydrogel nano-particles. Recently nanogels have emerged as very important materials for use in advanced technologies. The crosslinking in nanogels is achieved either by physical associations such as hydrophobic interactions, hydrogen bonding, and ionic interactions between the polymers or blends and interpenetrating networks of two dissimilar polymers or by covalent chemical cross-linkage, or combination of both [1]. The nano-size systems have many advantages owing to the small size and large surface area. The later attribute offers the advantage of multiple functional groups which makes nanogels potential entities for the functionalization to be carried out to obtain stimuli-sensitive materials. The availability of large number of these groups act as untapped reserves of potential binders for drugs, enzymes and bio-separates [2-4] and sensors [5,6]. Thus the nano-sized systems or nanogels become tools for the targeted applications. The size of the nanogel plays a great role in exploiting its applicability in drug delivery. The ability of the nanogel to penetrate into the cell and...
release the drug without any harmful side effects is its greatest merit. These characteristic properties make such materials the first choice for use in drug delivery to the cells and can be explored further for various applications. Nano-systems based on polymers can be engineered to achieve a controlled drug release or burst release, they can be made stimuli responsive to intracellular environment, directly released to the target site through injection and above all the small size makes them feasible for entry into the target cells [7]. The use of nanogels as drug delivery devices [8-15] to carry drugs to the innermost of the area in the human body and for controlled drug release [10,16] is appreciably explored. Work has been reported on the use of nanogels for loading anti-cancer drugs [17-24] and tumor cell imaging [25,26]. Chitosan-based nanogels have been synthesized and used as devices for controlled anti-cancer drug delivery [10]. These nanogels exhibited colloidal plus structural stability and pH responsiveness. These were found to be less cytotoxic and capable of releasing the anti-cancer drug at a low pH range of 5 to 7.4. Chitin-based nanogels have been studied for uptake and release of doxorubicin, an anti-cancer drug, to the cancerous cells [17].

Keeping in view the forgone discussion a series of poly(AAc)-based nanogels were synthesized using four different crosslinkers. These nanogels were subjected to swelling studies to determine their applicability and subsequently used for loading and release study of doxorubicin.

2. MATERIALS AND METHODS

2.1 Materials

Acrylic acid (AAc), ethylene glycol dimethacrylate (EGDMA) (Merck, India), divinyl benzene (DVB), N,N-methylene bisacrylamide (N,N-MBAAm), tri-propylene glycol di-methacrylate (TPGDA), ammonium persulphate (APS), 1-dodecanol, potassium dihydrogen phosphate, dipotassium hydrogen phosphate (S.D Fine, Mumbai, India), sodium hydroxide, sulphuric acid (S.D Fine, Mumbai, India) and Doxorubicin hydrochloride injection I.P. (50 mg, CYTOMED, ALKEM, Alkem Laboratories Limited) were used as received.

2.2 Synthesis of nanogel

The poly(AAc)-nanogels were synthesized by surfactant free emulsification method. The synthesis was achieved by first synthesizing the poly(AAc) hydrogel which was later converted to nanogel by modifying an earlier reported method [28]. The synthesized nanogels were designated as poly(AAc)-cl-DVB, poly(AAc)-cl-N,N-MBAAm, poly(AAc)-cl-EGDMA and poly(AAc)-cl-TPGDA where -cl– stands for crosslinked.

2.3 Characterization

The characterization of the synthesized poly(AAc)-nanogels was carried out by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and particle size analysis to get evidence for determination of reaction progress and the nanogel formation. FTIR spectra
of the nanogels was recorded on Perkin Elmer in transmittance mode in KBr. X-ray diffraction patterns were recorded on Philips PANANALYTICAL X'PERT-PRO X-ray diffractometer using a wavelength of 1.54060 Å (Cu- Kα radiation). The diffraction angle 2θ was varied from 10 to 70º. The particle size analysis of the synthesized nanogels was carried out on the Nanotrac wave (Microtrac), particle size analyzer, Metrohm, USA.

2.4. Doxorubicin Loading on the Nanogels

The loading of doxorubicin onto the poly(AAc)-cl-DVB, poly(AAc)-cl-N,N-MBAAm, poly(AAc)-cl-EGDMA and poly(AAc)-cl-TPGDA nanogels was carried out in 7.4 pH saline phosphate buffer solution at 37 °C. This pH was chosen as the drug shows maximum solubility at this pH. 1000 ppm solution of doxorubicin was prepared in 7.4 pH saline phosphate buffer and the loading was achieved by immersing 150 mg of the nanogel in 20 mL of 1000 ppm doxorubicin solution at 37 °C for 5h. The O.D values of doxorubicin were read from the standard curve prepared at 490 nm. Doxorubicin uptake was expressed as % uptake (PU):

\[ P_U = \frac{C_o - C_e}{C_o} \times 100 \]

Where \( C_o \) is the initial concentration of doxorubicin and \( C_e \) is the final or reject solution concentration.

The amount of doxorubicin loaded was calculated using the following formula:

\[ q_e = \frac{C_o - C_e}{W} \times V \]

\( q_e \) is the amount of the drug loaded onto the nanogel, \( W \) is the weight of the nanogel taken and \( V \) is the volume of the doxorubicin solution taken.

2.5. Doxorubicin Release from the Nanogels

The doxorubicin loaded nanogels dox-lod-poly(AAc)-cl-DVB, dox-lod-poly(AAc)-cl-N,N-MBAAm, dox-lod-poly(AAc)-cl-EGDMA and dox-lod-poly(AAc)-cl-TPGDA were subjected to release studies at 37 °C in 6.8 pH saline phosphate buffer for a period of 10 days (240 h). The release study was carried out by immersing 20 mg of the dox-lod-nanogel in 10 mL of 6.8 pH saline phosphate buffer at 37 °C for a particular time and taking the reading at 490 nm after particular time intervals. The % release (Pr) was calculated using the following formula:

\[ P_r = \frac{C_e}{C_o} \times 100 \]
Where $C_o$ is the total concentration of doxorubicin loaded and $C_e$ is the concentration of drug released.

The amount of doxorubicin released was calculated using the following formula:

$$q_e = \frac{C_e}{W} \times V$$

$q_e$ is the mg the drug released/g from the doxorubicin-loaded nanogel, $W$ is the amount of drug loaded onto the nanogel, $C_e$ is the concentration of drug released and $V$ is the volume of the solution taken.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Nanogels

The Fourier transform infra spectroscopy (FTIR) analysis of the nanogels revealed a crosslinked nanogel polymer synthesis [28-30]. The XRD pattern of the poly(AAc)-cl-TPGDA nanogel revealed the crystalline nature. In the SEM images of the nanogels, we get larger size images giving details of micro-gel morphology. The particle size analysis of the nanogels was carried out using hexane as the dispersion medium with observed nanogels being less than 10 nm in size [30]. The particle size analysis data has been presented in Figure 1.
3.2. Doxorubicin Loading on the Nanogels

The uptake studies were carried out over a time period of 5h with the nanogels showing maximum uptake after 2.30 h. A very high loading % of 98% was observed for poly(AAc)-cl-EGDMA whereas poly(AAc)-cl-DVB, poly(AAc)-cl-N,N-MBAAm, and poly(AAc)-cl-TPGDA nanogels exhibited an uptake of 89%, 96% and 80% respectively. The data obtained has been plotted in Figure 2. These results indicated that the synthesized nanogels are excellent materials for loading of doxorubicin (Scheme 1).
Figure 2: $P_u$ (Percent Uptake) of doxorubicin by poly(AAc)-$cl$-DVB, poly(AAc)-$cl$-$N,N$-MBAAm, poly(AAc)-$cl$-EGDMA and poly(AAc)-$cl$-TPGDA nanogels.
Scheme 1: Graphical representation of doxorubicin loading and release from the nanogels.

3.3. Doxorubicin Release from the Nanogels

The doxorubicin loaded nanogels dox-lod-poly(AAc)-cl-DVB, dox-lod-poly(AAc)-cl-N,N-MBAAm, dox-lod-poly(AAc)-cl-EGDMA and dox-lod-poly(AAc)-cl-TPGDA exhibited a sustained release over a period of 10 days (240 h). The release study revealed that the dox-lod-poly(AAc)-cl-TPGDA and dox-lod-poly(AAc)-cl-EGDMA showed a better pattern for sustained release of the drug from the matrix whereas the overall percent release remained almost comparable for all the nanogels with a high % release of 95% (Figure 3). The high release ratio indicates that the synthesized materials are capable of releasing the drug at a desired pH in a sustained manner and this makes them an excellent material of choice for uptake and release of doxorubicin.
Figure 3: % Release of doxorubicin from dox-lod-poly(AAc)-cl-DVB, dox-lod-poly(AAc)-cl-N,N-MBAAm, dox-lod-poly(AAc)-cl-EGDMA and dox-lod-poly(AAc)-cl-TPGDA nanogels.

4. CONCLUSION

A new series of nanogels are reported which act as an excellent material for uptake and release of doxorubicin. Doxorubicin in an anticancer drug requiring releases at a specific pH and these material exhibits the capability to do exactly the same. The high percent of uptake and release exhibited by dox-lod-poly(AAc)-cl-TPGDA and dox-lod-poly(AAc)-cl-EGDMA make them potential candidate for sustained release of doxorubicin.

REFERENCES


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