Original Research Article **Novel drug delivery system**

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PREPARATION AND CHARACTERIZATION OF CHITOSAN/ POLYETHYLENE GLYCOL DENDRIMERIC POLYMER COMPOSITE FOR CONTROLLED RELEASE OF CYCLOPHOSPHAMIDE DRUG

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ABSTRACT

Despite advances in polymeric nanoparticles (NPs) as effective delivery systems for anticancer drugs, rapid clearance from blood and poor penetration capacity in heterogeneous tumors still remain to be addressed. Here, novel chitosan hydrogels were successfully prepared by chitosan /polyethylene glycol with G (5)-NH_2 PAMAM dendrimer. The structure and morphology of hydrogels were characterized by XRD and scanning electron microscopy (SEM). The swelling properties of the hydrogels were investigated in solutions of pH 1.0 and 7.4. The hydrogels showed good swelling capacities and pH-sensitive swelling properties. The swelling studies have been carried out at different drug loading. Swelling study is an important parameter to predict the diffusion of the drugs from the matrix. The drug release was investigated at different pH medium, and it was found that the drug release depends upon the pH medium as well as the nature of matrix.

KEYWORD: Chitosan, PEG, Polyamidoamine, dendrimer, Cyclophosphamide, drug delivery.

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INTRODUCTION

Out of many routes for drug delivery, the oral route is the most preferred one[1-3] probably because of patience preference. For many existing and new drugs such as therapeutic peptides, peptidomimetics, oligonucleotides and others, oral bioavailability is in many cases below acceptable levels. To overcome this problem and to guarantee a sufficient high oral uptake, the use of efficient oral drug delivery systems is important. Transport of a dendrimer through the epithelial layer of the gastrointestinal tract depends upon the dendrimer's characteristics. Housing a drug inside a soluble dendrimer host not only solubilizes it but also allows it to bypass using a transporter protein for movement from the intestinal tract into the blood. Often drugs are not compatible with use of the protein transporter system that is designed to pass nutrients. The oral route using dendrimers looks very promising especially with anticancer and antihypertensive drugs[4-8] .To achieve positive results in the encapsulation and release of a guest drug, suitable dendrimer-guest partners must be carefully selected. For example, the complexation of opposite charged PEG block copolymers with cationic amino methacrylates or anionic styrene sulfonates has been explored[9]. Polymer-drug partners with specific acid-base interactions between hydrophobic drug molecules (R1–COOH) and polymer segments (NH_2–R_2) improved the drug loading capacity[10]. Hydrogen bonding formation between the guest drug and the host polymer has also been explored[11]. Another innovative way to deliver a drug conjugated to or adhering to a dendrimer is to further conjugate the dendrimer to aptamers[12]. The aptamers can be selected to bind to specific cell types, such as cancer or other disease cells with different cell-surface biomarkers. For example, carboxy-coated PAMAM dendrimers were conjugated to amino groups of the aptamers by forming activated esters from the carboxy groups[13] . Such an approach could easily be adapted to carboxy-coated polyester dendrimers that would have the advantage of having low toxicity and biocompatibility associated with polyester dendrimers.Therefore, in this study preparation and characterization of chitosan/PEG- G (5)-NH_2 PAMAM for controlled release of anticancer drug Cyclophosphamide.

MATERIALS & METHODS

Cyclophosphamide (CPA) was purchased from Biogenix India, PAMAM G(5)-NH_2 (Polyamidoamine generation 5 dendrimers) was purchased from Sigma Aldrich, USA. Chitosan (CH), polyethylene glycol (PEG), Gelatin (G), acetic acid and methanol were and other chemicals were used as analytical grade and purchased from Sigma Aldrich Company.

Preparation of CH-PEG-PAMAM

The preparation of chitosan solution as reported earlier. CH was purified by dissolving it in 2% acetic acid solution under constant stirring for 24 h. For CH-PEG bleand combinations, PEG was added to the CH solution under continuous stirring. The amount of PEG was kept constant as 50% of the CH content in the blended compositions. PAMAM dendrimers were prepared at room temperature, 20 mg of generations G (5)-NH_2 PAMAM of 0.1 M phosphate buffer (pH 9.0) were added to CH-PEG blend and stirred for 24 h[14] .

Drug loading

Required amount of denrimer was taken in 5 ml of acetic acid. The mixture was continuously stirred with a mechanical stirrer. Cyclophosphamide of different loadings, i.e., 10 wt %, 20 wt %, 30 wt %, 40 wt %, 50 wt % were then added to the above mixture and stirred for 1 h and then the composites were kept at room temperature for drying[15] .

Dissolution experiments

Dissolution experiments were performed at 37^0C using the dissolution tester (Disso test, Lab India, Mumbai, India) equipped with six paddles at a paddle speed of 100 rpm. About 900 ml of phosphate buffer solution (pH 3.4 and 7.4) was used as the dissolution media to stimulate gastrointestinal tract (GIT) conditions. A 5 ml aliquot was used each time for analyzing the Cyclophosphamide content at a fixed time interval. The dissolution media was replenished with a fresh stock solution. The amount of Cyclophosphamide released was analyzed using a UV spectrophotometer (Systronics, India) at the k max value of 287 nm.

Characterization

X-Ray Diffraction (XRD)

The change in gallery height of the blend was investigated by WAXD experiments, which were carried out using an X-ray diffractometer (BEDE D-3 system) with Cu Kα radiation at a generator voltage of 40 kV and a generator current of 100 mA. Samples were scanned from 2θ = 1-10^o at a scanning rate of 2^o /min.

Scanning Electron Microscopy (SEM)

The nanocomposite containing different concentrations was characterized using SEM (440, Leica Cambridge Ltd., Cambridge, UK). The powdered specimens were placed on the Cambridge standard aluminium specimen mounts (pin type) with double-sided adhesive electrically conductive carbon tape (SPI Supplies, West Chester, PA).

Swelling studies

Water absorption of the polymer-drug conjugates was measured following absorption standard test method ASTM D 570-81. The samples were preconditioned at 50^0C for 24 h and then cooled in a desiccator before being weighed. The preconditioned samples were submerged in distilled water at 25^0C for 24 h.The samples were removed and dried . Water absorption was calculated as a percentage of initial weight. The soluble material loss was checked by weighting the specimens after drying them in an oven at 50^0C for another 24 h. The total water absorption for 24 h was calculated including the soluble material loss.

$$\% \text{ swelling} = \frac{W_1 - W_2}{W_2} \times 100$$

Where, W_1 = weight of swollen composite after 24 h, W_2 = weight of dry composite

RESULT AND DISCUSSION

XRD

The reflections for PEG were at 2θ = 19.5°, 24°, 26.7° and 33.3°.The peaks around 2θ = 11.7°, 14.2°, 17°, 18.6° and 23° should be assigned to PEG/ G (5)-NH_2 PAMAM /CH: the peak around 2θ = 11.7° corresponding to crystalline structure. As indicated, the gradual disappearance of amorphous for samples PEG/ G (5)-NH_2 PAMAM /CH would promote the crystallization of chitosan polymer complex[16].

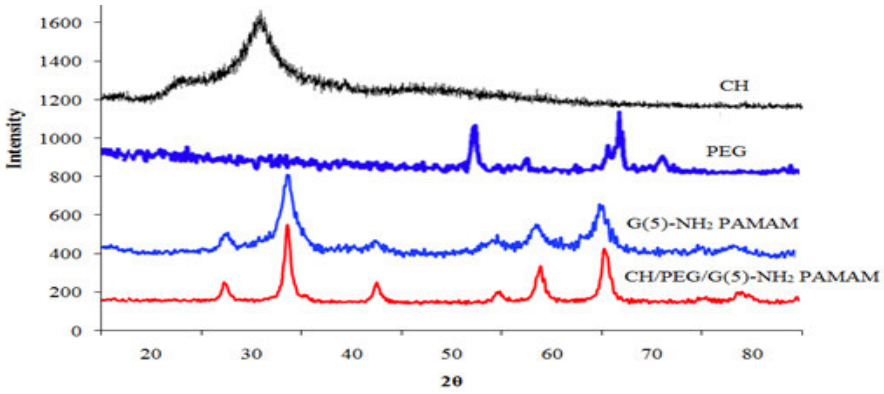

Figure 1
XRD of PEG,CH and CH/PEG/ G (5)-NH_2 PAMAM

SEM

In figure 3 shows SEM analysis of CH, CH-PEG and CH/PEG/ G (5)-NH_2 PAMAM. It was found that the polyelectrolyte complex and appears homogenous, indicating a uniform distribution and a good compatibility between CH-PEG and CH/PEG/ G (5)-NH_2 PAMAM. CH/PEG/ G (5)-NH_2 PAMAM showed the highest tapped densities due to their compact morphology.

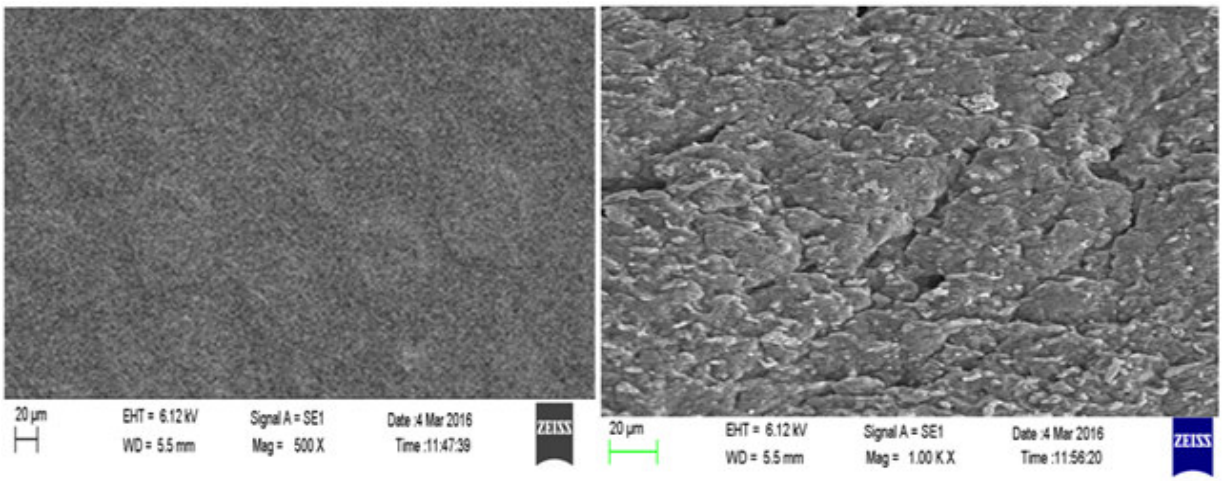

Figure 2
SEM of PEG, CH and CH/PEG/ G (5)-NH_2 PAMAM.

Swelling studies

The swelling behaviour of any polymer network depends upon the nature of the polymer, polymer solvent compatibility and degree of cross-linking. However, in the case of ionic networks, swelling behavior depends upon mass transfer limitations, ion exchange and ionic interaction. Fig. 3 represents the percentage of swelling (in terms of bar representation) for the CH/PEG/ G (5)-NH_2 PAMAM blend with different drug loadings (pH 7.4) at 37^{0}C. This indicates that the percentage swelling increases with increase of drug loading in the CH/PEG/ G (5)-NH_2 PAMAM composite[15].

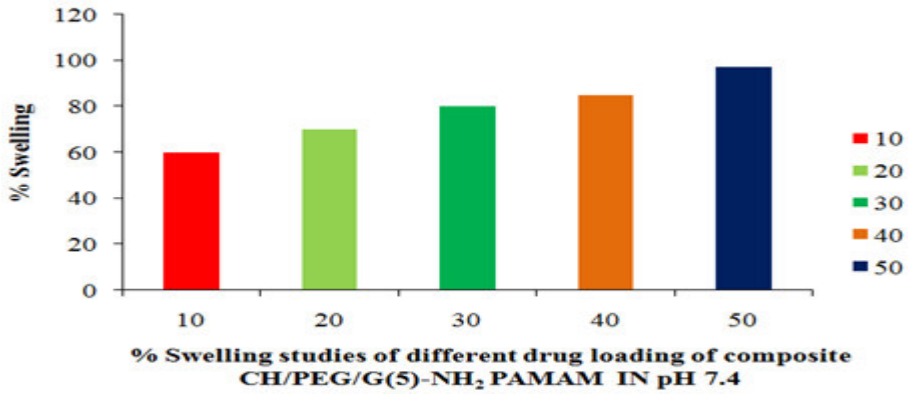

Figure 3
Water absorption of the CH/PEG/ G (5)-NH_2 PAMAM blend composite with different % drug loadings

In-vitro drug release
Effect of pH

In order to investigate the effect of pH on the swelling of composite CH/PEG/ G (5)-NH_2 PAMAM, we have measured the % cumulative release in both pH 3.4 and 7.4 media. Cumulative release data presented in Fig. 4 indicate that by increasing the pH from 3.4 to 7.4, a considerable increase in the cumulative release is observed for all composites. From Fig. 4a and b, it is seen that the 50% drug– polymer composites have shown longer drug release rates than the other composites. Thus, drug release depends upon the nature of the polymer matrix as well as pH of the media. This suggests that the drugs in the blend can be used to be suitable for the basic environment of the large intestine, colon and rectal mucosa for which there are different emptying times.

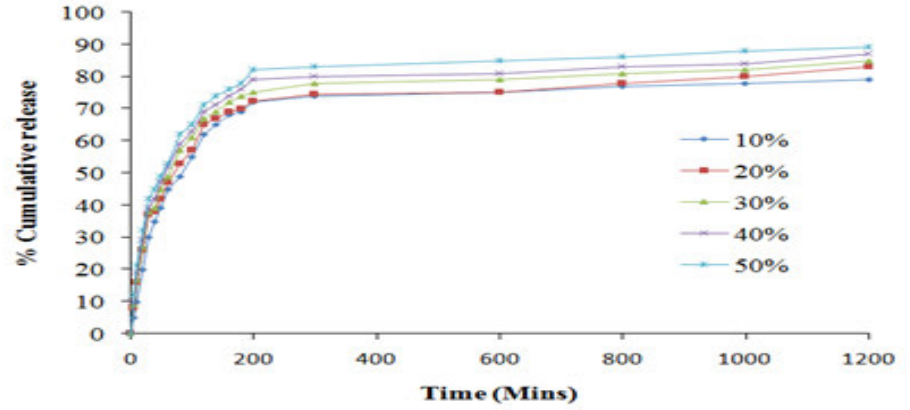

Figure 4a
% Cumulative release vs. time for different formulation loaded with CS CH/PEG/ G (5)-NH_2 PAMAM in pH 7.4

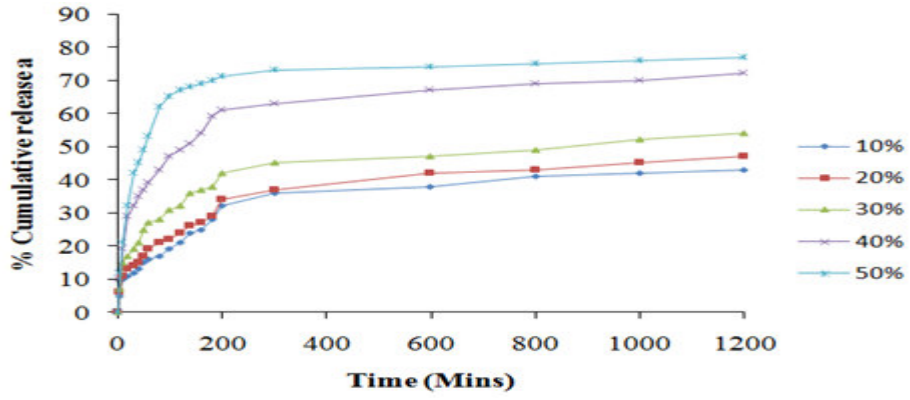

Figure 4b
% Cumulative release vs. time for different formulation loaded with CS CH/PEG/ G (5)-NH_2 PAMAM in pH 3.4

CONCLUSION

The interaction of components in the chitosan/PEG/ G (5)-NH_2 PAMAM films had been investigated. There is a strong molecular interaction between chitosan and PEG, so that the original crystal structures of chitosan and PEG have been changed. From these studies the homogeneity of the blends has been predicted. Swelling study is an important parameter to predict the diffusion of the drugs from the matrix. The percentage of swelling increases with increase in the percentage of drug loading. The drug release depends upon the nature of the polymer matrix as well as pH of the media. Further studies should focus on the interaction between drugs and gene agents, as well as the interaction between therapeutic agents and carriers. Continuous development of such combination delivery systems will ultimately lead toward availability of effective therapies for cancer.

CONFLICT OF INTEREST

Conflict of interest declared none.

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