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Development of chitosan–poly(acrylic acid) nanoparticles for delivery of 5-fluorouracil

Deepak Kakde, A. Parashar ,V. Chadhar, R. Kakde , A. T. Patil

Deepak Kakde, R. Kakde , A. T. Patil
Department of Pharmaceutical Science, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur- 440033.

Deepak Kakde, A. Parashar , V. Chadhar
Bhopal Institute of Technology and Sciences- Pharmacy, Bhopal

Correspondence:
Deepak Kakde
Department of Pharmaceutical Science, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur- 440033.
E mail: dipakkakde31@gmail.com

ABSTRACT

The present study was aimed at developing and exploring the use of chitosan–poly(acrylic acid) nanoparticles for delivery of anti-cancer drug 5-fluorouracil. Chitosan (CS)–poly(acrylic acid) (PAA) complex nanoparticles, have been prepared by dropping method. The physicochemical properties of nanoparticles were investigated by using,FT-IR,dynamic light scattering, transmission electron microscope and zeta potential. TEM study revealed surface properties of the systems. It was also observed that the prepared nanoparticles carried a positive charge with the size in the range from 100 to 210 nm at pH 4.5. The system was found to have good drug-loading capacity with reduced drug release rate. The encapsulation efficiency of nanoparticle was found to be 78% at pH 4.5. The release rate of drug was pH dependent and found to be more sustaining in pH 4.5 while at pH 7.4, nanoparticles have core shell fuzzy structure. However, the system was suitable for prolonged delivery of an anti-cancer drug by in vitro.

Keywords: Drug delivery; Chitosan; Poly(acrylic acid); Nanoparticles

1. INTRODUCTION

Chitosan (CS), a kind of nature polysaccharide, having structural characteristics similar to glycosaminoglycans, is non-toxic and biodegradable[1], which has wide applications in the pharmaceutical and biomedical fields [2–4]. Chitosan (CS), a (1-4)2-amino 2-deoxy b-D-glucan, is a deacetylated form of chitin, an abundant polysaccharide present in crustacean shells. Recently the use of complexation of oppositely charged macromolecules to prepare Chitosan (CS) complexes and nanoparticulate structures as controlled drug release formulations has attracted much attention [5–9], because this process is simple, feasible, and can usually be performed under mild conditions. Further, these hydrophilic nanoparticles are good carrier for hydrophilic drug (e.g. 5- fluorouracil (5FU), protein and peptides. 5- fluorouracil (5FU) has been widely used in the therapy of different solid tumor types such as cancer of the stomach, liver, intestine, and so on. Because of the short plasma half-life of 10–20 min, high doses, e.g. 400–600 mg/m^2, have to be administered weekly, to reach a therapeutic drug level [10]. To overcome the delivery problems and limitations, 5FU-Chitosan (CS)–poly(acrylic acid) (PAA) nanoparticulate system has been designed. The purpose of present study was to obtain stable complexes of nanoparticles from Chitosan (CS) and poly(acrylic acid) (PAA) which could embed 5FU with a relatively load capacity. Furthermore, the preliminary loading capacity, encapsulation efficiency and release properties of the CS–PAA nanoparticles have been studied in vitro.

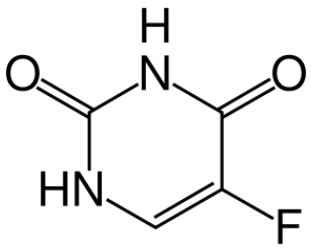

Fig 1: Chemical structure of 5-Fluorouracil (5FU)

2. MATERIALS AND METHODS

Materials

Chitosan and Acrylic acid (AA) were obtained from Sigma Chemical Co. (St. Louis, MO) and the drug 5-FU was obtained as gift sample from Roche, Switzerland. Cellulose dialysis tubing of MWCO 12000–14000 was purchased from HiMedia Lab, India. Rest all the chemicals were purchased from CDH, India.

Preparation of CS–PAA nanoparticles

CS–PAA nanoparticles were prepared by mixing positively charged CS and negatively charged PAA with dropping method reported by Hu et al. [11]. Briefly,1 ml 0.02% CS solution (CS with a molecular weight being 80 kDa was dissolved in 1% (w/v) acetic acid solution) was added dropwise into 5ml 0.02% PAA (Mn=100 kDa) aqueous solution under magnetic stirring. The opalescent suspension was formed which was then filtered by paper filter and incubated in a buffer solution of pH=4.5 for 24 hr.

FT-IR spectrum analysis

FT-IR spectra were measured by a Bruke IFS 66V vacuum-type spectrometer to determine the chemical interaction between CS and PAA. The CS–PAA nanoparticles were frozen and lyophilized to obtain dried CS–PAA nanoparticles. These nanoparticles were mixed with KBr and pressed to a plate for measurement.

Transmission electron microscopy

Transmission electron microscopy (TEM) (JEOL TEM-100, Japan) was used to observe the morphology of the CS–PAA nanoparticles. Samples were placed onto copper grill covered with nitrocellulose. They were dried at room temperature and then were examined using a TEM without being negative stained.

Particle size and zeta potential of CS–PAA nanoparticles

The zeta potential of the CS–PAA nanoparticles were measured on Zetasize 3000 HS. (Malvern,UK) . The mean size and size distribution of the CS–PAA nanoparticles were measured by dynamic light scattering (DLS) (Zetasize; 3000 HS,Malver n,UK). All DLS measurements were done with a wavelength of 633.0 nm at 25°C with an angle detection of 90°.

Preparation of drug loaded CS–PAA

The drug-loaded nanoparticles were prepared by dissolving 50 mg of 5-FU in 50 ml CS–PAA nanoparticlate with the help of magnetic stirrer and incubated for 48 h. Then, these nanoparticles were separated from the aqueous phase by ultracentrifugation (Ultra ProTM 80, Du Pont) with 50,000 rpm at 41^{o}C for 40 min. Next, the gained 5-FU loaded CS–PAA nanoparticles were washed by acetone three times, frozen and lyophilized to obtain dried 5-FU loaded CS–PAA nanoparticles.

Determination of encapsulation efficiency and loading capacity

To determine the encapsulation efficiency (EE) and loading capacity (LC), nanoparticles suspension was twice dialyzed under strict sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically to determine indirectly the amount of drug bound with the system. The dialyzed formulations were lyophilized and used for further characterization. 5FU encapsulation efficiency (AE) and loading capacity were calculated with the following equation:

$$EE = \frac{\text{The amount of 5FU in the nanoparticles}}{\text{Total amount of 5FU}} \times 100$$

$$LC = \frac{\text{The amount of 5FU in the nanoparticles}}{\text{Total amount of nanoparticle weight}} \times 100$$

In vitro drug release from the nanoparticles

A known amount (50mg) of 5FU-loaded CS–PAA nanoparticles were redispersed in 10 ml distilled water and placed in a dialysis membrane bag with a molecular cut-off of 10 kDa, tied and placed into 250 ml of water medium with various pH (3 and 7.4) values on sink conditions. The entire system was kept at 37^{o}C with continuous magnetic stirring. After a predetermined period, sample was removed and the amount of 5FU was analyzed by spectrophotometer.

3. RESULTS AND DISCUSSION

The CS–PAA nanoparticles were prepared by mixing positively charged CS and negatively charged PAA with dropping method. The complex was confirmed by IR analysis where, the intensities of amide band I at 1660 cm^{-1} and amide band II at 1580 cm^{-1}, which has clearly present in pure chitosan, decrease significantly, and two new absorption bands at 1731 and 1628 cm^{-1}, of the carboxyl groups of PAA (the absorption peak of carboxyl groups in pure PAA appears at 1740 cm^{-1}), and the NH_3+ absorption of CS, respectively, were observed. The broad peaks appeared at 2500 and 1900 cm^{-1}also confirmed the presence of -NH_3+ in CS–PAA nanoparticles (Fig. 2). When dropping CS into PAA solution,CS–PAA nanoparticles with a CS core and PAA membrane was formed. In acetic buffer solution at a pH value of 4.5, CS does not swell, hence there are no cavities formed in CS–PAA nanoparticles resulted in spherical CS–PAA nanoparticles (Fig. 3 (a) have a matrix like structure. Table 1 shows mean particle size and zeta potential of CS–PAA complex nanoparticles indicate a positive zeta potential with mean particle diameter of 208±30 nm. However, these nanoparticles in PBS at a pH of 7.4 shown in Fig. 3(b) reveal a compact core bounded by a spread and fuzzy coat having mean diameter of 665±112nm. This results indicate that as pH increases, the size of the nanoparticles also increases (Table 2). At pH 4.5, CS and PAA are partly ionized. The partly ionized CS and PAA can form compact polyelectrolytes complex by ionic interaction,which results in a solid matrix structure. At pH values of 7.4, the morphology of nanoparticles was changed because of the difference in the solubility of CS and PAA. At this pH value, CS was insoluble while PAA was highly swollen, which results in the phase separation of nanoparticles,result in core-shell-like structure where CS just physically coated with PAA. These results also help to understand the release of the drug from the nanoparticles. The encapsulation efficiency of nanoparticle was found to be 78% at pH 4.5. Fig. 4 shows the release profiles of 5FU from CS–PAA nanoparticles with various time intervals in various pH values release media at 37°C. An initial fast release followed by a slow release of 5FU occurred in pH values of 4.5 whereas at pH 7.4, initial burst followed by sustain release was observed. When compare both the pH value for drug release, pH 4.5 was found to be more sustain than pH 7.4 due to more stable nanoparticle formulation at this pH

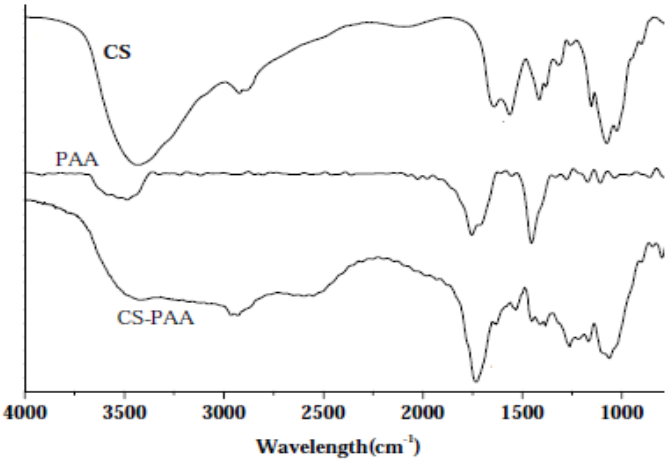

Fig 2: FT-IR spectra of CS, PAA and CS–PAA

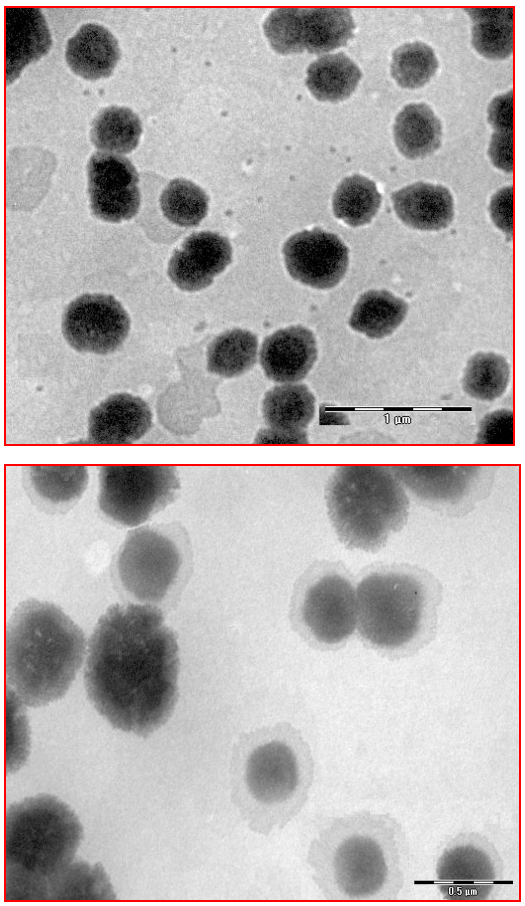

Fig 3: Electron transmission microphotography of CS–PAA nanoparticles at (a) pH=4.5 and (b) at pH=7.4.

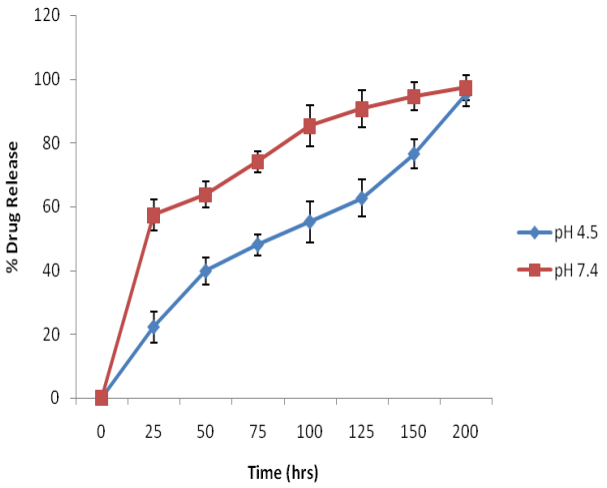

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Fig 4: Release profiles of 5-FU from CS–PAA nanoparticles at various pH values at 37°C (n = 3).

Table 1: Mean particle size and zeta potential of CS–PAA complex nanoparticles

S. No.	Parameters	Value
1.	Mean diametera (nm)	208±30
2.	Polydispersity	0.161±0.008
3.	Zeta potential (mV)	+24.2±2.9

Table 2: The mean diameter of CS–PAA nanoparticles under various pH values

S. No.	pH	Mean diametera (nm)
1.	4.5	208±30
2.	7.4	665±112

4. CONCLUSION

The system shows remarkable advantage of being solely made of hydrophilic polymers: chitosan and poly(acrylic acid),which are non-toxic, and biodegradable. Release experiments indicate that this system seems to be a very promising vehicle for the administration of hydrophilic drugs. Furthermore, these nanoparticles are stable under acidic and neutral conditions and can be made under mild conditions.

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