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Design and Evaluation of Cellulose-Alginate Nanoparticles as an Effective Delivery System for Controlled Release of Doxorubicin

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ABSTRACT

The main purpose of the present study was to synthesize cellulose nanoparticles with gelation of alginate core to provide excellent solubility in aqueous solution and to and characterize the resulting nanomedicine after loading with Doxorubicin, an anticancer agent. Cellulose exhibits the property of biodegradation and compatibility with body tissues and in combination with doxorubicin provides toxicity to cancer tissues. These nanoparticles were characterized under scanning electron microscopy, Fourier transform infrared spectroscopy to find topology, crystal structure and chemical purity of nanoparticles. The release of doxorubicin from cellulose-alginate nanoparticles was studied by *in vitro* method using a dialysis membrane in the presence of phosphate buffer. Under suitable conditions, the cellulose-alginate nanoparticles obtained were in size ranging between 10 and 100 nm. The *in vitro* drug release revealed the controlled release of drug from the polymer in a sustained manner. The percentage release of drug released from the dialysis bag was found to be 78% at the end of 72 hours.

KEYWORDS: Cellulose, Doxorubicin, Polysaccharide, Biocompatibility, PBS, FTIR, SEM.

Introduction

One of the effective areas of drug delivery is the design of nanoparticles that are capable to deliver drug to the specific target tissues at correct dosage and at right time (Simarpreet Kaur et al., 2011). These nanoparticles based drug delivery systems have the potential to increase drug stability and have toxic effects to cancerous cells. The drug can be loaded in to the polymeric particle which act as a carrier vehicle and it prevents drug degradation.

A polymeric nanoparticle consists mainly of synthetic polymer or natural polymer like polymer of amino acids or polymers of sugars. Natural polymers have been developed to deliver drugs as they are easily degradable and also biocompatible. Among them, cellulose is widely used in nanomedicine or controlled delivery of anticancer drugs in a sustained manner (Robert Moon et al., 2011). Cellulose is a water insoluble polysaccharide obtained from plants and it consists of linked D-Glucose units. Cellulose is also the structural component of the plant cell wall (Shoda and Sugano, 2005). These cellulose chains are hydrolyzed to produce cellulose nanoparticles of size 520nm followed by application of ultrasound to reduce particle size to 70 nm (Roderick Slavcev et al., 2011). These cellulose nanoparticles are water soluble, adhesive, biodegradable and compatible with body tissues and hence it plays a vital role as a drug delivery carrier system.

Various drugs (nifedipine, adriamycin and aromasin) have been incorporated with natural polymers which show enhanced delivery of drugs to target site, while doxorubicin shows extensive application in targeting breast cancer cells (Ahmed et al., 2003). The polymeric carriers have short biological half life period. It can be easily soluble in aqueous medium; hence it is widely used in cancer studies. While alginate, a non-toxic linear polysaccharide, is used to deliver drug in a controlled fashion (Alexis et al., 2005). The objective of this study is to investigate the cellulose alginate as a carrier vehicle or delivery of anticancer drug (doxorubicin). The hydrophobic drug is loaded in to hydrophilic polymer to enhance the stability of drug.

Materials

Sodium Alginate, cellulose was purchased from LOBA Chemicals, India. All other reagents and chemicals used are of analytical grade. All aqueous solutions were prepared by double distilled deionized water.

Preparation Methods

Preparation of nanoparticles by Desolvation method

The sodium alginate and calcium chloride solutions were prepared by double distilled deionized water. To maintain the pH level (5.1) of sodium Alginate, 0.1N hydrochloric acid was used. 1 mL of doxorubicin (1 mg/mL) was added with 5 ml of calcium chloride (3.5 mg/mL) and then the mixture of calcium chloride-Dox was added drop wise to 50 ml of aqueous sodium alginate (3 mg/mL) under stirred condition at 500 rpm or 1 hr. After 1 hr, 20 mL of cellulose was added and then stirred at 1 hr. The resultant substance was equilibrated overnight to allow nanoparticles to form uniform particle size.

Purification of Cellulose-Alginate Nanoparticles

After the preparation of cellulose-alginate nanoparticles it was purified by double distilled water. The pellet of the unpurified sample was taken out and the purification process was done by addition of water followed by centrifugation. The nanoparticle sample was centrifuged 3 times to ensure that no drug was loaded outside the polymer surface.

Characterization

SEM (Scanning Electron Microscopy)

SEM was performed to analyze the structure and chemical information about the sample and also imaging of Cellulose-alginate nanoparticles feasible. This finds application finding the loading of doxorubicin drug into the polymeric surface by coating the sample with carbon. The composition of materials was found using EPS analysis at controlled conditions.

FTIR (Fourier Transform Infrared Spectroscopy)

FTIR was done to check the purity of formulated nanoparticles. As the name indicates it uses infrared rays to analyze the samples. When the IR radiation is allowed to pass through the sample, the sample absorbs some radiation reveals the spectrum; absorption o the nanoparticles. This helps to find the purity of nanoparticles.

In Vitro Drug Release

In order to ensure the release of drug outside the body, *in vitro* drug release study was carried out using a dialysis membrane. The dialysis membrane of molecular weight 12k to 14k was used or drug release studies (Moghimi et al., 2011). First, the dialysis membrane bag was pretreated with phosphate buffer and allowed undisturbed for 15 minutes. After that 50 mg of the drug was added to the dialysis bag followed by addition of 1ml of phosphate buffer. Then the dialysis bag was kept in a 50 mL of phosphate buffer solution and the entire setup was kept in a magnetic stirrer at 100 rpm with pH 7.4. After 1:30 hrs, 2 mL of sample solution was taken out from the medium and the absorbance was checked at 266 nm. The entire setup was kept at 37 ºC to match with body temperature. The samples were withdrawn at different intervals followed by addition of fresh buffer.

Results and Disscusion

SEM (Scanning Electron Microscopy)

Scanning electron microscopy helps to observe the surface, structure and topology. The prepared nanoparticles was first coated on the carbon sheet in crystalline form and then examined under SEM. This ensures that the nanoparticle surface is smooth and it is spherical in shape and also drug is loaded into the polymeric surface as shown below with size ranges between 100-150 nm (Figure 1).

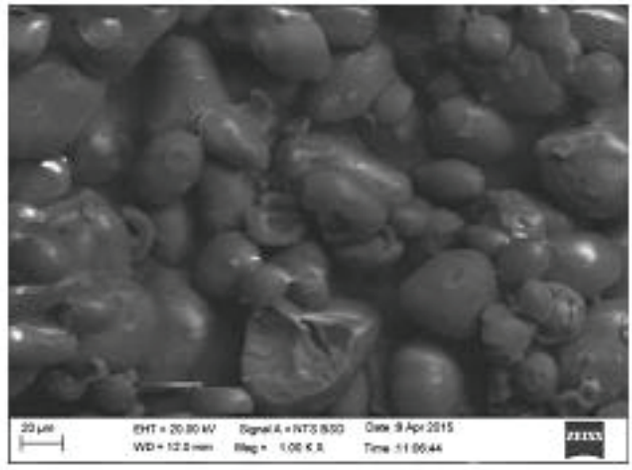

Fig. 1 Microscopic image of Cellulose-Dox under SEM.

FTIR (Fourier Transform Infrared Spectroscopy)

Fourier Transform Infrared Spectroscopy (FTIR) finds the purity of the prepared nanoparticles by determining the molecular characteristics of the nanoparticle sample. The graph below shows the purity of the nanoparticles before and after purification and also the absorption of the polymer particles alone. It describes that the samples were impure at 3305.28 cm^{-1} and pure at 2167.6 cm^{-1}, 2108.76 cm^{-1} (Figure 2).

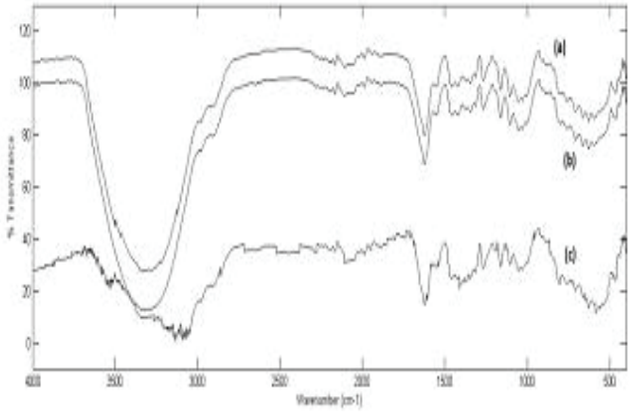

Fig. 2 FTIR result of Cellulose-Dox Nanoparticles: (a) Native Cellulose, (b) Cellulose-DoxNPs with purification (c) Cellulose-Dox NPs without purification.

In vitro drug release

The *in vitro* drug release study was carried out by using PBS (phosphate buffer solution) of pH 7.4 at 37 ºC. The cumulative absorbance of the solution ranges from 0.352 to 1.693, this indicates that the samples were released in a slow manner at initial conditions and gets increased with time due to its solubility in aqueous solution and the results were tabulated in Table 1. The Figure 3 represents the absorbance of the sample at various time intervals, which simply implies that drug release was increased over the period of time.

TABLE 1

In vitro drug release of Cellulose-Dox Nanoparticles.

Time (hrs)	Absorbance at 266 nm
1:30	0.352
3	0.269
4:30	0.396
6	0.674
48	1.218
72	1.693

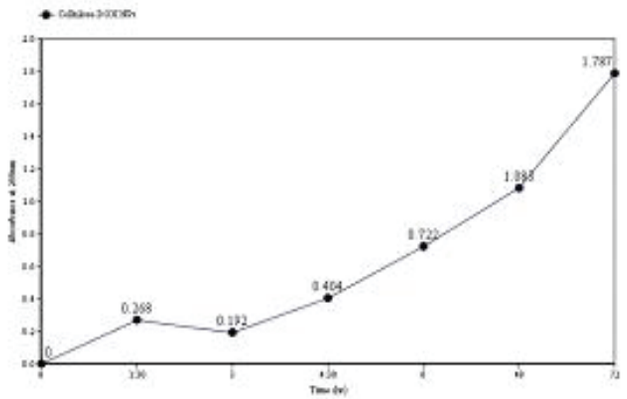

Fig. 3 *In vitro* drug release of Cellulose-Dox Nanoparticles.

Figure 3 represents the absorbance of the drug in the PBS solution. Various parameters like drug molecular weight, concentration; polymer drug composition affects the release of drug from the membrane. Cellulose due to its high solubility in aqueous solution shows an efficient release of drug from its polymeric surface. The percentage release of drug released from the dialysis bag was found to be 78% at the end of 72 hours.

Conclusions

Doxorubicin loaded cellulose nanoparticles were prepared using emulsification using calcium chloride and aqueous sodium alginate. The size of these nanoparticles ranges between 100-250 nm and also these nanoparticles was smooth and has a spherical cavity inside the surface. *In vitro* drug release studies were performed and it is estimated that 78% of the sample was released. This reveals that doxorubicin concentration, molecular weight of the polymer affects the release of nanoparticles in to the aqueous medium. Finally this cellulose polymeric particle proves that it is an excellent tool for loading anticancer drug.

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