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Formulation and characterization of starch nanoparticles for controlled release of doxorubicin

Siva Samraj Raveendran, Nagaraj Subramani, Shiek Abdul Rahman, Sasikumar Palsamy*
Department of Biomedical Engineering, PSNA College of Engineering and Technology, Kothandaraman nagar, Dindigul-624622, Tamilnadu, (INDIA)
E-mail: sasibiology@gmail.com

ABSTRACT

Nanoparticles synthesised from starch provides enhanced solubility of Doxorubicin in the aqueous solution. Various parameters like presence of organic solvent, surfactant concentration, loading efficiency, doxorubicin concentration affects the size of doxorubicin-starch nanoparticle. Under controlled conditions, doxorubicin loaded *in situ* on to the starch polymer with particle size ranging between 10nm to 100nm was achieved by nanoprecipitation method. These nanoparticles were characterized under Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) to find the surface topology, particle size, drug loaded inside the polymer, structure of DOX-starch nanoparticles. The doxorubicin loaded starch nanoparticle was observed for *in vitro* drug release study in a buffer solution mainly phosphate buffer solution for 5-7 days. This reveals that the starch polymer acts as an efficient carrier vehicle for the anticancer agents.

KEYWORDS

Desolvation;
Starch;
Doxorubicin;
Nanocapsules;
Surfactant;
Phosphate buffer.

INTRODUCTION

Nanoparticles are ultrafine particles ranging between 1 and 100 nm in size. The chemical nature of nanoparticles may be metallic or polymeric. Polymeric nanoparticles may be synthetic or natural and exhibit nanosized colloidal structures. Based on the preparation of nanoparticles, drugs can be loaded in to, on to the surface[1, 2]. In nanocapsules, the drug is entrapped by the polymeric surface surrounding the molecule. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) display the structure of these finest nanoparticles. Techniques like desolvation, emulsification and evaporation are the recent techniques used for the preparation of nanoparticles[3]. Among these, nanoprecipitation process is widely used process for the preparation of starch-doxorubicin nanoparticles. The nanoparticles must be prepared in such a way that it should be biodegradable and biocompatible to body tissues.

Natural polymers like starch exhibit biodegradability and also starch is a versatile and well known polymer which has a good drug delivery application with cells and tissues[4]. These starch nanoparticles range between 10-100nm in size. Since these are hydrophilic, easily degradable and compatible with body tissues, starch is a well preferred

polymer for this work. For instance, starch nanoparticles can be loaded with different drugs like testosterone, nifedipine etc[5]. Starch is a polysaccharide occurs mainly in plants in which it acts as a storage material. Starch mainly composed of glycopyranose which yields monosaccharide on hydrolysis[6]. Many investigations used starch nanoparticles as a transdermal drug delivery system. Starch nanoparticles are mainly prepared by nanoprecipitation, emulsification and homogenitization. Due to its simplicity and reproducibility nanoprecipitation is preferred for preparation of starch nanoparticles under controlled particle size by controlled use of surfactants and ethanol for purification[7].

In this study, starch nanoparticles were prepared by nanoprecipitation method. The loading of Doxorubicin *in situ* on to starch to form Doxorubicin loaded starch nanoparticles was alone by precipitation of the preformed polymer particles and by water oil emulsion method[8, 9]. This provides a major advantage that there is no need of any organic solvent and no rising temperature for preparation of nanoparticles. It requires only small amount of organic solvent like ethanol to remove oily residues and also surfactants during precipitation process. The type of surfactant used plays a major role in the loading efficiency of doxorubicin-starch nanoparticles as these surfactants should be non-ionic and non-toxic in nature. Also surfactant concentration determines the particle diameter[11, 12]. The anticancer drug loaded *in situ* in to starch is doxorubicin which is a widely used drug for chemotherapy in treating leukaemia, lymphomas, soft tissue carcinomas, bladder, and breast cancers. Recent investigations in anticancer research have coupled doxorubicin with monoclonal antibody to eliminate HIV-1 in mice. Doxorubicin is a vesicant (i.e.) it is a chemical that causes tissue damage if it blisters out from vein. Doxorubicin should be carefully encapsulated in to starch so that when it targets cancer tissue it does not cause damage to normal cells[13].

MATERIALS

Ethanol (98 %), Tween 80 (polysorbate 80), Starch were procured from LOBA Chemicals, India. Oil used was sunflower oil. All chemicals and reagents were used without further purification and were of analytical grade. The aqueous solutions were prepared by double distilled deionized water.

METHODS

Preparations by nanoprecipitation

Starch-Doxorubicin nanoparticles were prepared by nanoprecipitation technique. The surfactant (Tween 80- an emulsifying agent) was initially added to ethanol to form ethanolic surfactant. Then 1 ml of doxorubicin was added with 15 ml of ethanolic surfactant solution, followed by the addition of 5 ml of oil and the mixture was stirred for 2 hrs at room temperature. Then 1% starch solution was prepared by using double distilled deionized water. From that 1 ml of starch solution was added drop wise to the mixer and stirred continuously for 2 hrs, doxorubicin was loaded *insitu* with starch nanoparticles. The medium containing nanoparticles as fine aggregates was centrifugated at 15000rpm in a research centrifuge so that the doxorubicin loaded starch nanoparticles were formed as pellet leaving the supernatant above which has to be discarded. The pellet was taken out for purification followed by air dried in an incubator at 37ºC for 3 hours.

Purification of the prepared nanoparticles

The resultant pellet (unpurified) was taken out and then washed with ethanol for 5 times to remove the excess drug that gets loaded on the polymeric surface. After purification the pellet is air dried to form powdered nanoparticles.

CHARACTERIZATION

SEM

SEM was performed using ZEISS SEM. This helps to focus the sample to extract structural and chemical information of the nanoparticles and also helps to image the Starch-Doxorubicin combination. It helps to ensure the loading of drug in to the polymer. In this study the sample is coated with carbon

coating cressington units. EDS analysis can be used to find the comparison of different materials in the starch-doxorubicin nanoparticle combination.

FTIR

FTIR spectroscopy incorporates the use of infra-red radiation to find the structure of the resulting nanoparticle in the range of 50-4000cm^{-1}. This helps to find the structure of molecules and also its absorbance spectrum. By using FTIR the amount of starch present in the starch present in the nanoparticles can be investigated and the resulting spectrum reveals the characteristics of the starch-doxorubicin nanoparticles.

***In vitro* drug release**

The drug release studies were carried out in phosphate buffer solution using dialysis membrane under controlled conditions. The dialysis membrane of molecular weight ranges between 12000 and 14000 was used. Initially the dialysis bag was treated with phosphate buffer for above 15 minutes. Then 50mg of the formulated NPs were added to the dialysis bag followed by addition of 1ml buffer solution and the setup is immersed in a 50ml phosphate buffer saline solution and the pH of the phosphate buffer is 7.4. The entire setup kept at a magnetic stirrer at 100 rpm at 37ºC. After 1:30 hrs 2 ml of sample solution was withdrawn and the absorbance is measured using UV Spectrophotometer at 266 nm. After the sample is withdrawn from the setup 2ml of fresh buffer was added to the medium. The absorbance is measured at different time intervals to ensure the release of drug from the dialysis bag.

RESULTS AND DISSCUSION

SEM (Scanning electron microscopy)

Analysis of the prepared nanoparticles reveals the structure and size of nanoparticles and also loading of drug in to the polymeric surface. The nanoparticles were first coated in a carbon sheet in a crystalline form for examination under SEM. This images the loading of drug in to the polymer as shown in the figure given below with size range between 100-150nm with a smooth surface having spherical space inside.

FTIR

FTIR (Fourier Transform Infrared Spectroscopy) is done to check the purity of the prepared nanoparticles by using infrared light. The absorption spectrum of the nanoparticle sample was obtained, from that the purity was checked by plotting a graph between the wave number and transmittance. The graph shows the sample remains impure at 3013.11cm^{1} and it is pure after the purification process at 2190.74cm^{-1}, 2049.06cm^{-1}, 1550.49cm^{1}.

***In vitro* drug release**

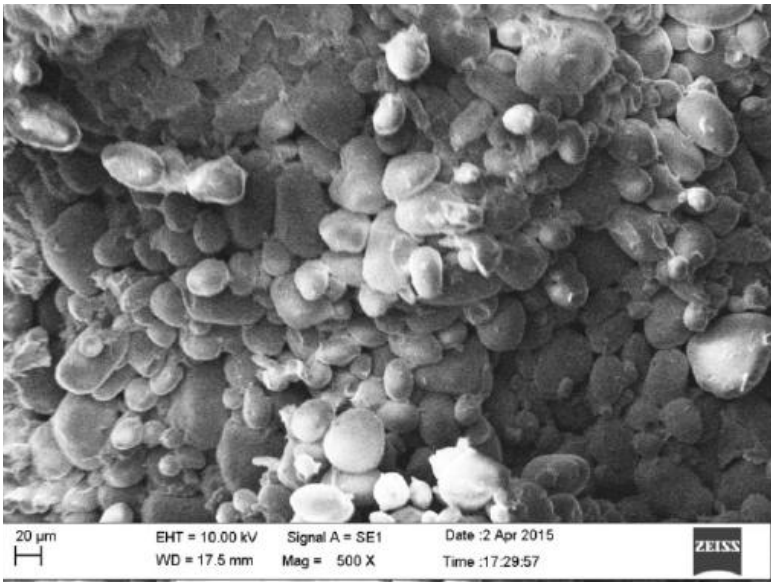

Figure 1 : Microscopic image of starch-dox under SEM

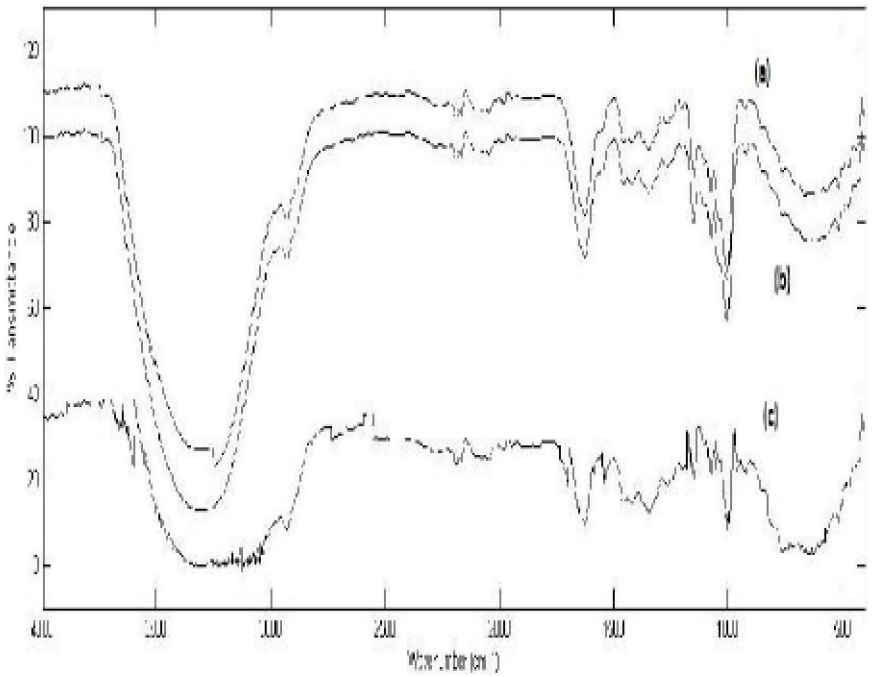

Figure 2 : FTIR result of starch-dox nanoparticles :(a) native starch, (b) starch-dox NPs with purification, (c) starch-Dox NPs without purification

TABLE 1 : *In vitro* release of starch-dox nanoparticles

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

Drug release measurement of the sample for the period of 72 hours was in the TABLE 1. The nanoparticle is put in a dialysis bag and then kept in PBS (Phosphate Buffer Solution) of pH 7.4 at 37°c and then stirred in a magnetic stirrer. The absorbance is low (0.352) at the initial period and it increases over the period of time; this indicates that the drug releases in a controlled fashion.

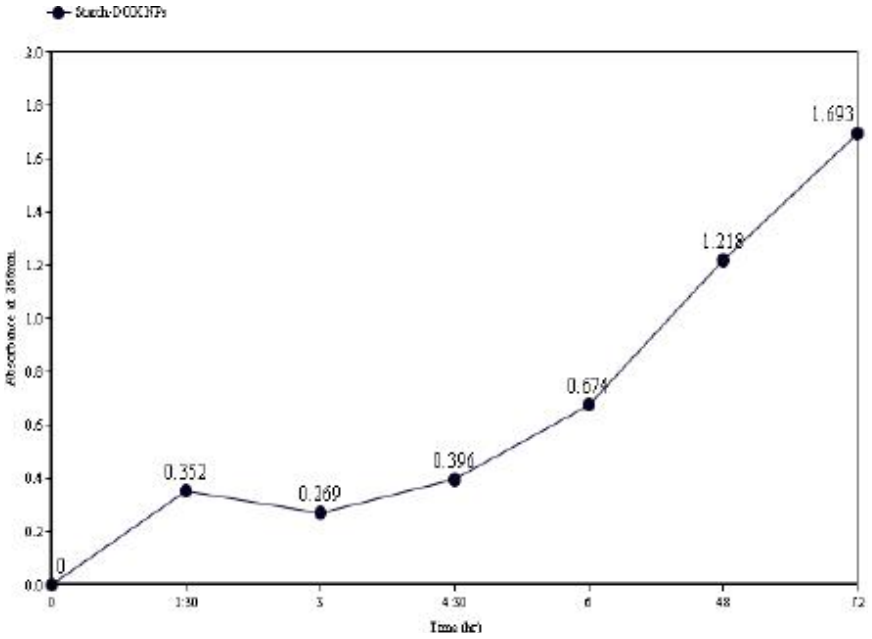

Figure 3 : *In vitro* release of starch-dox nanoparticles

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Various parameters like drug concentration, molecular weight, polymeric composition and the nature of nanoparticle affects the release of drug from the polymer. As starch is highly soluble in aqueous medium it possesses efficient release in a sustained manner. The percentage release of sample from the membrane was found to be about 85% at the end of 72 hours.

CONCLUSION

In this report we successfully loaded doxorubicin on to the starch polymeric nanoparticles using nanoprecipitation method and its size range between 250 t0 300 nm with smooth surface having spherical cavity inside which contains the anticancer drug (Doxorubicin). Various parameters like loading efficiency, molecular weight of the drug as well as the concentration o the drug affects release of nanoparticles from the dialysis bag. The absorbance was checked periodically after 1hr of treating with the phosphate buffer solution of pH 74 or the drug release studies. FTIR was carried out to check the purity of the prepared nanoparticle sample. At the last, we concluded that starch nanoparticle serves as an excellent carrier for drug delivery systems.

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