International Journal of Drug Delivery 9 (2017) 107-111
http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

# Synthesis of Alginate/Nanocellulose bionanocomposite for *in vitro* delivery of Ampicillin

R Poonguzhali[1], S Khaleel Basha[2], V Sugantha Kumari[1*]

***Corresponding author:**

V. Sugantha Kumari

[1] Department of Chemistry, Auxilium College, Vellore 632 006, India
[2] Department of Chemistry, C. Abdul Hakeem College, Melvisharam 632 509, India

Received: 17 July 2017
Accepted: 23 August 2017
Published: 23 January 2018

## Abstract

In this study Ampicillin drug loaded with alginate and nanocellulose film was prepared by solution casting method. Nanocellulose and ampicillin incorporated into alginate to improve both mechanical and swelling property. The formulated ampicillin loaded Alg/NC film gave acceptable physicochemical properties compared with Alg-amp film and was able to deliver the drug in a prolonged release pattern. *In vitro* drug release showed that alginate, could provide an immediate release of ampicillin with further enhanced nanocellulose, and followed by a sustained release over 500 min of the remaining drug. The present study exhibited a simple and useful approach to systematically design for providing drug release profiles.

**Keywords** Alginate . Nanocellulose . Ampicillin . Drug release. Swelling property

## Introduction

Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both *in vivo* and *in vitro* biomedical research and applications [1]. Nanocellulose has undergone rapid development in recent years as promising biomedical material because of their excellent physical and biological properties, in particular their tissue repair [2], wound healing [3] and drug delivery [4]. Among these applications, drug delivery draws tremendous attentions during past decades. Combining the advantage of nanocellulose and the smart biopolymer by blending method creates a nanocellulose film with better controllability.

Alginate has a potential for a broad range of applications as a biomaterial and especially as the supporting material or delivery system for tissue engineering [5]. Alginate is a naturally occurring biopolymer extracted from brown seaweeds that has many possible applications in the area of drug delivery. Chemically, alginate is a linear polymeric acid composed of 1, 4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. Biocompatibility and immunogenicity of alginate are two cardial issues for successful application in carriers for drug delivery [6]. The most important advantage of using alginate for controlled release formulations is its biodegradability, good mechanical strength and non-toxicity property [7-9]. Sodium ampicilin was chosen as a topical long acting antibiotic which also has good water solubility in an aqueous solution and it is broad spectrum antibiotic which means that it kills more kinds of bacteria than other penicillin family and gentamicin antibiotics. Sodium ampicilin was utilized as an antibiotic instead of gentamicin sulphate salt which is loaded with PVA-dextran membranes [10]. We here aim to develop a novel material made of sodium alginate with nanocellulose loaded with various concentration of sodium ampicillin by solution casting method. Properties of scaffolds such as mechanical and swelling behaviour and ampicilin release studies were investigated.

## Experimental

### Materials

Sodium alginate (Mw = 300000 g/mol) and ampicillin was supplied by Sigma Aldrich Mumbai. The *Hibiscus sabdariffa* used as raw material was obtained from local farming community.

### Preparation of Nanocellulose (NC) from *Hibiscus sabdariffa*

Isolation of nanocellulose from *Hibiscus sabdariffa* fibers usually achieved by acid hydrolysis, during which the acid hydrolysis involve the diffusion of acid molecules into fibers, followed by cleavage of glycosidic bonds. After cutting the cellulose fiber in small piece approximately 2 cm to sterilize the fiber treated with 2% NaOH in an autoclave with a pressure of 15 lbs and at a temperature of 120 C for a period of 1h. Then sterilized fibers neutralized by washing with water for several water. The dried

DOI:10.5138/09750215.2168

cellulose fiber was bleached with a mixture of sodium hydroxide, acetic acid and sodium hypochlorite in 1:3 ratios for an hour and repeated for several times followed by washing with water till the smell of bleaching agent was removed. Bleached cellulose fibers are acid hydrolyzed with oxalic acid for 5h in an autoclave in the pressure of 20 lb. The fibers were neutralized and the suspension was diluted with water, kept for stirring in a magnetic stirrer for 8h. The pH of the suspension was around 6.7. The nanocellulose was obtained from the suspension by drying it at room temperature.

## Film preparation

Alg/NC nanocomposite films were prepared by employing solution casting method. 1 g of Alginate was dissolved in 100 mL of deionised water; stirring was conducted at room temperature at 250 rpm by a homogenizer for 2 h. The pure Alginate film was prepared by the above solution. To this alginate solution 0.5% of nanocellulose was added and stirring was taking place for 2 h. The solutions were cast on the Teflon coated plates and the required time for film forming was 48 h. The dried films were removed from the plates and placed inside the oven for two further days to evaporate residual solvents completely.
Preparation of corresponding drug-loaded Alginate and Alginate/NC nanocomposite films was similarly performed. In addition, 1% of ampicillin was dissolved in 50 mL of the above prepared Alginate and Alginate/NC. The mixture was thoroughly stirred and left overnight to remove air bubbles before being cast on to Teflon plates at room temperature.

## Characterization studies

## Morphology studies

The morphology of nanocellulose was investigated by means of TEM (model Philips CM 200). A drop of a diluted suspension of nanocellulose was deposited on the surface of a clean copper grid and coated with a thin carbon film and dried at room temperature.
The surface morphology of the films was examined using SEM. This was performed using a scanning electron microscope (SEM), model JOEL 5410LV (Tokyo, Japan), equipped with a system INCA dispersive X-ray detector (Oxford Instruments, Austin, TX, USA), operated at a voltage of 20 kV, and membranes were coated with gold in a sputter coater.

## Swelling property

The swelling ratio was measured by immersing a preweighed dry sample in appropriate swelling medium at a given interval. Excess surface water was blotted out with filter paper before weighing. Highly swollen samples were placed between two sieves and then blotted with filter paper. Percentage swelling of prepared dressing at equilibrium was calculated from the formula.

$$\text{Swelling ratio (\%)} = W_w\text{-}W_d/W_d \times 100 \quad \ldots\ldots \quad (1)$$

Where, $W_w$ and $W_d$ are weights of wet and dry scaffolds respectively. The swelling medium was buffer solutions of Phosphate buffer saline (PBS) pH 3-12. The pH values were precisely checked by a pH-meter previously standardized with buffer solutions of pH 3 and 9.

## Mechanical property

Tensile testing was carried out using MTS Criterion 5 kN testing Machine according to ASTM D-638-2010 with a crosshead speed of 50 mm/min. Test was performed until tensile failure occurred. The measurements were done at 25 ºC. Samples dimensions were 4mm x 10mm. At least five sample specimens for each set were tested to get the average value.

## *In vitro* drug release

To examine the release profile of Ampicilin, the films containing ampicillin were incubated in 400 ml of phosphate-buffered saline (0.03 M, pH = 7.4) with constant stirring (200 rpm) at 37 ºC. Sodium ampicillin was chosen as a topical long acting antibiotic which also has good water solubility in an aqueous solution. At specific time points, 3 mL of solution was taken out for analysis and replaced with the same volume of fresh PBS solution. The concentration of ampicillin in each aliquot was determined by UV spectrophotometer at 355 nm (Shimadzu UV Visible Spectrophotometer, UVmini-1240). Released ampicillin was determined by the following equation.

$$\text{Drug release (\%)} = (\text{Released drug}) / (\text{Total drug}) \times 100 \quad \ldots(2)$$

Where released drug was calculated from the drug concentration measured in the total volume and total drug was the amount loaded in the obtained sample. All experiments were carried out in triplicate.

# Results and Discussion

## Morphological studies

The morphology of the nanocellulose was studied by Transition Electron Microscopy (TEM). As shown in the Figure. 1a needle shaped nanocellulose crystals were observed for synthesized nanocellulose and also revealed a broad range of nanoparticles in the range of 2-4 nm width and 10-15 nm length. The surface morphology of Alginate, Alg/NC and drug loaded films were investigated by SEM (Figure .1b-e). The nanocellulose can be seen

in the SEM image of Alg/NC film in the form of small white spots, which revealed the presence of nanocellulose, were well dispersed throughout the film. Whereas, the Alginate did not contain any small white spots and shows smooth surface. For drug loaded films a multiphase morphology was observed and the surface roughness of the film was enhanced by loading the ampicillin. Furthermore, there is a uniform surface roughness observed for Alg/NC-Amp film when compared with Alg-Amp film.

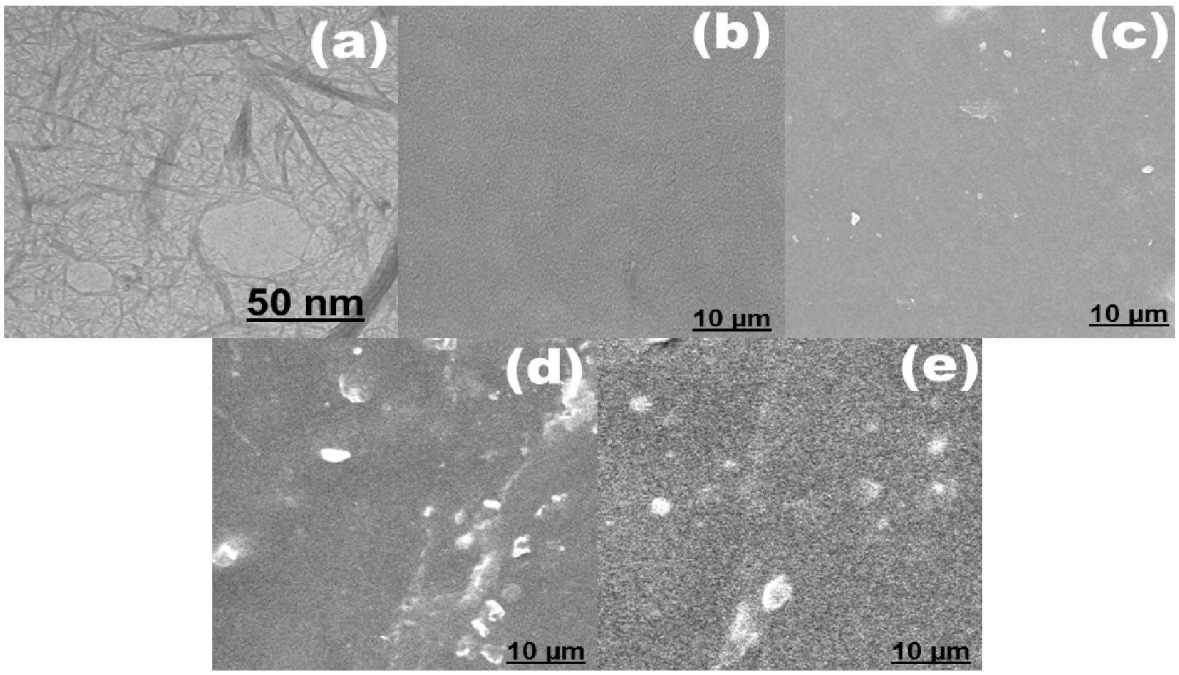


**Figure 1.** TEM micrograph of nanocellulose (a) SEM image of Alginate (b) Alg/NC (c) Alg-amp (d) and Alg/NC-amp (e) films

## Swelling property

The swelling ratio versus pH study of the film (Figure. 2) shows the maximum swelling rate, from 500% to 570% at pH 10.0 and lowest from 300% to 380% at pH 2.0. At pH 10.0 the swelling is increased for the Alg/NC and Alg/NC-amp film due to nanocellulose is soluble at alkaline pH, which might have dissolved some of the nanocellulose present in the film creating voids. The voids so formed are responsible for the high swelling ratio at pH 10.0 [11]. Alg-amp film has higher swelling when compared with Alginate and Alg/NC film due to higher solubility of ampicillin drug than nanocellulose so easily created voids thus swelling was higher for Alg-amp film.

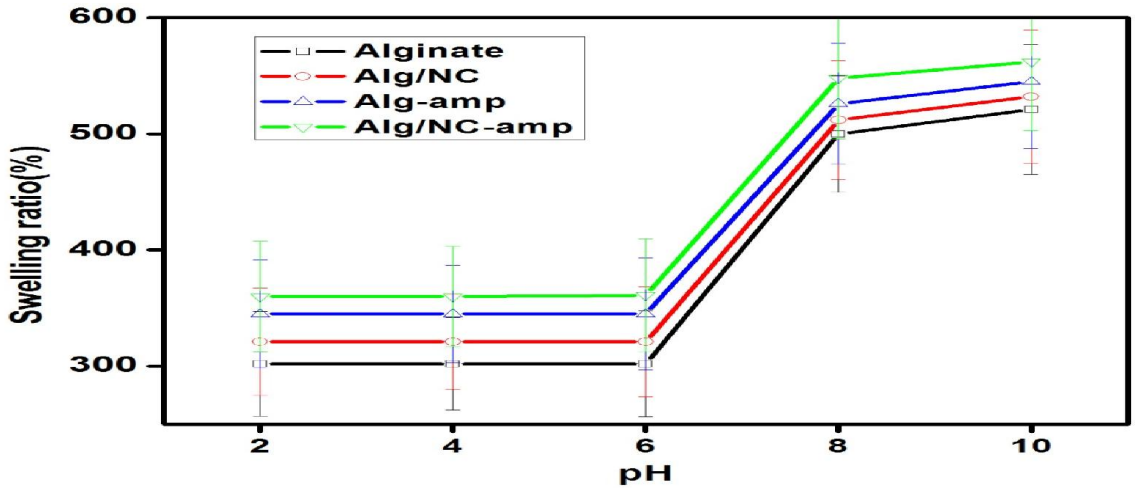


**Figure 2.** Swelling ratio of Alginate, Alg/NC, Alg-amp and Alg/NC-amp films

## Mechanical property

A suitable material for biomedical application should be strong and to some extent flexible. The tensile strength (TS) and elongation at break (EB) of the scaffold are shown in Figure. 3. The nanocellulose obtained from *Hibiscus cannabinus* was very promising reinforcing material for alginate film because of their high stiffness and strength [12]. The increase in the tensile strength of the Alg/NC film shows the reinforcing effect of nanocellulose with alginate. It can be seen that the tensile strength of the drug loaded alginate film increased slightly when compared to the Alg/NC film due to higher incorporation of ampicillin with alginate. However, addition of nanocellulose as well as ampicillin decreased the elongation at break and increased the tensile strength. These results show that nanocellulose and ampicillin has a positive impact on the mechanical stability of the drug loaded film.

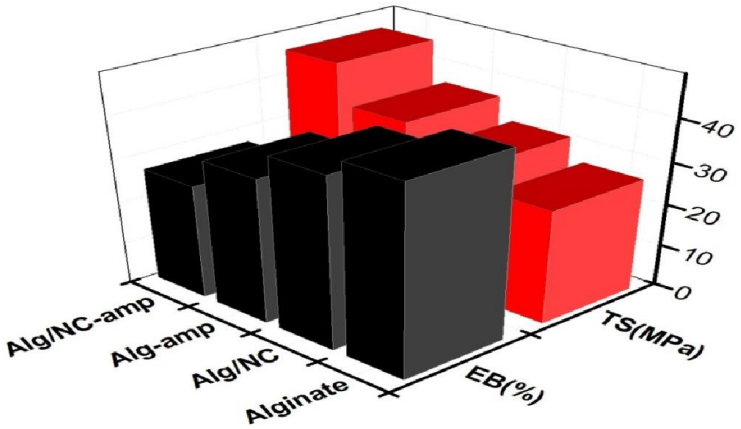


**Figure 3.** Mechanical properties of Alginate, Alg/NC, Alg-amp and Alg/NC-amp films

## *In vitro* drug release

The release behavior of ampicillin from the Alginate, Alg/NC and drug loaded films were presented in Figure. 4. The release profile for ampicillin from alginate film with nanocellulose was higher than that of without nanocellulose. This could be due to the less binding affinity between alginate and ampicillin. On the other hand presence of nanocellulose in the scaffold leaves free space for the ampicillin causing fast release of the drug in the PBS. The release of ampicillin from alginate occurs mainly by diffusion through film and due to erosion mechanism. As it is hydrophilic, it is expected that ampicillin molecules will significantly penetrate the voids of the Alg/NC scaffold [13]. This suggests that the Alginate/NC film with of ampicilin can be used to be suitable for the *in vitro* drug release application.

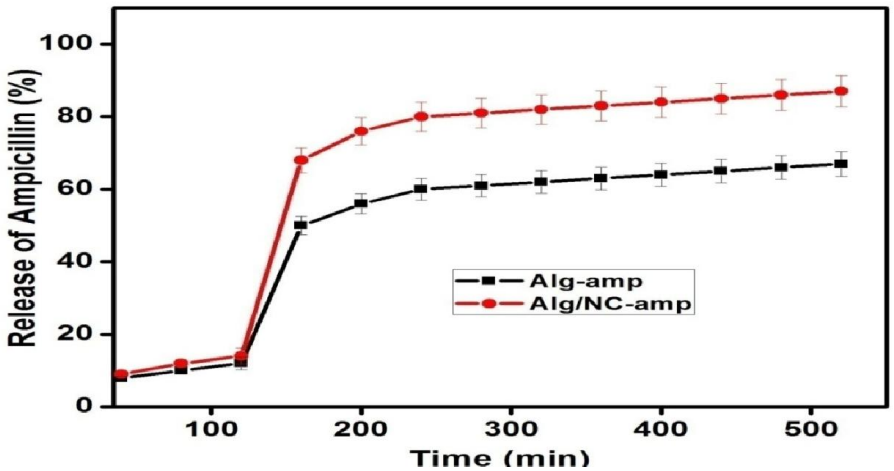


**Figure 4.** *In vitro* drug release of Alg/NC-Amp Scaffolds

## Conclusion

The present study investigated the combined activity of Alginate and nanocellulose loaded with ampicilin on *in vitro* drug release. The film of alginate, nanocellulose and ampicilin shows improved swelling property at basic medium and showed good mechanical property. It was concluded that the drug loaded films incorporated nanocellulose exhibit a good drug delivery system with the sustained drug release.

## References


[1]. Andronescu E, Brown JM, Oktar FN. Nanomaterials for Medical Applications: Benefits and Risks. J Nanomater. 2016; 10(4):1154.

[2]. Markstedt K, Mantas K, Tournier I. 3D Bioprinting Human Chondrocytes with Nanocellulose-Alginate Bioink for Cartilage Tissue Engineering Applications. Biomacromolecules. 2015; 14(25):1-27.

[3]. Hakkarainen T, Koivuniemi R, Kosonen M. Nanofibrillar cellulose wound dressing in skin graft donor site treatment. J. Control. Release. 2016; 244(23): 292-301.

[4]. Plackett DV, Letchford K. A review of nanocellulose as a novel vehicle for drug delivery. NPPRJ. 2014; 29(1): 1-14.

[5]. Venkatesan J, Bhatnagar I, Manivasagan P. Alginate composites for bone tissue engineering: A review. Int. J. Biol. Macromol. 2014; 72(57): 269-281.

[6]. Hjorth H, Karlsen J. Alginate in Drug Delivery Systems. Drug Dev Ind Pharm. 2002; 28(6): 621-630.

[7]. Jeon O, Bouhadir KH. Photocrosslinked alginate hydrogels with tunable biodegradation rates and mechanical properties. Biomaterials. 2009; 30(12): 2724-2734.

[8]. Kaklamania G, Chenelerd D. (2014) Mechanical properties of alginate hydrogels manufactured using external gelation. Journal of the mechanical behaviour of biomedical materials. 2014; 4(36): 135-142.

[9]. Borges O, Cordeiro-da-Silva A. Uptake studies in rat Peyer's patches, cytotoxicity and release studies of alginate coated chitosan nanoparticles for mucosal vaccination. J. Control. Release. 2006; 6(114): 348-358.

[10]. Hwang M, Kim J. Gentamicin-Loaded Wound Dressing With Polyvinyl Alcohol/Dextran Hydrogel: Gel Characterization and In Vivo Healing Evaluation. Pharm Sci Tech. 2012; 10(11): 1-12.

[11]. Cha R, He Z, Ni Y. Preparation and characterization of thermal/pH-sensitive hydrogel from carboxylated nanocrystalline cellulose. Carbohydr. Polym. 2012; 88(25): 713-718.

[12]. Tang S, Zhao Z, Chen G. Fabrication of ampicillin/starch/polymer composite nanofibers with controlled drug release properties by electrospinning. J Sol-Gel Sci Technol. 2015; 15(10): 1-10.