



## Evaluation of *Bombax costatum* Calyx Hydrogel as a Potential Insulin Delivery System

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### Authors' contributions

*This work was carried out in collaboration between both authors. Both authors designed the study. Author JTB wrote the protocol. Author UDA managed the analysis, literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.*

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### ABSTRACT

Hydrogel are a class of polymer materials that can absorb large amount of water without dissolving. The property of hydrogels to absorb water arises from hydrophilic functional groups attached to the polymeric backbone, while resistance to dissolution is due to physical or chemical crosslinking of the hydrophilic polymer chains. They are capable of responding to physical and chemical stimuli such as temperature pressure, pH, ionic strength, etc. In this study *Bombax costatum* calyx hydrogel was prepared to explore its potential in drug delivery. The study involves the encapsulation of insulin using swelling equilibrium method. The hydrogel was characterised using FT-IR; SEM techniques to study the structure of the networks. The swelling behaviour was studied in three different mediums (distilled water (pH6.9-7, pH 7.4 and pH 1.2) and at two temperatures (room temperature and 37°C). The hydrogel swell more in distilled water (pH 6.9-7) than in pH 7.4 and pH 1.2. Contrary to the trends observed in the swelling behaviour, the in vitro drug release in pH 7.4 was greater than that released in distilled water and pH 1.2. In conclusion, the smart

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behaviour exhibited by the hydrogel makes it a promising carrier in the site specific delivery of protein and peptide drugs to the colonic region.

*Keywords: Bombax costatum; calyx; hydrogel; swelling; drug delivery.*

## 1. INTRODUCTION

Recent advances in the field of biotechnology have led to the discovery of numerous drugs among which are protein and peptide based therapeutics. However their clinical development faces great challenge due to poor delivery properties of peptide and protein based product. Today, the most common means for administering these protein drugs remain injection (i.e. intravenous, intramuscular, or subcutaneous administration). Patient compliance with drug administration regimens by any of these parenteral routes is generally poor and severely restricts the therapeutic value of the drug, particularly for diseases such as diabetes [1,2]. The main challenge is to improve the oral bioavailability from less than 1% to at least 30-50% [3].

The safety and efficacy of protein and peptide therapeutics are limited by three inter-related pharmaceutical issues, in vitro and in vivo instability, immunogenicity and short half-lives. Novel drug modifications for overcoming these issues are under investigation and include covalent attachment of poly(ethylene glycol)(PEG), polysialic acid or glycolic acid, as well as developing new formulations containing Nano-particulate or colloidal systems(e.g. liposomes, polymeric microspheres, and polymeric nanoparticles.). Such strategies have the potential to be developed as next generation protein therapeutics [4].

Many drug delivery systems can be synthesized with controlled composition, shape, size and morphology. Their surface properties can be manipulated to increase solubility, immunocompatibility and cellular uptake. Promising and versatile nano-scale drug delivery systems include nanoparticles, nanotubes, Nanogels and dendrimers. They can be used to deliver both small-molecules such as peptides, protein, plastid, DNA, and synthetic oligodeoxynucleotides etc [5].

Extensive applications of polymers in drug delivery have been realized because polymers offer unique properties which so far have not been attained by any other material [6]. Various

polymers have been used in drug delivery research as they can effectively deliver the drug to a target site and thus increase the therapeutic benefit, while minimizing side effects [7]. Polymeric delivery systems are mainly intended to achieve either a temporal or spatial control of drug delivery, The introduction of the first synthetic polymer-based (polyglycolic acid) drug delivery systems led to an increased interest in the design and synthesis of novel biodegradable polymers that obviated the need to remove the drug delivery systems after delivery, unlike the non-degradable polymeric systems [8].

Among the numerous polymers used in the drug delivery systems naturally occurring polysaccharides are extremely advantageous compared to synthetic polymers being widely present in living organism and often produced by recombinant DNA techniques. Coming from renewable sources, polysaccharides also have frequently economic advantages over synthetic polymers. Polysaccharides are usually non-toxic, biocompatible and show a number of peculiar physico-chemical properties that make them suitable for different applications in drug delivery systems [9].

[10] Stated that Polysaccharides and their derivatives can be used as a rate controller in sustained release formulations due to their gelling property. They are biodegradable and their chemical composition varies greatly. For instance, they contain hydroxyl groups that allow direct reaction with drugs with carboxylic acid functions, thereby producing ester linkages that are biodegradable and thus facilitate the release of the drug in the body.

*Bombax costatum* occurs from Senegal eastward to Cameroon, southern Chad and the Central African Republic [11]. In Nigeria, different parts of *Bombax costatum* are employed for various purposes. The immature fruits are prepared as an emollient; decoction of young leaves is used as a warm bath for febrile children; the ground bark is taken by pregnant women to increase lactation; the extract from the bark is drunk or applied on the head for dizziness; and the gum resin from the bark is pulverized, mixed with oil and used to manage skin diseases such as

“craw-craw” [12]. [13] reported that the analysis of monosaccharide of mucilage and pectin of *Bombax costatum* sepal shows high content of glucuronic acid, arabinose, rhamnose and mannose. In view of the medicinal importance and gelling nature of *Bombax costatum* calyx this research focused on the modification of *Bombax costatum* calyx in to a hydrogel for delivery of protein and peptide drug adopting Insulin as model drug.

## 2. MATERIALS AND METHODS

### 2.1 Materials

*Bombax costatum* calyx was collected from GIREI LOCAL GOVERNMENT AREA OF ADAMAWA STATE, Nigeria which was then decoated, air dried in the laboratory and ground into a fine powder. Hydrochloric acid was obtained from Jinhuada chemicals, while Borax ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ), sodium hydroxide, Sodium carbonate, Sodium-Potassium tartrate, potassium chloride, potassiumdihydrogenOrtho phosphate, Copper sulphate penta hydrate, and Folin reagent were of British drug house (BDH) and Insulin drug was bought from TORRENT. The chemicals were of analytical grade and were used as provided.

### 2.2 Preparation of *Bombax costatum* Calyx Hydrogel

Physical cross-linking Method reported by [14,15] was adopted with slight modification. 6 g of *Bombax costatum* calyx powder was dissolved in 50 ml of distilled water in a glass beaker of 250 ml capacity and stirred thoroughly for one hour to obtain a viscous solution. Then 2 g of  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  was added to this solution and stirred with glass rod to form a gel. The gel was then allowed to stand for one hour to cure before casting on a clean glass surface and dried over a period of 72 hours (three days) at 25°C.

### 2.3 Drug Loading into the Polymer Matrix

The loading of drug into hydrogels was carried out by swelling equilibrium method [16]. The hydrogels were allowed to swell in an insulin drug solution of 60 IU (1.8 mg) concentration for 24 h and then dried to obtain the release device.

### 2.4 Preparation of Buffer Solution

The dissolution media of various pH values (1.2, Distilled water and 7.4) were prepared according to [17] by combining HCl, KCl,  $\text{KH}_2\text{PO}_4$  and

NaOH. A pH buffer 1.2 was prepared by taking 50 ml of 0.2 M KCl and 85ml of 0.2 M HCl in a volumetric flask and making it up to 200ml with distilled water. 0.2 M KCl solution was prepared by dissolving 14.911 g of KCl in distilled water to make volume 1000mL with distilled water. pH buffer 7.4 was also prepared by taking 100ml of 0.1M  $\text{KH}_2\text{PO}_4$  and 78.2ml of 0.1M NaOH in a volumetric flask and making it up to 200ml with distilled water. 0.1 m  $\text{KH}_2\text{PO}_4$  was prepared by dissolving 13.609 g of  $\text{KH}_2\text{PO}_4$  in distilled water to make volume to 1000ml with distilled water.

### 2.5 Preparation Calibration Curves

In this procedure, the absorbance of a number of standard solutions of the reference substance (soluble insulin) at concentrations encompassing the sample concentrations was measured on the UV Visible Spectrophotometer at 660 nm and calibration graph was constructed. The concentration of the drug (insulin) in the sample solution was read from the graph as the concentration corresponding to the absorbance of the solution. Three calibration graphs were made in distilled water, pH 1.2 buffer and pH 7.4 buffers to determine the amount of drug released from the drug loaded polymeric matrix in these mediums.

### 2.6 Swelling Behaviour of Hydrogels

The swelling behaviour of the hydrogel was measured as described by [18]. Accurately weighed amount of hydrogel (ranging from 0.4 to 0.5 g) was immersed in excess of solution medium and at fixed time intervals the hydrogel was separated from the medium using a stainless steel spatula. Immediately, they were wiped gently with paper and weighed. The dynamic weight change of the hydrogel with respect to time was calculated according to the formula:

$$\% \text{ weight change} = [(W_s - W_i) / W_i] \times 100\% \quad (1)$$

Where  $W_s$  is the weight of the hydrogel in the swollen state and  $W_i$  is the initial weight of the hydrogel.

### 2.7 Drug Release Studies

The *in vitro* drug release was carried out using Distilled water, simulated gastric and colonic medium with modification as reported by [19]. 1 g of the drug loaded hydrogel was placed in 30 mL medium of pH 1.2, distilled water (pH 6.9-7) and pH 7.4 in a water bath at 37°C. At predetermined

time intervals of 30 min, 3 mL of the solution was taken and 3 mL of the fresh buffer solution was added to maintain a constant volume. The concentration of drug released was determined by Lowry's method using UV/Vis spectrophotometer, and then the cumulative percentage of drug released was calculated.

Two percent sodium carbonates (2% Na<sub>2</sub>CO<sub>3</sub>) in 0.1N sodium hydroxide (NaOH), One per cent sodium-potassium (1% Na-K) Tartrate in water and 0.5% CuSO<sub>4</sub>.5 H<sub>2</sub>O in H<sub>2</sub>O was prepared for the analysis.

Then 48 ml of (2% Na<sub>2</sub>CO<sub>3</sub>) in 0.1N (NaOH), 1 ml of (1% Na-K) Tartrate, 1 ml 0.5% CuSO<sub>4</sub>.5 H<sub>2</sub>O in H<sub>2</sub>O as mixed as reagent I and 1 part Folin-Phenol [2 N]: 1 part water as reagent II.

### 2.7.1 Procedure

- 0.2 ml of insulin working standard in 5 test tubes and make up to 1ml using distilled water.
- The test tube with 1 ml distilled water serves as blank.
- 4.5 ml of Reagent I was added and incubated for 10 minutes.
- After incubation 0.5 ml of reagent II was added and incubated for another 30 minutes for the blue colour to develop.
- The absorbance was measured at 660nm and the absorbance was used to plot the standard graph (calibration curve).
- The estimated amount of protein present in the given sample was determined from the standard calibration curve prepared.

The release percent of insulin was calculated from the following equation:

$$\text{Release (\%)} = (W_t/W_\infty) \times 100 \quad (2)$$

Where  $W_t$  is the amount of the released insulin at time  $t$  and  $W_\infty$  is the total adsorbed insulin in the hydrogel structure.

### 2.8 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra were recorded using (FTIR 8400S Shimadzu). The FT-IR was used to study the modification of *Bombax costatum* calyx hydrogel.

### 2.9 Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy was carried out to investigate and compare the surface morphology and pore sizes of *Bombax costatum* calyx hydrogels. PHENOM WORLD model of SEM machine was used in this analysis. The pores sizes were automatically calculated by the instrument using the PoroMetric software.

## 3. RESULTS AND DISCUSSION

### 3.1 Swelling Behaviour

The swelling behaviour was studied to know the response of the hydrogel in Distilled water, Simulated Gastric Fluid (pH 1.2) and Simulated Intestinal Fluid (pH 7.4) at room temperature (25°C) and at 37°C and is presented in Figs. 1 and 2 showing similar behaviour. The hydrogels percentage swelling in distilled water was more pronounced compared to pH 7.4 and pH 1.2. The polymer-polymer and the polymer-solvent interactions (solvent that in biomedical applications will be usually water) show an abrupt re-adjustment in small ranges of pH or temperature, and this is translated to a chain transition between extended and compacted coil states [20]. The polymer backbone of *Bombax costatum* calyx contains about fifty eight percent glucuronic acids as reported by [13]. The swelling phenomena can be due to the fact that at lower pH values the -COOH groups do not ionize and keep the network in its collapse state. At high pH value, hydrogels get partially ionize, and the charged -COOH groups repel each other, leading to the more pronounced swelling of the polymer [21]. That is to say in aqueous solutions of pH 6.9-7 the activated -COO<sup>-</sup> attracts more water thereby increasing percentage swelling of the hydrogel in distilled water and reduces as the solution become more basic as seen in (Fig. 1).

Temperature-responding polymers present a fine hydrophobic-hydrophilic balance in their structure, and small temperature changes around the critical  $T_c$ , make the chains to collapse or to expand responding to the new adjustments of the hydrophobic and hydrophilic interactions between the polymeric chains and the aqueous media [20].

From Figs. 1 and 2 the swelling behaviour of polymer showed significant increase as the temperature was increase from room temperature to 37°C. This observed increase can be attributed to entropic increase as a result of increase in temperature, as the polymer chains in the cross linked polymer network elongates, it results in increase in the pore size of polymer matrix thereby absorbing more solvents.

### 3.2 In vitro Drug Release

In vitro release dynamics of insulin from per gram of the drug loaded hydrogels has been studied in distilled water, pH 1.2 buffer and pH 7.4 buffer and results are presented in Figs. 3 and 4. It can be observed from Fig. 3 that the amount of insulin released in pH 7.4 was greater than that released in distilled water (pH 6.9-7) and pH 1.2.

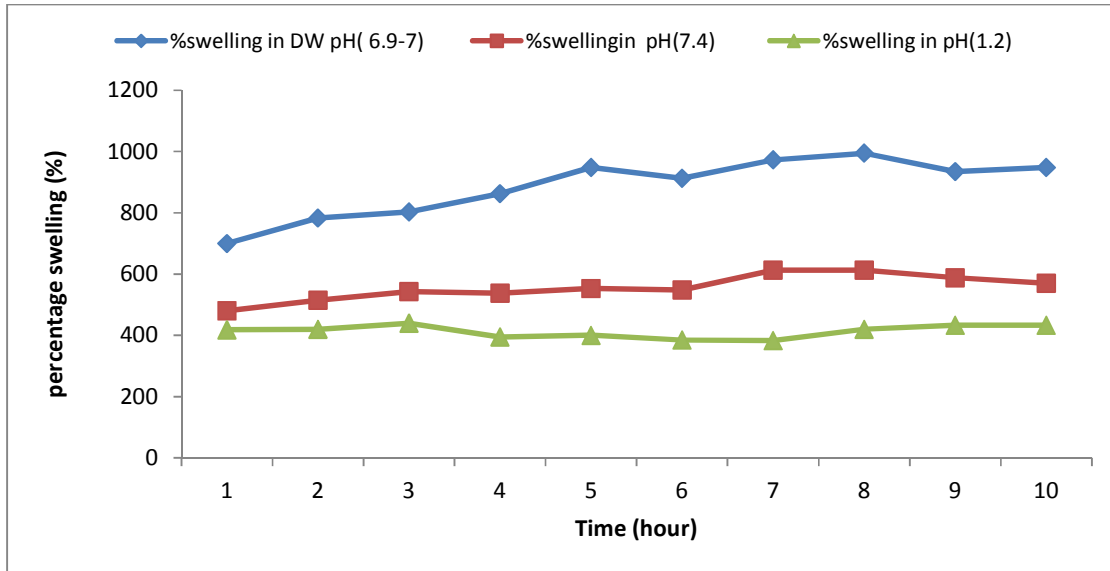


Fig. 1. Swelling behaviour of *Bombax costatum* hydrogel in different media at room temperature (25°C)

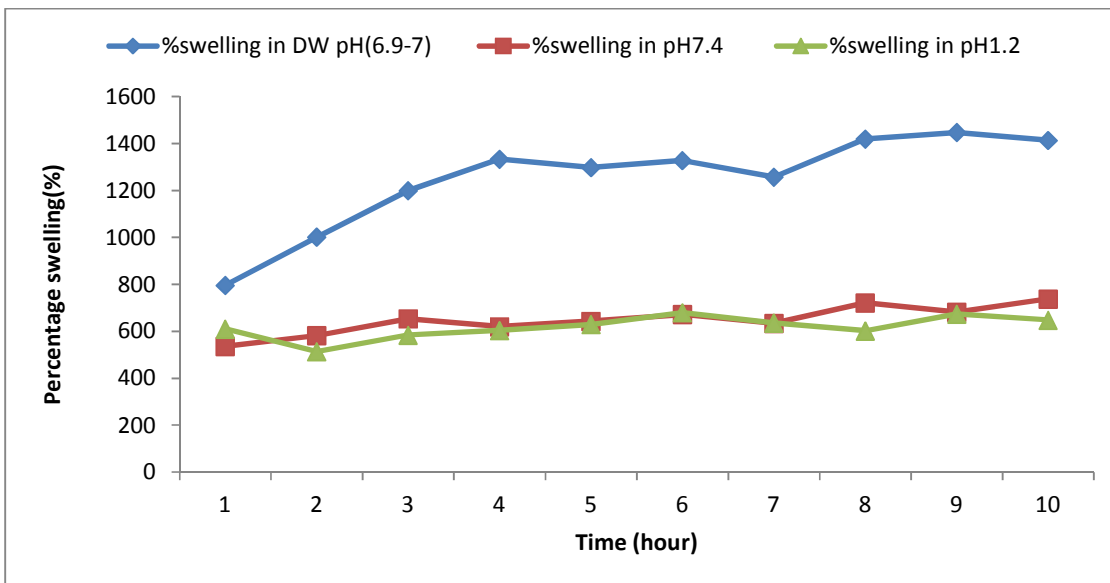


Fig. 2. Swelling behaviour of *Bombax costatum* hydrogel in different media at (37°C)

The trends obtained were also contrary to the swelling behaviour of *Bombax costatum* calyx hydrogels which showed greater swelling in distilled water than pH 7.4 from all the swelling graphs, a situation which can be attributed to the drug or other additives which may act as effective osmolytes [22]. From Fig. 4 percentage cumulative release showed that fifty per cent of total release in distilled water, pH 1.2 and pH 7.4 occurred at 30, 100 and 25 min respectively. It can also be seen that in the first 30-50 min there was burst release of insulin followed by a sustained pattern; this indicates that the hydrogel can also be used for extended release formulations according to [23].

### 3.3 Fourier Transform Infrared Spectroscopy

The FT-IR spectra of the polymers were recorded to observe the nature of bonding and interaction of the polymers and to also check the interaction of the polymer with the drug after encapsulation within the matrices. The observed absorption bands of  $2922\text{ cm}^{-1}$  and  $2851\text{ cm}^{-1}$  are due to  $\text{CH}_2$  asymmetric stretching and symmetric stretching respectively [24]. The band at  $3429.2\text{ cm}^{-1}$  can be said to be due to  $-\text{OH}$  stretching on the polymer backbone of the cross-linked *Bombax costatum* hydrogel. Bands observed from  $1241\text{ cm}^{-1}$  to  $1028\text{ cm}^{-1}$  are attributed to

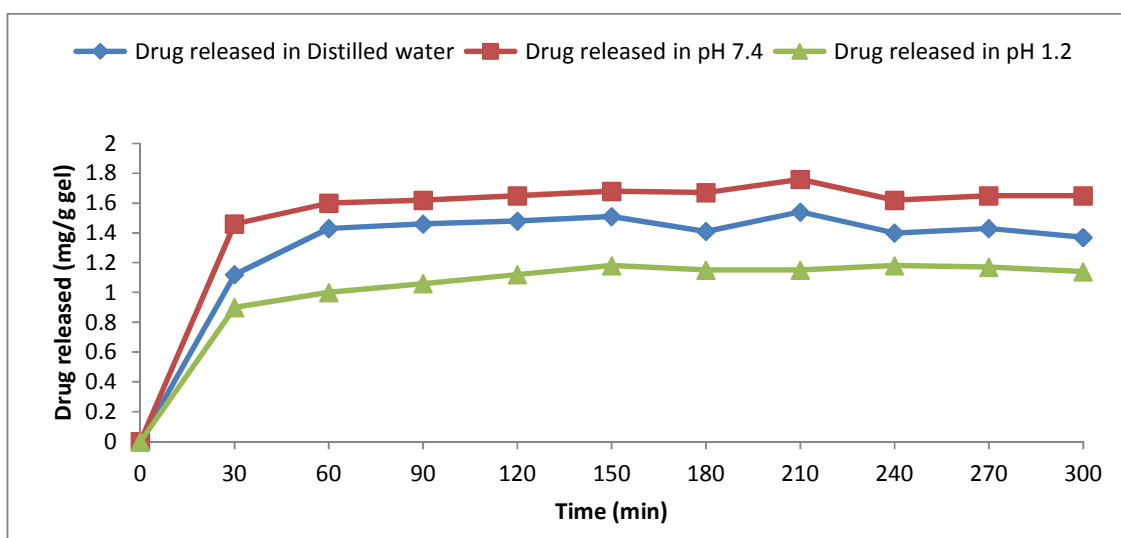


Fig. 3. Drug released from hydrogel in different media

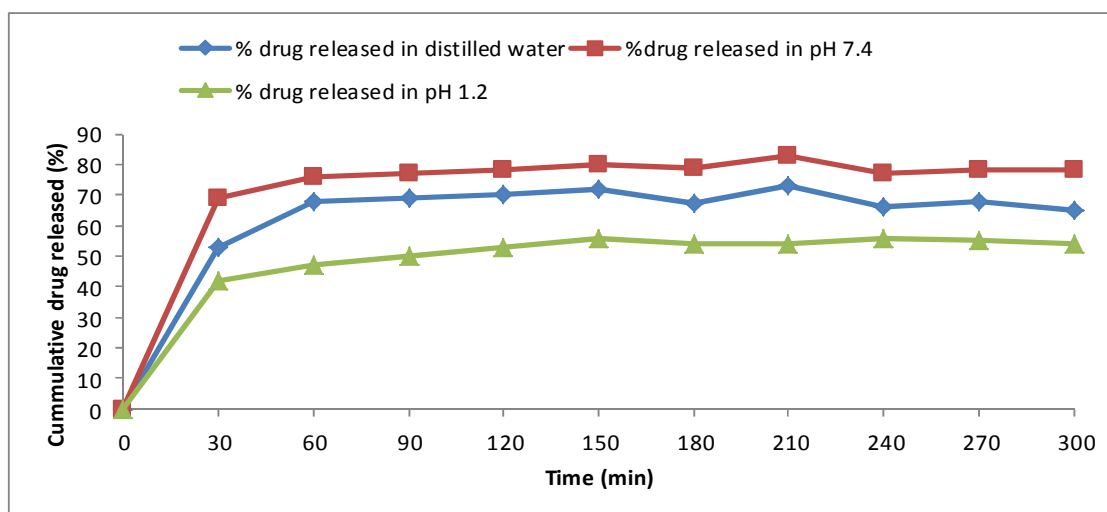


Fig. 4. Cumulative drug release from hydrogel in different media

C-O and C-O-C stretching vibrations which are typical for natural polysaccharides. From Fig. 5 the spectra of the *Bombax costatum* hydrogel polymer impregnated with drug (insulin) it can be observed that there is no any significant change from that of the polymer without drug except the broadening of the peaks around  $3429\text{ cm}^{-1}$  which indicate that the drug was only absorbed into the polymer matrices without creating any new chemical bonds.

### 3.4 Scanning Electron Micrograph (SEM)

The morphology of *Bombax costatum* calyx hydrogel in Fig. 6 shows a mixture of smooth, homogenous as well as rod like structures in the upper most part of the image with pore size ranging from  $0.41\text{ }\mu\text{m}^2$  to  $216.51\text{ }\mu\text{m}^2$ . While the analysis of *Bombax costatum* calyx hydrogel with drug (insulin) shows more of heterogeneous

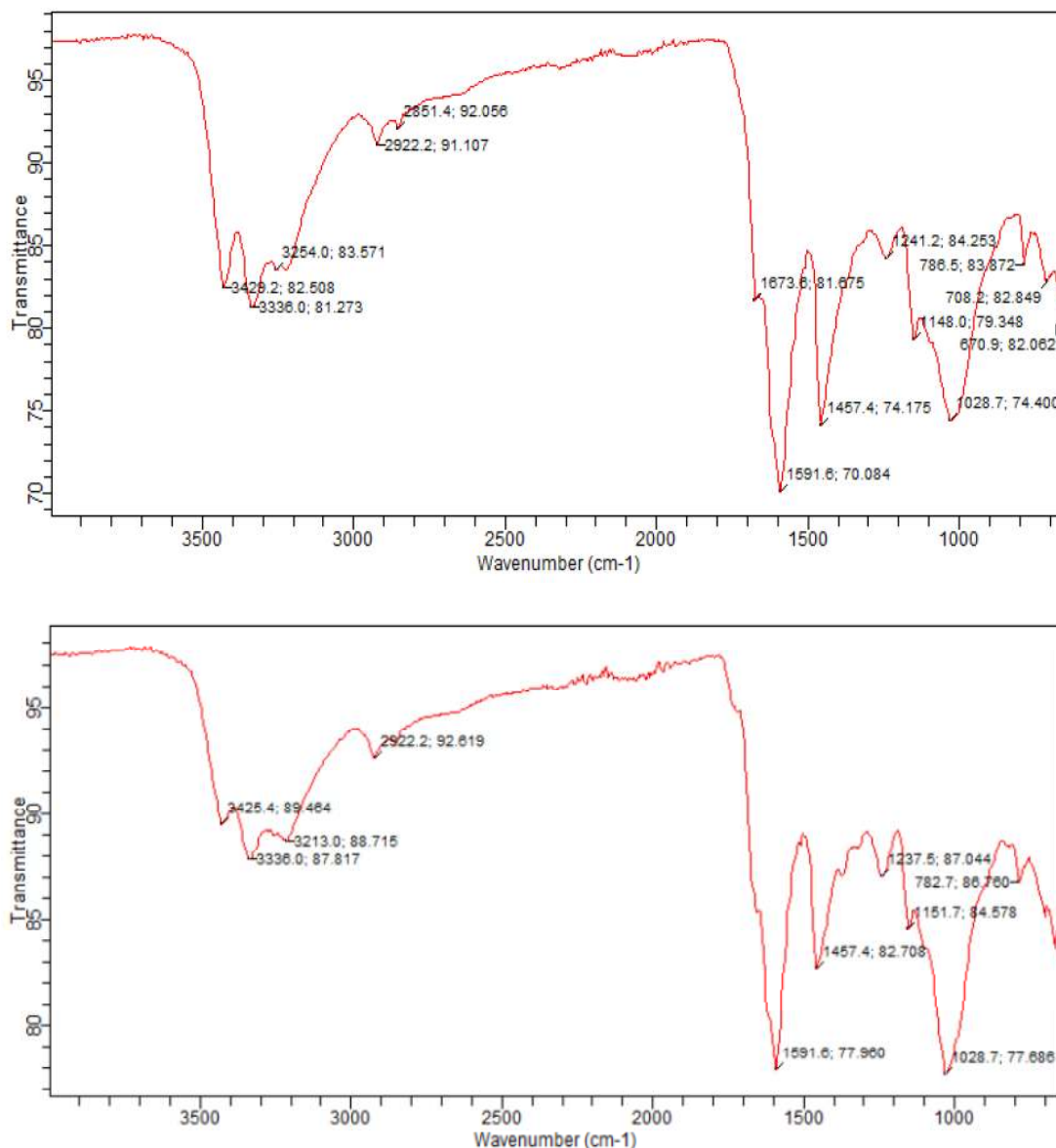
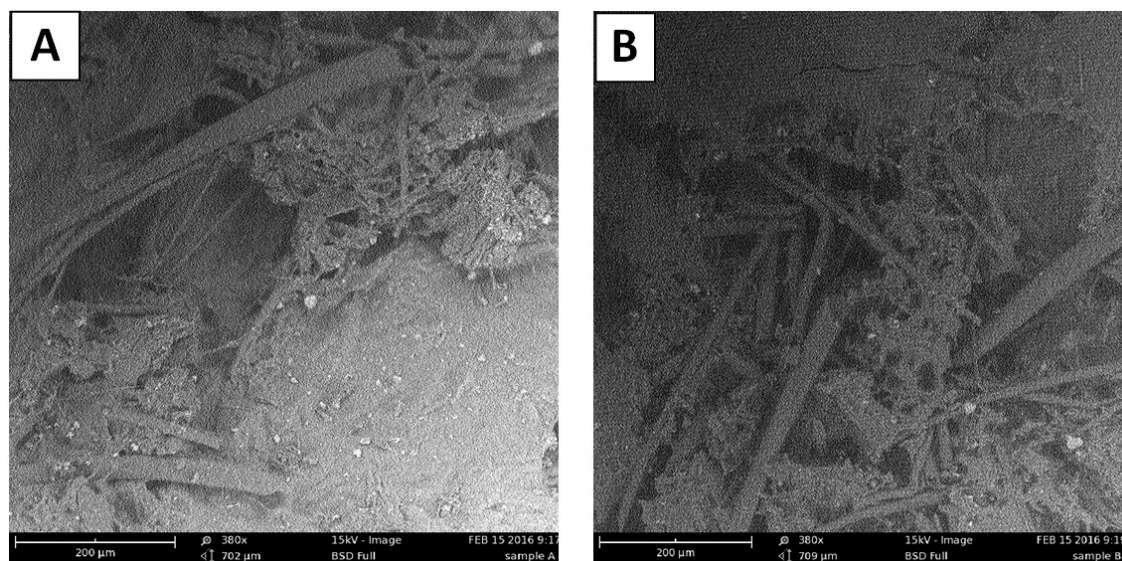


Fig. 5. FT-IR spectrum of plain *Bombax costatum* calyx hydrogel (uppermost) and *Bombax costatum* calyx hydrogel impregnated with insulin



**Fig. 6. Scanning electron micrograph of *Bombax costatum* calyx hydrogel (A) without insulin (B) with insulin**

structure consisting mostly of rod-shape morphology with pore size ranging between  $0.41 \mu\text{m}^2$  and  $4.25 \mu\text{m}^2$ . The decrease in pore size can be attributed to the drug being encapsulated in the large pores of the hydrogel.

#### 4. CONCLUSION

In conclusion, *Bombax costatum* calyx hydrogel have not been reported in any literature. Thus, hydrogel prepared from *Bombax Costatum* calyx has shown potential in the delivery of protein drug to the colonic region as shown by high release pH 7.4 followed by pH 6.9-7. *In vitro* release of insulin followed a sustained pattern after a burst effect and can be used for extended release formulation.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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