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Development and assessment of Thiolated galactomannan from seeds of *Caesalpinia pulcherrima* as a film former in drug delivery

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Abstract

Objective: Objective of present investigations is isolation and thiolation of galactomannan from seeds of Caesalpinia pulcherrima by esterification method and evaluate for film forming ability of isolated and modified polysaccharide. Galactomannan from seeds Caesalpinia pulcherrima were isolated by aqueous maceration method and thiolated by using thioglycollic acid Thiolation of polysaccharide was estimated by using Ellman's reagent. Zeta potential measurement, DSC thermogram, 1H NMR, and FT-IR were used to confirm the thiolation of Caesalpinia pulcherrima galactomannan. Film was casted by using isolated and modified seed galactomannan used for evaluation of film making potential for medication delivery. The results showed that thiolation of the isolated galactomannan significantly improved the film-making property. Chemically modified galactomannan was used to create a biodegradable film that showed enhanced mechanical, strength, folding endurance, drug release, and mucoadhesive properties. As a conclusion, it can be said that thiolated galactomannan from seeds of Caesalpinia pulcherrima is suitable film forming material for drug delivery system.

Key words : *Caesalpinia pulcherrima,* galactomannan, thiolation and biodegradable film.

Natural polysaccharides are increasingly being considered as biopolymers due to their low cost, availability, nontoxicity, ease of modification, biodegradability, and biocompatible properties. Because of their diverse structure and features, they have numerous applications in the pharmaceutical and food industries.[13,17] Galactomannans composed of a (1-4)-d-mannan cahain and a single d-galactose branch attached (Figure 1). Galactomannans are neutral heteropolysaccharides, which means they do not include uronic acid residues or other charged groups (such as sulfo groups) on their backbone. When hydrated in water,

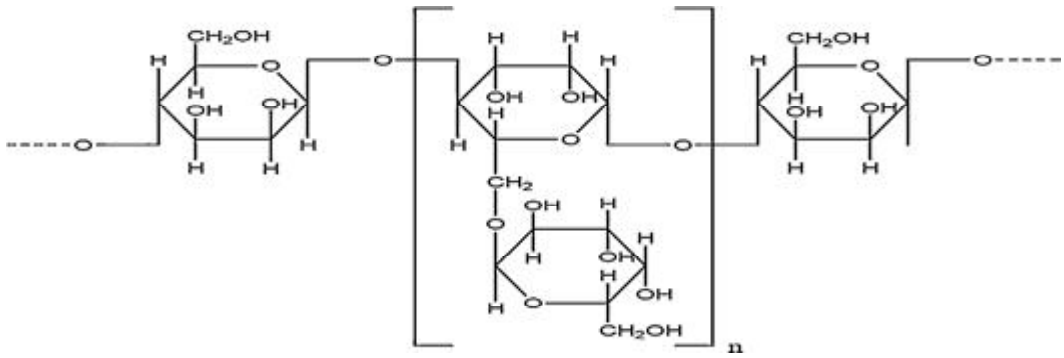

Figure 1. Structure of galactomannan.

galactomannan forms a viscous colloidal dispersion. It is utilized as a viscosity former and water binder in numerous industries, including textiles, food, paper, petroleum, and medicines.[22]

Thiolated polymers (thiomers) were introduced in the 1990s and have unique mucoadhesive properties. Their unique mucoadhesive feature stems from their ability to make strong covalent connections with cysteine-rich subdomains of mucus glycoproteins via a disulfide-thiol exchange mechanism.[15] *Caesalpinia pulcherrima* is an ancient medicine plant with prickly bushy legume commonly called as Pride of Barbados, Dwarf Poinciana, Dwarf Flamboyant, Barbados Pride, Barbados Flower-fence, Peacock Flower, Paradise Poinciana, and Red Bird-of Paradise. It is widely distributed in tropical and subtropical regions such as India, Myanmar, Vietnam, Sri Lanka, and the Malay Peninsula. Its seeds contain significant levels of galactomannan.[4]

Pharmaceutical specialists around the world are investigating film as a new formulation approach. Films have been identified as better altered formulation to standard dosing of medicament. Films are thought to be easy to swallow, self-administrable, and have immediate dissolving dosage form, making them a good platform for medicament delivery.[12]

In present investigations seed galactomannan was isolated from seeds of Caesalpinia pulcherrima by the method of extraction. Thiolation of extracted galactomannan was performed by using thioglycolic acid. Both natural and chemically modified seed polysaccharides were evaluated for film forming potential for drug delivery system.

Chemicals and excipients were received from research laboratory store department. Ondansetron hydrochloride was obtained as a kind gift from Intas Pharmaceuticals, Ahmadabad, India. Seeds from plant Caesalpinia pulcherrima were collected from suburban areas of Dhule, Maharashtra. Analytical grade reagents were used for laboratory procedure.

Isolation of galactomannan from seeds of Caesalpinia pulcherrima :

To release the seed galactomannan, the dried seeds of Caesalpinia pulcherrima

were steeped in filtered water for a whole day, heated for one hour, and then left for two hours. In order to separate the marc from the filtrate, the material was strained through a muslin bag. The mucilage was then precipitated by adding an equivalent volume of acetone to the filtrate. The mucilage underwent separation, desiccation at 50°C in an oven, pulverization, and filter mesh 80 passing. Until it was needed, the powder was kept in a desiccator.[10]

Esterification C. pulcherrima galactomannan :

Thiolation of isolated *C. pulcherrima* galactomannan was accomplished via esterification by using thioglycolic acid in acidic condition. 50 millilitres of distilled water were used to dissolve 6 grams of *C. pulcherrima* galactomannan. 3.6 millilitres of thioglycolic acid and 2 millilitres of 7N hydrochloric acid were poured to the solution mentioned above. For 180 minutes, the reactants were allowed to react at 80ºC. To precipitate the thiolated C. pulcherrima galactomannan, pour resulted solution in 500 milliliters of methyl alcohol. Three times, methanol was used to wash the resulting creamy white precipitate and spent four hours frozen at -80ºC.[24]

Phytochemical analysis and polysaccharide content estimation :

Isolated mucilage went through qualitative analysis to investigate various phytochemicals, including Ruthenium red test, Iodine test, Molisch's test, Ninhydrin test, Dragendorff 's test for glyacoside, Legal test for alkaloids and ferric chloride test for tannins. Using glucose as a standard, the phenol-sulphuric acid technique was applied for detection of polysaccharide content. In method, 1 ml mucilage solution (100 µg/ml), a ml of 5 % phenol was added and it is followed by incorporation of 5 ml of conc. H_2SO_4. Allow to interact for 10 minutes and absorbance was detected on 488 nm lambda max. Linear equation that resulted from the rotation against absorbance curve for various glucose concentrations (50–90 µg/ml) was used for calculation of polysaccharide content. The test solution and the reference polysaccharide were produced in the same way. Triplicates of the experiment were conducted.[5]

Confirmation of thiolation :

Ellman's reagent was used to spectrophotometrically measure the amount of thiol groups on CP galactomannan. Ellman's reagent, used to estimate free sulfhydryl groups and used to determine thiols in the structure. When this compound's produced solution combines with sulphhydryl groups, it creates a detectable yellow product, which was detected at 450nm. In a nutshell, 10 mL of distilled water were used to correctly weigh and dilute 20 mg of thiolated mucilage. 2.5 ml of the resulted solution were taken, and to which 5 ml of Ellman's reagent and 2.5 ml 0.5 M phosphate buffer (pH=8) were incorporated. Process methods for isolated CP galactomannan solution were the same. The products were subjected for reaction for two hours at room temperature. absorbance at 450 nm was measured. Using Ellman's reagent and the accompanying reference curve developed between 0.20 and 2.0 millimoles of thioglycolic acid solution in water, the amount of thiol substitution in galactomannan was determined, as previously mentioned.[9,23]

Characterisation of galactomannan by FTIR :

FTIR spectra were captured using an FTIR (Bruker Alpha) spectrophotometer. After that, the sample was placed onto the FTIR sensor (Bruker Alpha). The isolated and thiolated galactomannan was subjected to structural and functional group identification using the spectrum acquired within range of 4000–400 cm^{-1}. [6]

Characterisation of galactomannan by DSC :

The isolated and thiolated galactomannan was studied using DSC (DSC-7020, Hitachi High Tech Japan). 10 mg of each sample were put into an aluminum pan that was sealed. Sample were analysed at the rate of 20°C per minute between temperature 40°C and 400°C. In order to increase cooling effectiveness and baseline stability, controlled cool nitrogen gas was used.[14]

Zeta potential measurement of galactomannan :

Anton Par DLS Litesizer 500 was used to assess zeta potential of both isolated and modified galactomannan. Isolated and thiolated galactomannan was dispersed in distilled water to create the sample (0.1% v/v). After that, the sample was moved to an Omega cuvette for measurement. 170 degrees was the fixed back scattering angle, and the measurement was performed at 25°C.[1,19]

Preparation of films :

The casting procedure was followed in the preparation of the films. In brief, 50 ml of deionized water was heated to 60° C for an hour while magnetic stirring was used to dissolve 1g of Caesalpinia pulcherrima seed mucilage with specified quantity of model drug were added. After that, the resultant solution was homogenized for 15 minutes at 10,000 rpm using a high-speed homogenizer (Remi, India). Two millilitres of glycerol were added as a plasticizer to the mixture above. To release trapped air, the resulting solution was subjected to 20 minutes of sonication. After that, the solution was spread out across a petri dish with a radius of roughly 4.5 cm that had been coated with Teflon and baked at 40°C. After being peeled off, the dry films were kept in storage at relative humidity 55 ± 5%.[16]

Preparation of Calibration Curve :

Ondansetron HCl (25 mg) was accurately weighed and then transferred to a volumetric flask (25 mL). Dissolve it with a small amount of pH 6.8 phosphate buffer solution to increase the volume. The dilutions were studied using a UV-spectrophotometer with a maximum wavelength range of 310 nm.[16] The calibration curve was plotted by taking the concentration (micrograms/mL) of the drug on the x-axis and absorbance on the y-axis as shown in Figure 7.

Properties of CP and TCP film :

Film thickness :

For thickness measurement of CP and TCP film digital vernier caliper was used. Five distinct locations were used to measure each film, and the average was calculated.[18]

Weight of CP and TCP Film :

The dried films were carefully cut into rectangles measuring 2 X 2 cm with sharp scissors, and precisely weighed with weighing balance made by Shimadzu, Japan.

Folding endurance :

Manual measurement was used to estimate the folding endurance, film was folded repeatedly at fix spot. Films were divided into 2 X 2 cm rectangles, and they were repeatedly folded at the same spot while being held in place with forceps until they broke.[25]

Moisture content :

Film samples (with an average diameter of 2 cm) were desiccated for a full night and then roasted at 105°C for 30 minutes to get steady weight procedure repeated for thrice.[21]

The following formula was used to determine the moisture content value:

$$\%\ Moisture\ Content = \frac{Wa - Wb}{Wb} \times 100 \ldots\ldots\ldots \text{i}$$

Where-Wa= Weight before drying,
Wb= Weight after drying

Tensile strength of Film :

A texture analyzer (Brookfield Eng. Lab Inc. USA) was used to measure the strength of film. Tensile strength was measured by recording force displacement curves and stretching the films between two grips at a pace of 0.1 mm/s till it break.[11]

Mucoadhesive properties :

Force displacement transducer (BIOPAC Systems Inc. USA) attached to the student's Physio-graph assembly were used for estimation of mucoadhesion. Force necessary to separate the film from mucosa was measured in order to ascertain the mucoadhesive potential of the material. Equation was used to estimate the bioadhesive force, which is represented in dyne/cm2, based on the minimal weights required to separate the tissues from the film surface.[20]

$$Mucoadhasive\ force = \frac{M \times g}{A} \ldots\ldots\ldots ii$$

Where, M= weight needed for detachment (gm)
g = the gravity, A = surface area of mucosa in cm^2

Scanning electron microscopy :

Using a SEM (JEOL Ltd., Tokyo, Japan), the microstructure of the film surfaces was assessed. In order to visualize the surface at a higher magnification for SEM research, the film's surface was coated by gold particles using sputtering for 60 seconds, which allowed for surface scanning using voltage of 30 Kv.

Differential scanning calorimeter :

For assessment of thermal characteristics of film differential scanning calorimeter was used. Nitrogen (purge gas rate of 10 ml/ min) was utilized for glass transition temperature of the film sample[2]. An aluminium pan holding two milligrams of film material was charged from 40°C to 400°C at a rate of 10°C per minute.

In-vitro drug release :

For drug release study from film Franz

diffusion cell having dialysis membrane were used. Phosphate buffer pH-6.6 was filled in receiver chamber and maintained at 37±0.5 °C temperature. Each miiliter of sample was taken at pre-arranged intervals, filtered, and subjected absorbance detection by using UV-visible spectrometer (Shimadzu, UV-1900i, Japan). 26 The following exponential Korsemeyer-Peppas equation was used to analyse the drug release to ascertain the release profile of drug-loaded polymeric film.[8]

$$Mt/M . ktn \quad \ldots\ldots\ldots\ldots\ldots \text{iii}$$

$$Log\ Mt/M . \log k + n\ log\ t \quad \ldots\ldots \text{iv}$$

Where Mt/M is the amount of medicament released at time t,
k = rate constant
n = diffusion exponent for the release mechanism.

If n = 1 this indicates zero order release, n is equal to 0.5, the drug release is with a fickian diffusion. When 0.5 < n > 1 it indicates non-fickian release.

Isolation and thiolation of CP seed galactomannan :

Extracted Seed polysaccharide from CP was brown coloured amorphous powder. Percentage yield of extracted CP was 11.25%. Cream colour free flowing powder TCP galactomannan chemically synthesized and % yield of thiolated CP was found to be 60.55%.

Results of phytochemical screening :

Phytochemical screening for CP and TCP identified that tannins, alkaloids, glycosides, and starch were absent and test for mucilage and carbohydrates was present. Polysaccharides content for mucilage was determined to be 60 ± 4.1% w/w and 58 ± 2.5% w/w for CP and TCP respectively. The mucilage of CP galactomannan was esterified using a ratio of two moles of thioglycolic acid to one hydroxyl group. Thiolated samples decreased total polysaccharide content validates the chemical alterations.

Conformation of thiolation :

Esterification method was used to thiolate CP galactomannan. Hydroxyl group of galactomannans and the carboxyl group from thioglycolic acid linked to form ester allowed galactomannan to attach covalently with thioglycolic acid. Thiol content of Thiolated CP galactomannan was found to have 3.4 mmol of thiol groups/g by using Ellman's reagent and result validates thiol modification isolated natural galactomannan.[3]

FTIR study for CP and TCP Galactomannan :

Figure 2 displays the distinctive absorption bands for galactomannans as documented in the literature. The existence of the (polysaccharide) carbohydrate moiety in the mucilage is confirmed by these distinctive peaks. The region between 3062 and 2800 cm1 is indicative of -C-H stretching modes, while bands at 3746 and 3325 cm^{-1} are ascribed to O-H stretching. The broadband between 802 cm^{-1} and 1165 cm^{-1} is caused by the closely linked C-C-O and C-O-C stretching modes of the polysaccharide chain, whereas the region between 1395 cm1 and 1541 cm^{-1} relates to -CH_2 deformation modes.[7] Peak at

1165 cm1 showed bending vibration of C-O for pyranose while the bands at 802 cm1 and 871 cm^{-1} confirmed existence of a d-galacto-pyranose units and d-mannopyranose respectively.

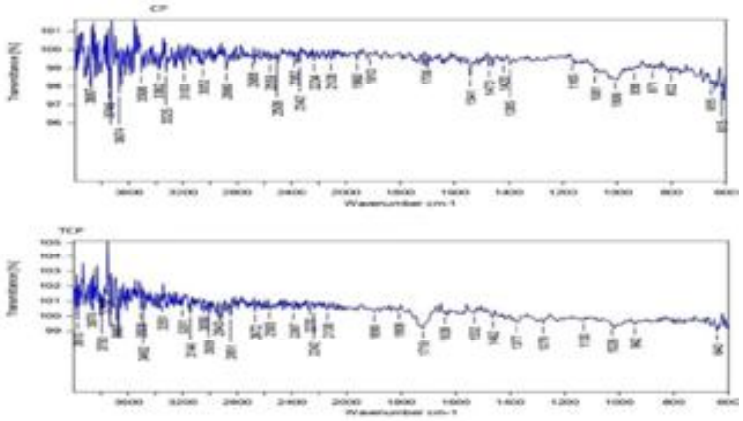

Figure 2. FTIR Spectra of TCP Galactomannan

Differential Scanning Calorimeter of modified mucilage :

DSC endotherm at 247.40°C and exotherm at 115.70°C with a heat of fusion of 81.23J/g were visible on the CP DSC curve (Figure 3). TCP's DSC thermogram revealed an endotherm of 95.70°C, a glass transition temperature of 226.20°C. Consequently, a drop in the heat of fusion and endothermic transition temperature along with modifications in thermal behaviour suggest that CP galactomannan undergone for thiolation.

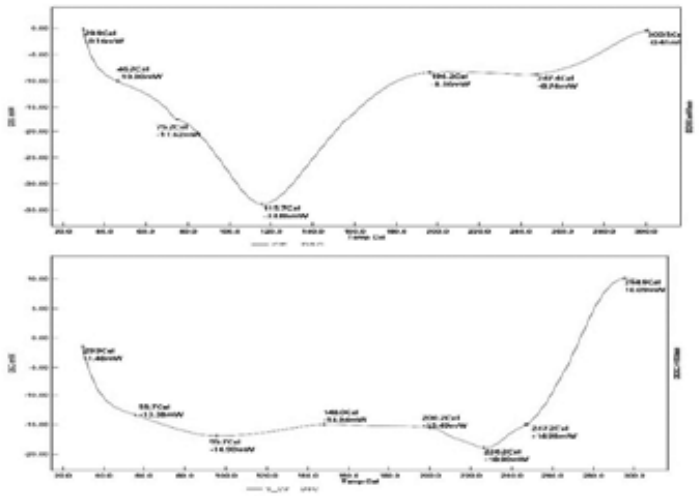

Figure 3. DSC Spectra of TCP and TCP Galactomannan

Zeta potential :

Zeta potential values of CP and TCP (Figure 4.) were -3.5 mV and −12.2 mV, respectively, suggesting anionic nature. The outcome demonstrated that the zeta potential changed to a more negative value could be because the anionic structure has a lot of -OH groups in it.

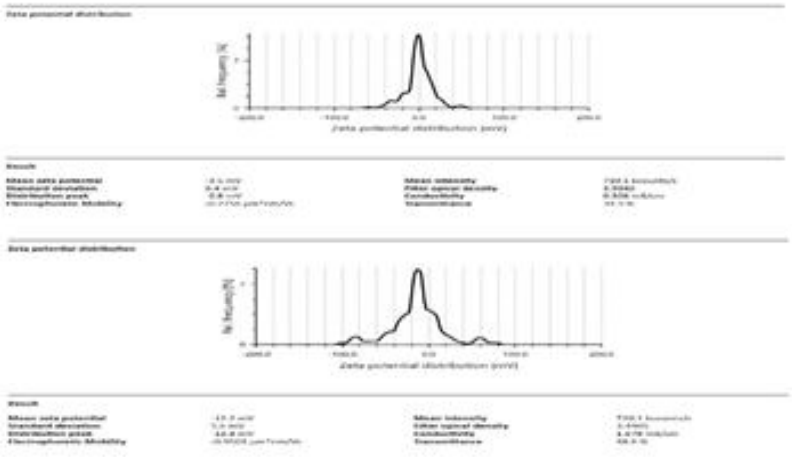

Figure 4. Zeta Potential Measurement of CP and TCP Galactomannan

SEM analysis :

From Figure 5. SEM revealed that CP galactomannan had irregular particle size due to amorphous nature while TCP galactomannan had rough and granular and crystalline surface.

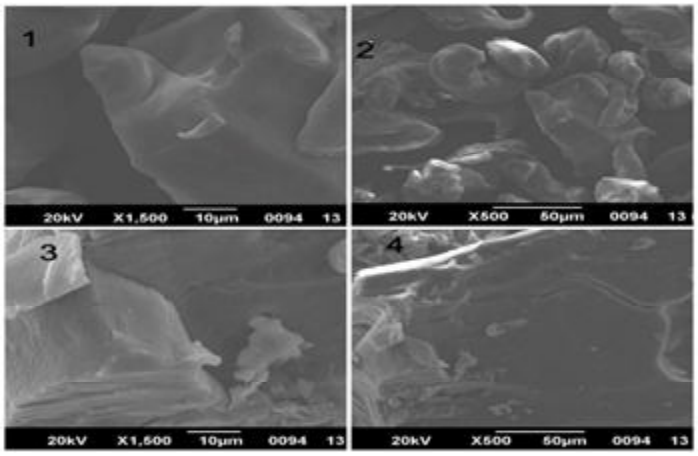

Figure 5. SEM Images 1-2 for CP and 3-4 for TCP Galactomannan

Table-1. Comparative evaluation of film forming agent property of CP and TCP

Physical Property	Results	
	CP Film	TCP Film
Weight (mg)	0.92 ± 0.02	0.93 ± 0.01
Film thickness (mm)	0.14 ± 0.01	0.14 ± 0.02
Moisture content (%)	0.92 ± 0.02	0.90 ± 0.02
pH	6.96 ± 0.05	6.92 ± 0.05
Folding endurance	More than 500	More than 500
Mechanical strength(kg/mm^2)	0.20	0.22
Mucoadhesive Force (dynes/cm^2)	2460.80 ± 89.40	2550.60 ± 89.45

Weight measurement :

Table-1 illustrates the weight range of the films, which was 0.90 to 0.95 mg. The primary need for ensuring drug dosage accuracy in a polymer matrix is weight uniformity.

Film thickness :

The films ranged in thickness from 0.12 to 0.16 mm (Table-1). Proper thickness is necessary for pharmaceutical films to meet drug consistency criteria and improve patient compliance.

Moisture content :

Table-1 displays the moisture content of the CP and TCP seed mucilage films. The decreased proportion of moisture content can be ascribed to the usage of less plasticizer (2% glycerol) during the film production process. Reduced moisture content is ideal since it affects film permeability, drug dissolving rate, chemical stability, and microbial proliferation.

Folding endurance :

Film's folding endurance was greater than 500 (Table-1). It is a measurement of the films' fatigue, stretch, and strength throughout handling, pack aging, and transit. The more flexible film produced by TCP seed polysaccharide compared to CP polysaccharide film proved to be appropriate for use in pharmaceutical applications.

Tensile strength of film :

Ability of the film to withstand with applied stretching is a crucial parameter. TCP film surpassed CP film in mechanical strength, as seen by the 0.22 kg/mm (Table-1).

Mucoadhesive properties :

TCP films had a more mucoadhesive force of 2550.60 ± 89.35 compared to CP film 2460.80 ± 89.40 dynes/cm^2. This strength is crucial factor, especially when it comes to mucosal distribution, including buccal, nasal, and rectal etc. Mucoadhesion of mucilage is due by the hydroxy group.

Scanning electron microscopy (SEM):

Images (Figure 6.) were collected in order to validate the microstructure qualities and assess the film's surface shape. Under a microscope, the surface of the TCP film seemed very smooth, uniform, and break-free, in contrast to the somewhat wrinkled appearance of the CP film.

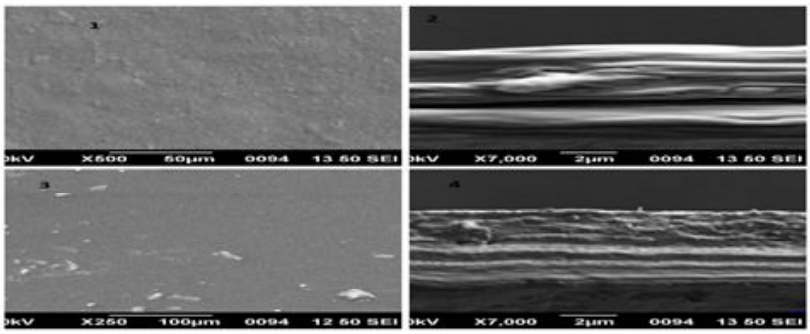

Figure 6. SEM Images 1-2 for CP and 3-4 for TCP galactomannan film

In vitro drug release :

Release up to 2 hours for CP and TCP galactomannan film was found to be nearly equal to 85-90 % demonstrating sustained drug release. The Korsmeyer-Peppa equation was used to examine the drug release data. The n value (0.660) more than 0.5 reflects release of model drug from the film followed non-Fickian release. (Figure 8).

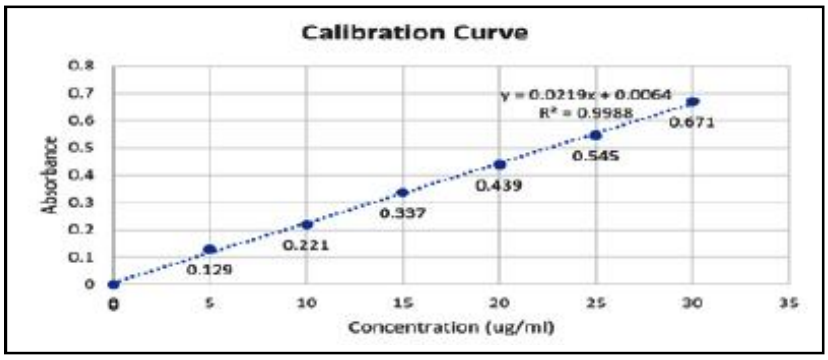

Figure 7. Calibration curve of Ondansetron hydrochloride

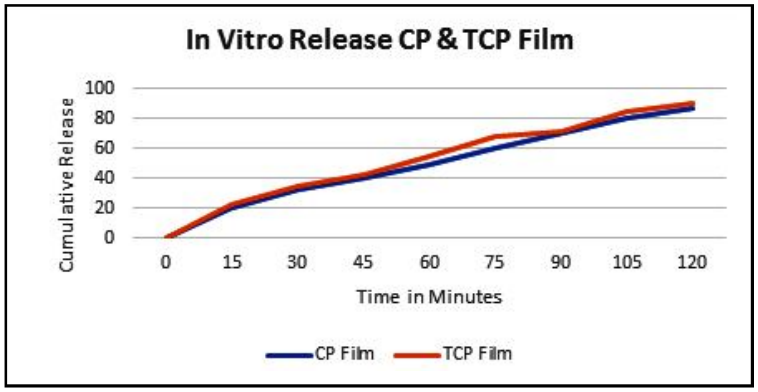

Figure 8. *In-Vitro* Drug release of CP & TCP Film

In this work, CP galactomannan was isolated from Caesalpinia pulcherrima seeds and thiolated using thioglycolic acid. A variety of spectral, thermal, microscopical, and crystallographic parameters, including DLS Particle size, zeta potential, NMR, FTIR, DSC, SEM, XRD, and other preformulation phytochemical investigation parameters, were assessed for both naturally occurring and chemically modified galactomannan. This study showed that CP galactomannan and its thiolated form can be used to create a degradable film for drug delivery applications. The thiolated form of *Caesalpinia pulcherrima* galactomannan film demonstrated improved drug release, folding endurance, mechanical strength, and mucoadhesive strength as compared to the original film. Together with improved texture and optimum characteristics, the TCP galactomannan film also shows good in-vitro degradation. This work revealed a detailed development and characterisation of isolated and modified *Caesalpinia pulcherrima* galactomannan and suggested its suitability of film forming property for drug delivery.

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