

## A Novel and Inexpensive Procedure for the Purification of Concanavalin a from Jack Bean (*Canavalia ensiformis*) Extract

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**Abstract:** In this paper, we have developed a novel method for purification of concanavalin A (Con A) from jack bean meal involving calcium alginate cellulose beads as a physical support. On introducing transition metal ions of Ni (II) the yield was further increased upto 5.25% as compared to 3.38% as obtained by traditional method of Sephadex G-50. The purified lectin was identical in all aspects to Con A as revealed by presence of  $\beta$ -sheet structure by FTIR. Its AFM and SEM images also exhibited the surface morphology of purified lectin. The SDS-PAGE ascertained the homogeneity of the isolated lectin. Thus, our findings lead us to conclusion that our novel method for the purification of Con A is better in terms of cost and protein yield than the classical method involving Sephadex.

**Abbreviations:** Con A, Concanavalin A; AFM, Atomic force microscopy; SEM, Scanning electron microscopy; FTIR, Fourier transform infrared spectroscopy; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis.

**Key words:** AFM • Calcium alginate cellulose beads • Concanavalin A • FTIR • SEM.

### INTRODUCTION

Lectins constitute a group of proteins or glycoproteins of non-immune origin, which bind reversibly to carbohydrates and usually agglutinate cells or precipitate polysaccharides and glycoconjugates [1]. The lectins were redefined by Peumans and Van Damme [2] as proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono or oligosaccharide. However, according to Cummings [3], antibodies and proteins with enzymatic activity related to carbohydrates cannot be considered as lectins. As a consequence of their chemical properties, they have become a useful tool in several fields of biological research (immunology, cell biology, membrane structure, cancer research and genetic engineering). Lectins are present in a wide range of organisms from bacteria to animals, being present in all classes and families, although not in all the kinds and species [4]. Lectin-carbohydrate complex usually involves hydrogen bonds, vander waals forces and hydrophobic interactions. Con A was the first among the lectins to be isolated in the pure form [5, 6]. Its

occurrence in high concentration in the jack bean (*Canavalia ensiformis*), ease of purification and ability to bind glucose and mannose, which widely occur in natural glycoconjugates. Hence, it accounts for the attention received by the lectin. Con A is composed of four identical protomers, held together by polar interactions, hydrogen bonds and electrostatic interactions [7-9]. Each protomer contains one saccharide binding site which appears to react with only one saccharide residue of di- and tri-saccharides [10, 11].

One of the most investigated biological properties of con A is its cell agglutinating ability and interaction with glycoproteins as well as glycolipids. Several studies have shown that cells transformed by RNA or DNA containing tumor viruses are agglutinated by Con A at the concentrations which fail to agglutinate non-transformed cells. Con A chromatography is suitable for baculovirus purification and may be used for purification of the viruses contain surface glycoproteins. The chromatography that harnesses the possible affinity interaction between gp64 and con A has been used for simple and effective baculovirus purification [12]. Another

simple and inexpensive procedure has been reported for the purification of lectin using magnetite and levan to synthesize a composite to which lectins from *Canavalia ensiformis* (Con A) and *Cratylia mollis* (Cramoll 1 and Cramoll 1, 4) got bound specifically [13]. One study describes that a novel, simple and convenient water-soluble biofunctional Au nanodots can be used for the detection of Con A and *Escherichia coli* [14]. Bitter melon peroxidase immobilized on the surface of Con A layered on calcium alginate-starch beads can be used for the successful and effective decolorization of environmentally hazardous textile industrial effluents [15].

Sephadex has been widely used as a support for the purification of Con A. Being biodegradable Sephadex is vulnerable to microbial degradation. Hence, it is necessary to include preservatives in the buffers used with sephadex gel to make it long lasting. On the other hand, it is very expensive as compared to calcium alginate-cellulose beads containing transition metal ions. Sometimes, the Sephadex column used for the purification of Con A gets blocked but calcium alginate-cellulose beads are large that the blocking problem is easily ruled out.

Thus, our present study is aimed to discover an inexpensive, simple and efficient procedure for the purification of Con A by introducing calcium alginate-cellulose beads with Ni (II).

## MATERIALS AND METHODS

**Materials:** Jack-bean meal, Sephadex G-50 and dextran were purchased from Sigma Chemicals Co. (MO, USA). Ovalbumin and SDS were brought from SRL Chemicals Co. (Mumbai, India), [2-amino-2-(hydroxyl methyl) propane-1, 3-diol] (Tris) was obtained from Qualigens Fine Chemicals Co. (Mumbai, India). All other chemicals used in this study were of analytical grade.

**Isolation and Purification of Con A:** Con A was isolated from 10% jack bean meal extract. Jack bean meal was soaked in 0.5 M NaCl at 4 °C for 4 h followed by filtration through four layers of cheese cloth. The homogenate thus obtained was centrifuged by Remi cooling centrifuge at 6000 rpm for 30 minutes. Solid ammonium sulphate was added to the supernatant to achieve 30% saturation followed by 80% saturation. After 12 h, the precipitate was collected by centrifugation and later, dialysed in 0.01 M Tris/HCl buffer containing sodium chloride, manganese chloride and magnesium acetate, pH 7.4. Further purification of Con A was carried out by using Sephadex G-50, calcium-

alginate cellulose beads and calcium-alginate cellulose beads containing nickel (II) as three separate batch processes.

**Preparation of Calcium Alginate Cellulose Beads Containing Metal Ions of Ni (II):** An aqueous mixture of sodium alginate (2.5%), nickel (0.25%) and cellulose (2.5%) was prepared in buffer. The resulting mixture was slowly extruded as droplets through a 5.0 ml syringe with attached needle into 0.2 mol/L calcium chloride solution. The formation of calcium alginate-cellulose beads was instantaneous and the solution was further gently stirred for 2 h. The beads were then washed and stored in 0.1 M acetate buffer of pH 5.6 at 4 °C, for further use.

**Hemagglutination:** Hemagglutinating activity of Con A was detected by using trypsinized erythrocytes. The lectin solution was serially diluted in microtiter V plate that further was mixed with 50 µl of 4% suspension of rabbit erythrocytes. Haemagglutination was observed after 1 h at room temperature. The titer of the tested lectin was expressed as the reciprocal of the highest dilution showing agglutination of trypsinized rabbit erythrocytes.

**FTIR Spectra of Purified Con A:** The FTIR spectrum of purified Con A was monitored by INTERSPEC 2020 model FTIR instrument, USA. The calibration was done by polystyrene film. The samples were injected by Hamiet 100 µL syringe in ATR box. The syringe was first washed by acetone followed by distilled water. FTIR analysis was done to monitor the functional groups of the compounds.

**AFM and SEM:** Tapping mode AFM of purified Con A from calcium-alginate cellulose beads containing nickel (II) was performed using commercial etched silicon tips as AFM probes with typical resonance frequency of ca. 300 Hz (RTESP, Veeco). SEM analysis of the surface and cross-section of freeze dried sample of purified Con A was performed with scanning electron microscope (Philips-515, USA). The membrane samples were mounted on an aluminium sample mount and the sputter was coated with gold to minimize surface charging. The specimens were observed at 15 kV accelerating voltage.

**Determination of Protein Concentration:** Protein concentration was determined by the method of Lowry *et al.* using bovine serum albumin as the standard [16].

**SDS-PAGE:** SDS-PAGE was carried out according to the method of Laemmli *et al.* in 10% acrylamide gel [17].

## RESULTS

**Purification of Con A:** Jack bean extract was precipitated to 80% by ammonium sulphate fractionation method. After that, dialysed homogenate was incubated with Sephadex G-50 or calcium alginate-cellulose beads or calcium alginate-cellulose beads containing nickel (II) separately in beaker. The precipitate was loaded on Sephadex G-50 or calcium alginate-cellulose beads or calcium alginate-cellulose beads containing transition metal ions. The unbound proteins were drained out during the washing of beads of Sephadex and cellulose. The bound protein on the Sephadex G-50 or the calcium alginate-cellulose beads or calcium alginate-cellulose beads containing transition metal ions was eluted by 0.2 M glucose solution. The solution of the eluted protein had bound glucose on its surface. Glucose was removed from the protein by extensive dialysis in Tris/HCl buffer pH 7.4, containing sodium chloride, manganese chloride and magnesium acetate. The protein yield and the degree of purification obtained at various stages of isolation of Con A from jack bean meal by various methods have been summarized in table 1. This Table represent the Con A purified with the help of Sephadex G-50 or calcium alginate-cellulose beads or calcium alginate-cellulose beads containing Ni (II). The protein yield of Con A purified by calcium alginate-cellulose beads containing transition metal ions (nickel) was 5.25% as compared to 3.38% achieved by sephadex G-50 procedure.

**SDS-PAGE:** SDS-PAGE of crude jack bean meal extract, Con A purified by Sephadex G-50 and lectin purified from calcium alginate-cellulose beads containing transition metal ions has been shown in figure 1. Lane 1 represents the unbound protein after incubation with Sephadex G-50 that appears like a smear due to presence of variety of proteins. Similar pattern was observed in lane 2

representing the crude extract after incubation with calcium alginate cellulose beads containing Ni (II) ions. A band of bound protein by sephadex G-50 and calcium alginate-cellulose beads containing nickel (II) has been represented in lane 3 and 4 respectively. The migratory behavior of bound protein was identical to commercially available Con A in lane 5. The similarity in the electrophoretic patterns of the purified and the commercially obtained Con A suggested that the purified Con A was homogenous with respect to commercial Con A.

**Hemagglutination Activity:** The crude homogenate of jack bean meal was tested for its agglutination activity to trypsinised rabbit RBC. The purified protein by calcium alginate-cellulose beads containing Ni (II) ions exhibited agglutination activity with higher titer (5712) with respect to Con A purified by sephadex G-50 (5266). Hence, the purified protein is a lectin and most probably Con A.

**FTIR Analysis:** The lectin purified is Con A was further confirmed by FTIR spectroscopy. FTIR analysis revealed the presence of amide I (1635.76), amide II (1535.04), bands as depicted by figure 2. Although the native-state Con A secondary structure is predominantly  $\beta$ -sheet with no  $\alpha$ -helix, the sequence must have some helical propensity. It manifests that the main structure is consisted of  $\beta$  sheet forms, thus it shows the structure of Con A [18].

**AFM and SEM Analysis:** Figure 3a presents the AFM image of Con A purified by calcium-alginate cellulose beads containing Ni (II) ions while its 3-dimensional image with corresponding height has been shown in Figure 3b. Clearly, the lateral size of the protein imaged by the AFM was found large due to the convolution effect of the tip, while the height probably reflects the protein size more accurately.

Table 1: Purification table of Con A with the help of Sephadex G-50, calcium alginate-cellulose beads and calcium alginate-cellulose beads containing Ni (II)

Step fraction	Total protein (mg)*	Total activity (titer)#	Specific activity (titer/mg)	Purification Fold	Protein Yield (%)
Dialysed homogenate (Common to all)	340	6399	18.8	1	100
Purification of Con A by using Sephadex G-50.					
Elution by 0.2 M glucose solution.	11.52	5266	457.1	24.3	3.38
Purification of Con A by using calcium alginate-cellulose beads.					
Elution by 0.2 M glucose solution.	19.2	5119	266.6	14.1	4.14
Purification of Con A by using calcium alginate-cellulose beads containing Ni (II) ions.					
Elution by 0.2 M glucose solution.	17.85	5712	320	17.02	5.25

# The titer of the tested lectin is expressed as the reciprocal of the highest dilution showing agglutination of trypsinized rabbit erythrocytes.

\* Determined by the method of lowry *et al* (1951).

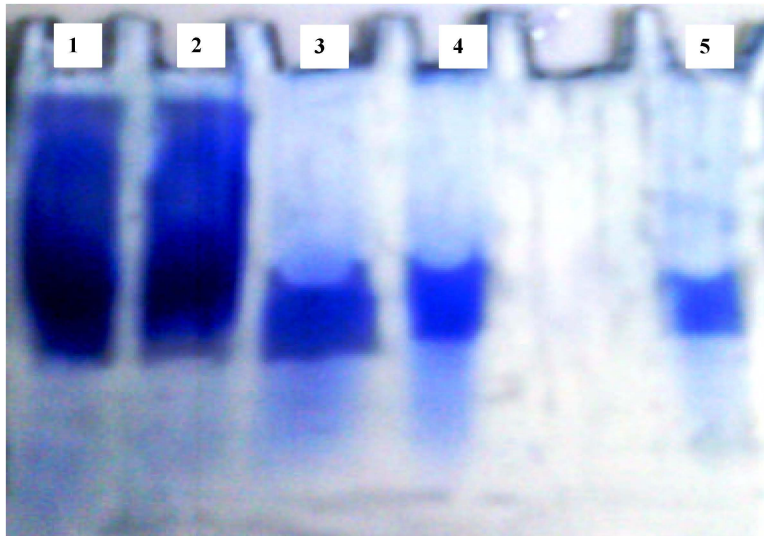


Fig. 1: SDS-PAGE of fraction produced during the purification of Con A (10% gel, stained with Coomassie Blue R250). Lane; 1 (Crude after incubation with sephadex), 2 (Crude after incubation with cellulose beads containing metal ions), 3 (Purified Con A from Sephadex), 4 (Purified Con A from cellulose beads containing metal ions). 5 (Commercially purified Con A).



Fig. 2: FTIR spectra of Con A purified by cellulose beads containing metal ions.

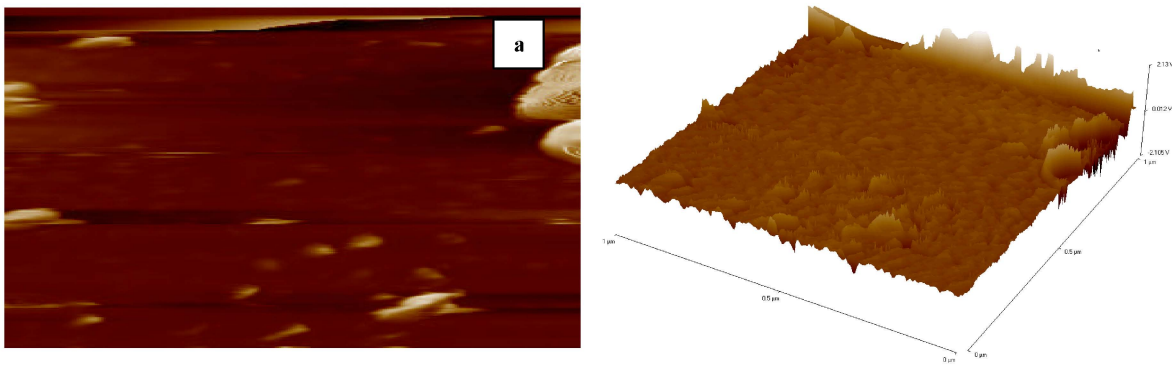


Fig. 3: AFM micrographs of Con A purified by cellulose beads containing metal ions (a) 2D image of Con A (b) 3D image of Con A.

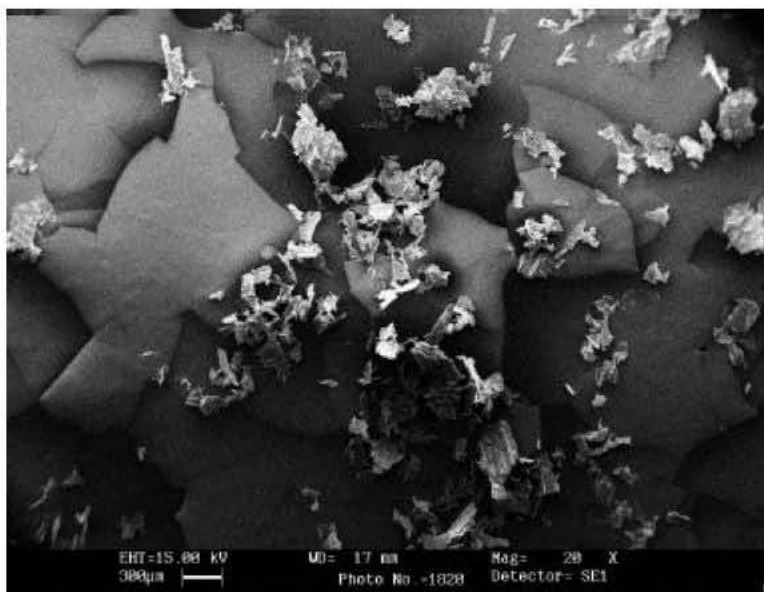


Fig. 4: SEM image of Con A purified by cellulose beads containing metal ions.

SEM observations further confirmed the AFM results. Figure 4 showed SEM image of the Con A that clearly demonstrates the Con A in clusters. Each bunch was composed of closely packed Con A forming a radiating structure resulting from freeze-drying of the sample. SEM and AFM showed the morphological homogeneity of the purified Con A.

#### DISCUSSION

The proposed novel method for Con A purification by calcium alginate-cellulose beads containing nickel showed to give higher protein yield as compared to the purified by Sephadex G-50 by batch process. The purified lectin was identical in all aspects to Con A as revealed by SDS PAGE and FTIR. Its AFM and SEM images demonstrate the morphology of Con A. Increase in the yield of Con A purified by calcium alginate-cellulose beads containing transition metal ions as compared to calcium alginate-cellulose beads (without Ni) may be due to the affinity of histidine and tryptophan occurring on Con A towards the transition metal ions [19]. Besides, the cost of purification of Con A by calcium alginate cellulose beads as the solid support is very low as compared to the cost of Sephadex G-50 used in classical Con A purification method. The novel method has superiority over Sephadex in terms of storage, simplicity, cost and protein yield.

Purified Con A can be used for glycoprotein enrichment by a nanoscale, chelating, Con A

monolithic capillary prepared by using GMA-EDMA (glycidyl methacrylate-co-ethylene dimethacrylate) as polymeric support. Con A was immobilized on Cu (II)-charged iminodiacetic acid (IDA) regenerable sorbents by forming a IDA:Cu (II):Con A sandwich. This immobilized lectin chromatography can be employed for glycoprotein enrichment [20]. Methods like one-step affinity-chromatography involve Con A and magnetic beads for separation of highly purified plasma membrane proteins from crude membrane preparations or cell lines. Con A was immobilized on magnetic beads by binding biotinylated concanavalin A to streptavidin magnetic beads [21]. A number of neurodegenerative diseases are known to involve protein aggregation. Common mechanisms and structural properties of amyloids are supposed to be involved in aggregation-related cytotoxicity. Study on Con A aggregation can be used as an ideal model to study the relationship between cell toxicity and aggregation processes [22].

Studies on pH exposure of Con A in the presence of methyl  $\alpha$ -D-glucopyranoside and methyl  $\alpha$ -D-galactopyranoside, the former acted as a protector preventing conformational alteration at different pH while the presence of latter induced a different stable conformational state and this state persists over the pH range from 2 to 10 [23]. Con A at 30%(v/v) PEGs exists as compact intermediate with molten-globule-like characteristics, viz., enhanced hydrophobic surface area, retention of compact secondary as well as tertiary structure and a considerable degree of carbohydrate

binding specificity and activity [24]. Con A has shown a remarkable antiproliferative effect on human melanoma A375 cells [25]. It is demonstrated that there was a link between the antiproliferative activity of Con A and its sugar-binding activity. An interesting aspect of this interaction is that Con A can induce human melanoma A375 cell apoptosis in a caspase-dependent manner [25].

Thus the purification procedure of Con A with the help of calcium alginate-cellulose beads containing transition metal ions is a better purification method than the Sephadex G-50 based classical method. This novel procedure is superior in terms of cost, storage and protein yield. Further studies are required to fine-tune this proposed Con A purification method to make it convenient and commercial.

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