

REVIEW PAPER

Gel filtration chromatography as a versatile tool in lectin purification - A review

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Abstract. Lectins are a diverse class of carbohydrate-binding proteins found throughout nature, known for their significant biological and therapeutic potential. They play crucial roles in immune modulation, pathogen recognition, antiviral defense, and cancer inhibition. Due to their biomedical importance, the purification of lectins in a pure and structurally intact form is essential for precise structural and functional studies. Among various purification methods, gel filtration chromatography (GFC) has proven to be a reliable and gentle technique for separating lectins according to molecular size while preserving their natural conformation and bioactivity. This review summarizes the use of GFC for lectin purification from diverse sources such as plants, fungi, algae, animals, and microorganisms. GFC effectively distinguishes monomeric, dimeric, and multimeric lectins, offering insights into their molecular weight, structural integrity, and homogeneity. Although the method maintains protein activity and compatibility with downstream analyses, it shows limited resolution for proteins of similar molecular size, often requiring additional purification steps. Overall, GFC remains a fundamental and versatile technique in lectin purification, contributing substantially to advancements in analytical chemistry, glycoscience, immunology, and pharmaceutical research. This review comprehensively summarizes the role of gel filtration chromatography as a gentle, size-based technique for purifying lectins from diverse biological sources while preserving their structural integrity and bioactivity.

Keywords: lectins, proteins, gel filtration, purification.

Classification numbers: 1.4, 1.2.

1. INTRODUCTION

Lectins constitute a unique group of proteins widely distributed in nature, characterized by their selective interaction with carbohydrates and glycoconjugates [1]. Lectins are well known for their roles in fighting viral infections, balancing blood sugar, protecting against pathogens, and slowing cancer growth. Lectins have gained considerable interest for their potential therapeutic applications [2–4]. Lectins are derived and isolated from plants, animals, and fungi. Although their complete range of functions and applications has yet to be fully identified, their widespread occurrence, remarkable thermal stability, and specific carbohydrate-binding

properties make them valuable tools in glycoscience, immunology, biotechnology, healthcare, and the pharmaceutical industry for diverse studies and applications [5]. Purification of lectins generally involves several techniques, including ammonium sulphate precipitation, followed by dialysis, gel filtration and affinity chromatography [6]. Gel Filtration Chromatography (GFC) is a common method for purifying proteins such as lectin based on molecular size. Smaller molecules enter the porous matrix more readily than larger ones, leading to their separation according to size differences [7, 8]. This method is particularly advantageous because the mild conditions of the mobile phase enable accurate protein characterization while maintaining their natural structural integrity and environmental context [9]. Several types of porous media are used in this method including dextran (Sephadex™), polyacrylamide (Bio-Gel P™), agarose (Sephacrose™ and Bio-Gel A™), and mixed matrices such as dextran–polyacrylamide (Sephacryl™). Each medium is produced with pores of varying sizes to facilitate the separation of molecules based on their molecular dimensions [8]. The continued refinement of chromatographic techniques and material design has significantly enhanced the efficiency and resolution of protein purification, making gel filtration one of the most reliable tools for studying lectin structure and function [9, 10].

This review examines studies published between 1975 and 2025, drawing on data from Scopus, ResearchGate, Google Scholar, and Directory of Open Access Journals (DOAJ). This review aims to highlight the purification of lectins with a specific focus on size exclusion/gel filtration chromatography. It discusses the principle, advantages, and applications of this technique in isolating lectins while preserving their structure and activity.

2. A GEL FILTRATION CHROMATOGRAPHIC METHOD FOR THE PURIFICATION OF LECTINS

Lectins are a diverse group of proteins capable of recognizing and binding specifically to carbohydrate components present in glyco-conjugates on cell membranes [11]. Plant lectins possess remarkable therapeutic properties, demonstrating antiviral, anticancer, and immune-regulating activities [8, 12]. Algal lectins exhibit promising antiviral properties, making them potential candidates for developing novel antiviral agents [13]. Similarly, animal lectins display a wide range of biological activities, including roles in immune regulation, cell signaling, and pathogen recognition, highlighting their importance in both physiological processes and therapeutic research [14]. Consequently, lectins have gained significant attention in biomedical research for their potential use as diagnostic biomarkers and as promising agents in cancer therapy [15]. For this reason, purifying lectins is a crucial step to ensure their suitability for biomedical applications and therapeutic use, as purity directly influences their specificity, safety, and biological effectiveness [16]. Several methods can be employed to purify lectins, including precipitation, affinity chromatography, and ion-exchange chromatography; however, GFC is often considered the most effective and efficient technique for obtaining high-purity lectin preparations [17]. Table 1 indicates summary of lectin purification studies using GFC.

Table 1. Analysis of lectin purification using gel filtration methods.

Source of lectin	Gel filtration matrix name	Purification outcome	Reference
<i>Arachis hypogaea</i> (Peanut agglutinin, PNA)	Sephadex	Confirmed tetrameric (110 kDa) structure; four identical 27.5 kDa subunits	[18]
<i>Clerodendrum infortunatum</i>	Sephadex G-200	Isolated galactose-specific lectin	[19]

seeds		prior to affinity step	
<i>Viscum album</i>	Sephadex G-150	Achieved 11.64-fold purification; HPLC single peak	[20]
<i>Inocybe umbrinella</i>	Superdex 75	Final step; 160-fold enrichment; 17 kDa monomer	[21]
<i>Phaseolus vulgaris</i> (French bean)	Superdex 200 FPLC	Final purification; dimeric (2 × 32 kDa) structure	[22]
<i>Penicillium duclauxii</i>	Sephadex G-100	Separated lectin post ion-exchange; homogeneous glycoprotein	[23]
<i>Pisum sativum</i> (Green split pea)	Superdex 200 FPLC	Isolated heterotetrameric native protein	[24]
<i>Russula lepida</i> (RLL)	Superdex 75 FPLC	Final step; monomeric, homogeneous lectin	[25]
<i>Acinetobacter baumannii</i>	Sephadex G-100	Isolated homogeneous lectin for assays	[26]
<i>Armillaria luteo-virens</i>	Superdex 75 FPLC	Final step; confirmed purity after multiple ion-exchange steps	[27]
<i>Typhonium divaricatum</i> (TDL)	Sephacryl S-200	Produced homogeneous 48 kDa lectin; bioactive	[28]
<i>Indigofera heterantha</i>	Sephadex G-100	Purity confirmed by PAGE & SDS-PAGE	[29]
<i>Heterometrus granulosomanus</i> (Scorpion)	Sephadex G-200	Final step after affinity chromatography	[30]
<i>Aristichthys nobilis</i>	Sephacryl S-200 HR / Superdex 200	Final step; homogeneous, intact lectin	[31]
<i>Phaseolus lunatus</i> (Lunatin)	Superdex 75	Final step; homogeneous glycoprotein	[32]
Lectins from various plant source	Sephadex G-100	Purified stable lectin; enhanced bioactivities	[33]
<i>Alisma orientale</i> (Sam.) Juzep	Sephacryl S-200	Yielded homogeneous monomeric lectin	[34]
<i>Caulerpa cupressoides</i> (CcL)	Sephadex G-100	Isolated active lectin for bioassays	[35]
<i>Tuber borchii</i>	Sephadex G-75	Verified purity & structure before interaction assays	[36]
<i>Terfezia sp.</i> tubers	Sephadex G-100	Achieved high purity & molecular homogeneity	[37]
<i>Capparis spinosa</i> seeds	Superdex 75	Final step; homogeneous lectin for activity tests	[38]
Microalga BTM 11	Sephadex G-75	Key step; homogeneous 17 kDa protein; antiviral activity	[39]

In the purification of peanut agglutinin (PNA), gel filtration chromatography played a crucial role in determining the molecular characteristics and homogeneity of the lectin. This technique effectively separated PNA based on molecular size, confirming its tetrameric nature with an estimated molecular weight of approximately 110 kDa. The gel filtration process also revealed the presence of four identical subunits (≈ 27.5 kDa each) when analyzed under denaturing conditions, indicating that the lectin exists as a stable, symmetric multimer. Overall, gel filtration proved essential not only for purification but also for assessing the structural integrity and oligomeric state of the lectin [18]. While studied by Sukumaran and Haridas, a galactose-specific lectin from *Clerodendrum infortunatum* seeds was successfully purified using

GFC on a Sephadex G-200 column, followed by affinity chromatography. The gel filtration step played a crucial role in isolating the lectin fraction prior to further purification, demonstrating its effectiveness as a key technique for lectin purification. In *Arachis hypogaea* lectin purification, gel filtration on Sephadex G-150 proved essential for achieving high purity and confirming structural homogeneity [20]. Lectin from *Inocybe umbrinella*, GFC using a Superdex 75 column was employed as the final purification step, ensuring the separation of the 17 kDa lectin from other protein contaminants. This size-based purification step significantly enhanced purity achieving nearly 160-fold enrichment and confirmed the monomeric nature of the lectin. The effectiveness of gel filtration in refining lectin purity highlights its importance in isolating bioactive fungal proteins for structural and functional studies [21]. Purification of *phaseolus vulgaris* (French bean) lectin by GFC using Superdex 200 (FPLC – Fast Protein Liquid Chromatography) served as the final and most critical step in achieving a highly purified and homogeneous protein. Following affinity and ion-exchange chromatography, the gel filtration stage effectively separated the lectin based on molecular size, confirming its dimeric structure composed of two 32 kDa subunits. This method ensured the removal of residual contaminants and allowed precise determination of the lectin's molecular composition, demonstrating the efficiency of gel filtration in lectin purification workflows [22]. In the purification process of *Penicillium duclauxii* lectin, GFC using Sephadex G-100 played a crucial role in refining the protein and confirming its molecular structure. After initial ion-exchange chromatography on DEAE-Sepharose, gel filtration effectively separated the lectin according to molecular size, yielding a purified glycoprotein with distinct subunits. This technique proved instrumental in obtaining a homogeneous lectin preparation for further biochemical and functional analyses [23]. In the purification study of the lectin from the red marine alga *Vidalia obtusiloba*, GFC was essential for assessing the protein's molecular characteristics and purity. This technique effectively separated the lectin based on molecular size, ensuring a homogeneous preparation and providing valuable insight into its structural composition. Gel filtration proved to be a critical step in validating the structural and biochemical identity of the purified algal lectin [40]. In the purification of *Bothrops leucurus* lectin, GFC was used after affinity purification to isolate the protein based on molecular size. This step yielded a pure, dimeric lectin and confirmed its structural uniformity, demonstrating the effectiveness of gel filtration in achieving homogeneity and precise molecular characterization [41]. Lectins from *Penicillium chrysogenum* lectin (PeCL), GFC was the primary technique used to isolate the mannose-specific protein from the fungal extract. This method effectively separated PeCL based on molecular size, yielding a pure lectin that was confirmed by SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis method). The gel filtration step provided a rapid and efficient means of obtaining a homogeneous lectin suitable for functional assays, highlighting its importance in producing bioactive fungal lectins for potential applications such as pest control [42]. In the purification of the lectin complex from *Clathrina clathrus*, GFC played a critical role as the final purification step. This technique effectively separated the lectin complex based on molecular size, ensuring the isolation of a homogeneous and active form. Followed by haemagglutination assays, gel filtration confirmed the multimeric nature and structural integrity of the lectin, demonstrating its importance in obtaining a purified, calcium-dependent protein complex suitable for biochemical characterization [43].

Purification of the green split pea (*Pisum sativum*) lectin, GFC on a Superdex 200 column using FPLC played a key role in obtaining a highly purified and structurally defined protein. This size-based separation step efficiently isolated the lectin, confirming its native molecular weight and heterotetrameric configuration. The use of gel filtration ensured the recovery of a

homogeneous and biologically active lectin preparation suitable for further structural and functional analyses [24].

Gel filtration chromatography served as the final step in purifying *Pleurotus flabellatus* lectin (PFL-L), following DEAE-cellulose anion-exchange chromatography. It efficiently separated PFL-L by molecular size, confirming its homogeneity as an 18 kDa monomer. This step was essential for removing residual impurities and obtaining a pure, biologically active lectin for further analyses [44]. In kidney bean (*Phaseolus vulgaris* L.) lectins, size exclusion chromatography technique ensures high purity and homogeneity, effectively removing impurities and verifying the protein's structural integrity. The purified lectin obtained through gel filtration is essential for accurate allergen detection and functional characterization [45]. Purification of the novel lectin from *Russula lepida* (RLL), GFC served as a critical final step to achieve molecular homogeneity. Following ammonium sulfate precipitation and ion-exchange chromatography on DEAE-cellulose and SP-Sepharose, FPLC using a Superdex 75 column effectively separated the lectin based on molecular size. This gel filtration step confirmed the purity and monomeric nature of RLL, ensuring its structural integrity for subsequent analyses of hemagglutination and antitumor activities [25]. In *Acinetobacter baumannii*, lectin purification involved sequential chromatographic techniques following extraction with glass beads. GFC on a Sephadex G-100 matrix served as a key step for separating the lectin based on molecular size, enabling the isolation of a homogeneous protein suitable for functional assays [26]. In the purification of the *Armillaria luteo-virens* lectin, GFC played a crucial role in achieving molecular homogeneity. Following ammonium sulfate precipitation and successive ion-exchange steps on DEAE-cellulose, CM-cellulose, and Q-Sepharose, FPLC on a Superdex 75 column effectively separated the lectin based on molecular size. This step ensured its purity for subsequent characterization [27]. During the purification of the mannose-binding lectin from *Typhonium divaricatum* (TDL), GFC played a pivotal role in obtaining a highly pure and structurally uniform protein. After ammonium sulfate precipitation and ion-exchange purification on DEAE-Sepharose and CM-Sepharose, the final separation on a Sephacryl S-200 column efficiently resolved the lectin according to molecular size. This step produced a homogeneous 48 kDa protein, confirming its purity and suitability for subsequent analyses of its hemagglutinating, antiviral, and antiproliferative properties [28]. *Indigofera heterantha* lectin was purified using Sephadex G-100 gel filtration, and its purity was confirmed by PAGE and SDS-PAGE [29]. In contrast, the lectin from *Trilepisium madagascariense* underwent partial purification through Sephadex G-75 gel filtration, whereas purification of a sialic acid-specific lectin from the Indian scorpion *Heterometrus granulosomanus* involved affinity chromatography followed by Sephadex G-200 gel filtration [30, 46]. In *Aristichthys nobilis*, purification of the fucose-binding lectin involved ion-exchange chromatography followed by gel filtration on Sephacryl S-200 HR and Superdex 200 columns. This step was essential for obtaining a homogeneous, structurally intact lectin suitable for functional and biochemical characterization [31]. In the purification of the lectin Lunatin from *Phaseolus lunatus*, GFC on a Superdex 75 column was used as the final step following ammonium sulfate precipitation, affinity, and ion-exchange chromatography. This step efficiently separated the lectin by molecular size, producing a homogeneous glycoprotein suitable for biochemical and bioactivity analyses [32]. A novel lectin from various plant sources can be purified using ammonium sulfate precipitation, ion-exchange chromatography on DEAE-cellulose, yielding a purified protein. Gel filtration effectively separated the lectin, which was stable and gel filtration on Sephadex G-100 under heat and showed enhanced antibacterial, antiviral, and cytotoxic activities after purification [33]. A novel lectin was purified from the fresh rhizome of *Alisma orientale* (Sam.) Juzep using ammonium sulfate precipitation, affinity chromatography on Affi-

Gel Blue, and GFC on a Sephacryl S-200 column. Gel filtration enabled separation based on molecular size, yielding a homogeneous monomeric protein [34].

The lectin from *Caulerpa cupressoides* (CcL) was purified using a chromatographic process that included GFC on a Sephadex G-100 column. This step was crucial for isolating the active lectin fraction used in subsequent biological assays evaluating its antinociceptive and anti-inflammatory properties [35]. The fruiting body-specific protein TBF-1, a novel lectin from *Tuber borchii*, was purified through a multistep process that included GFC on a Sephadex G-75 column. Gel filtration was essential for confirming the purity and structural integrity of TBF-1 prior to studying its specific interactions with *Rhizobium* species associated with the truffle [36]. Lectin from *Terfezia* sp. tubers was extracted and purified through ammonium sulfate precipitation, ion-exchange chromatography, and GFC on a Sephadex G-100 column. The gel filtration step was crucial for achieving high purity and confirming the molecular homogeneity of the lectin [37]. The purification of the *Burkholderia cenocepacia* lectin included a crucial gel filtration chromatography step to separate the protein based on molecular size and confirm its oligomeric state. Using Superdex 200 gel filtration, researchers demonstrated that it exists as a hexameric complex composed of mannose/heptose-specific dimers and fucose-specific trimers. This gel filtration step was essential for verifying the protein's quaternary structure and purity prior to subsequent structural and functional analyses, including electron microscopy and X-ray scattering [46]. Purification of the *Capparis spinosa* seed lectin-like protein, GFC on a Superdex 75 column served as the final and crucial step. The gel filtration step was essential for confirming the protein's purity and structural uniformity, ensuring its suitability for subsequent analyses of its antiproliferative, antifungal, and HIV-1 reverse transcriptase inhibitory activities [38].

In the purification of lectin from microalgae BTM 11, GFC on a Sephadex G-75 column was employed as a key step to achieve size-based separation and obtain a homogeneous 17 kDa protein. This step was crucial in confirming the purity and structural uniformity of the lectin, enabling accurate characterization of its antiviral and antibacterial properties against pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* [39].

3. ADVANTAGES AND LIMITATIONS OF GEL FILTRATION CHROMATOGRAPHY DURING PURIFICATION OF LECTINS

Table 2 indicates advantages and limitations of gel filtration chromatography during purification of lectins.

Table 2. Advantages and limitations of GFC during purification of lectins.

Aspect	Advantages	Limitation	References
Gentle, non-binding separation	Maintains the native structure and biological activity of lectins because separation occurs under mild buffer conditions without strong matrix interactions	Low resolution between lectin and proteins of similar molecular size; may not achieve high purity if contaminants have comparable hydrodynamic radii.	[47]
Size-based fractionation	Efficiently separates monomeric, dimeric, and aggregated lectin forms.	Cannot differentiate proteins of similar molecular size but different charge or shape; anomalous elution possible for non-globular lectins.	[48]

Preservation of carbohydrate-binding activity	Avoids harsh elution conditions (no high salt or pH extremes), thus preserving lectin's hemagglutination or sugar-binding properties,	Yields moderate purification only; affinity chromatography is usually required for high specificity. [22–47]
Molecular weight and oligomer estimation	Provides a reliable estimation of lectin oligomeric structure	Gives approximate MW; deviations occur due to glycosylation or non-spherical shape of lectins. [49]
Compatibility with functional assays	Eluates in a buffer suitable for direct downstream assays (e.g., hemagglutination, sugar binding).	Lectin activity can be lost upon sample concentration post-filtration due to dilution. [50]

Gel filtration chromatography offers several advantages in lectin purification due to its gentle, non-binding separation mechanism that preserves the native structure and biological activity of lectins under mild buffer conditions. However, it provides low resolution when separating proteins of similar molecular size [47]. Its size-based fractionation efficiently separates monomeric, dimeric, and aggregated lectin forms, though it cannot distinguish proteins of similar size but different shape or charge [48]. The method also helps preserve carbohydrate-binding activity, avoiding harsh conditions that could inactivate lectins, but often yields only moderate purification, requiring additional affinity steps [22, 47]. It enables estimation of molecular weight and oligomeric state, though values may be approximate due to glycosylation or molecular shape variations [49]. Furthermore, it is compatible with functional assays, as eluates can be directly used for biological testing, but dilution during elution may cause partial loss of activity upon concentration [50].

4. FUTURE SCOPE OF GEL FILTRATION CHROMATOGRAPHY IN THE PURIFICATION OF LECTINS

The future scope of GFC in lectin purification is promising and closely tied to ongoing innovations in chromatographic technologies. Advances in stationary phase design, such as the development of matrices with uniform pore size distribution, enhanced surface chemistry, and improved mechanical stability, are expected to greatly enhance separation efficiency and column longevity. Furthermore, coupling GFC with advanced analytical detectors such as multi-angle light scattering (MALS), dynamic light scattering (DLS), and online UV–Vis spectroscopy facilitates simultaneous analysis of molecular weight, aggregation state, and conformational stability during purification [51, 52]. Automation and miniaturization of GFC systems will enable high-throughput purification, particularly beneficial for recombinant lectins used in therapeutic and diagnostic research. Additionally, the integration of GFC with hybrid purification platforms, combining it with affinity or hydrophobic interaction chromatography, is anticipated to achieve superior selectivity, resolution, and homogeneity in lectin preparations. On an industrial scale, the emergence of continuous flow and membrane-based size-exclusion systems will further enhance scalability and reproducibility. As lectins continue to gain importance in biomedical research, pharmaceuticals, and biosensor technologies, GFC is projected to evolve from a mere polishing step into a pivotal component of integrated purification and analytical quality control systems. This progression will ensure reproducibility, structural stability, and regulatory compliance in lectin-based product development [53, 54].

5. CONCLUSION

Gel filtration chromatography (GFC) remains a cornerstone in the purification of lectins due to its ability to achieve gentle, size-based separation while maintaining the native structural and functional integrity of these carbohydrate-binding proteins. Across studies from 1975 to 2025, GFC has consistently demonstrated its effectiveness in isolating homogeneous lectins from diverse biological sources, including plants, fungi, algae, and microorganisms. It not only facilitates purification but also enables determination of lectin molecular weight, quaternary structure, and oligomeric state, which are critical for functional and structural characterization. When applied as a final or intermediate purification step, GFC complements techniques such as affinity and ion-exchange chromatography to yield high-purity preparations suitable for biomedical, diagnostic, and therapeutic applications.

Despite its advantages, GFC faces challenges such as low resolution among proteins of similar molecular size, moderate purification capacity, and variability across experimental conditions, which limit scalability and standardization. Future advancements are expected to refine these limitations through innovations in stationary phase design, introduction of uniform-pore matrices, enhanced mechanical stability, and real-time integrated analytics such as MALS, DLS, and UV-Vis detection. The integration of GFC with automated and continuous-flow systems, as well as hybrid separation platforms, will promote high-throughput, reproducible, and industrial-scale purification processes.

As lectin-based research expands within pharmaceuticals, nanomedicine, and biosensor applications, GFC is poised to evolve from a conventional separation method into a versatile, analytically integrated platform ensuring structural consistency, purity, and reproducibility. This evolution will play a vital role in advancing lectin-based product development and ensuring that these biomolecules continue to serve as valuable tools in medicine and biotechnology.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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