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Review

Lectin Purification through Affinity Chromatography Exploiting Macroporous Monolithic Adsorbents

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Citation: Silva, J.F.d.; Lima, C.M.G.; da Silva, D.L.; do Nascimento, I.S.; Rodrigues, S.d.O.; Gonçalves, L.A.; Santana, R.F.; Khalid, W.; Verruck, S.; Emran, T.B.; et al. Lectin Purification through Affinity Chromatography Exploiting Macroporous Monolithic Adsorbents. *Separations* **2023**, *10*, 36. https://doi.org/10.3390/separations10010036

Academic Editor: Danilo Sciarrone

Received: 28 October 2022
Revised: 23 December 2022
Accepted: 30 December 2022
Published: 6 January 2023

Abstract: Growing medical, engineering, biochemical, and biological interest has led to a steady pace of research and development into polymeric monolithic structures with densely interconnected pores for purifying bio compounds. Cryogels, which are generated by freezing a reactive polymerization mixture, are highlighted due to their versatility and low relative cost as macroporous, polymeric, monolithic adsorbents. The conversion of cryogels into affinity adsorbents is one possible alternative to their optimal application. Some of the most often utilized supports for immobilizing particular ligands are monolithic columns manufactured with epoxy radicals on their surfaces. The purification of biomolecules with a high degree of specificity, such as lectins and glycoproteins with an affinity for glycosylated groups, has garnered interest in the use of fixed non-traditional beds functionalized with ligands of particular interest. The interaction is both robust enough to permit the adsorption of glycoproteins and reversible enough to permit the dissociation of molecules in response to changes in the solution's pH. When compared to other protein A-based approaches, this one has been shown to be more advantageous than its counterparts in terms of specificity, ease of use, and cost-effectiveness. Information on polymeric, macroporous, monolithic adsorbents used in the affinity chromatographic purification of lectins has been published and explored.

Keywords: purification of bio compounds; macromolecules; affinity chromatography; monolithic cryogels; glycoproteins; versatile applications

1. Introduction

Developing techniques and methods for separating and purifying biological macromolecules such as proteins has been an important prerequisite for many of the advances

made in several areas of knowledge. In the field of chromatography, the development of new porous resin supports, new cross-linking agaroses, and new porous silicas allowed rapid growth in high-resolution techniques such as high-efficiency liquid chromatography, analytical and laboratory preparative scales, as well as for industrial chromatography in columns with several-hundred-liter bed volumes [1]. Macroporous monoliths, called cryogels, are produced from a solution of monomers and hydrophilic polymers using cryofreezing techniques previously used for biomedical applications. Cryogeleification is a specific type of synthesis of polymeric gels in which the formation begins in the cryogenic treatment of systems capable of forming gels [2]. The use of cryogels that incorporate free epoxy radicals on their surfaces is one of the most frequent ways to immobilize ligands of interest. This is due to the ease with which amine-, thiol-, or hydroxyl-group molecules can react to form a stable covalent bond. This is one of the most popular choices among the possibilities available. The glutaraldehyde (GLU) strategy has been highlighted as one of the other mechanisms likely to be employed with these reactive epoxy radicals. The essential advantage it offers is the ability to create a long spacer arm between the ligand and the adsorbing surface. Because of this, it can avoid the effect of steric hindrance, which can enhance the destabilizing power of the ligand as well as purify the target molecule. It is possible to purify lectins using macroporous adsorbents. Lectins are glycoproteins of immune origin which lack catalytic activity and have activity and specificity mediated by mono- and oligosaccharides; this method has a good chance of success. When different types of carbohydrates are immobilized, adsorbents with other properties are produced. Consequently, there is now a distinct possibility that these adsorbents can be used in processes intended to separate lectins [3,4].

The gel formation process in a semi-freezing zone allows the preparation of mechanically stable monolithic matrices composed of large interconnected macropores, since the pores formed in this process are 100 to 1000 times larger than those in other gels, which have pores in the range of 0.03–0.4 μm. This characteristic reveals the attractiveness and potential of these materials because the flow through these pores is purely convective and the resistance to mass transfer is low, which makes them permeable to aqueous solutions of proteins and even cell suspension [3–5].

Protein purification is grounded in studies of the physical–chemical characteristics, and structural and biological properties, being stimulated by its potential use in several areas of medicine, chemistry, biochemistry, and biology [6]. The most used technique to mediate such purification is affinity chromatography [7,8], based on highly specific and reversible interactions between pairs of biological materials (enzyme–substrate, enzyme–inhibitor, antigen–antibody) as well as studies using interactions with N-acetyl glucosamine [9], galactose derivatives [7], mannose [10], and proteins [11], among others, which can guarantee greater selectivity due to the specific stereochemical and typological characteristics presented by proteins. The principle of the method in question is to improve the separation capacity of biomolecules from specific, non-covalent bonds to insoluble supports, favoring the obtaining of bio-separations with high selectivity [12–14]. It is known that the use of monolithic, macroporous matrices applied in the purification of bio compounds is in constant growth and development [15]. In this sense, this study aimed to report and discuss information from the literature on polymeric, macroporous, monolithic adsorbents applied in the purification of lectins using affinity chromatography.

2. Lectin Activity

Conceptually, lectins are proteins of non-immune origin that recognize and are associated with carbohydrates or glycoconjugates reversibly, with high affinity and specificity. Due to this ability, these biomolecules have important biological effects, such as insecticide, bactericidal, antitumor, and fungicide, in addition to an injunction on HIV-I protease, and became essential instruments in the diagnosis of diseases, identification of microorganism strains, and in studies related to blood types. Plant lectins have been used in cell biology and immunology as diagnostic and immunomodulatory agents, as well as for therapeutic

purposes [16]. In addition, they can be used in the production of biosensors for the food industry, verifying the presence of microorganisms to ensure the quality of raw materials and industrialized products [17,18].

It is worth noting that the study of Matoba et al. [19] increased the antiviral activity of lectins. Such proteins have as characteristics the recognition and maintenance of specific and reversible bonds to mono- or oligosaccharides and other substances containing sugars, maintaining the covalent structure of these glycosidic ligands [19]. They can precipitate cells, glycoconjugates, and polysaccharides from animal, plant, virus, and bacterial sources [20,21].

The binding of lectins with sugars is attributed to a carbohydrate recognition domain (CRD) within their polypeptide structure. The interaction of lectins with certain carbohydrates can be as specific as the interaction between antigen and antibody or substrate and enzyme. Some are metalloproteins, in other words, they require the presence of metal cations at their specific binding sites with carbohydrates in connection with them, resembling metalloproteases; but lectins do not present catalytic activity [20,22]. Generally, lectins have at least two binding sites for carbohydrates, which allow cross-linking between cells through combinations with sugars on the surface or between sugars contained in macromolecules, justifying their ability to agglutinate particles and precipitate glycoconjugates. The lectin–carbohydrate interaction is due to covalent bonds, in which water molecules, associated with the polar group of proteins and also around the carbohydrate, are displaced (Figure 1). This modification results in the formation of new hydrogen bonding networks, which, together with van der Waals forces, stabilize this interaction [7,23].

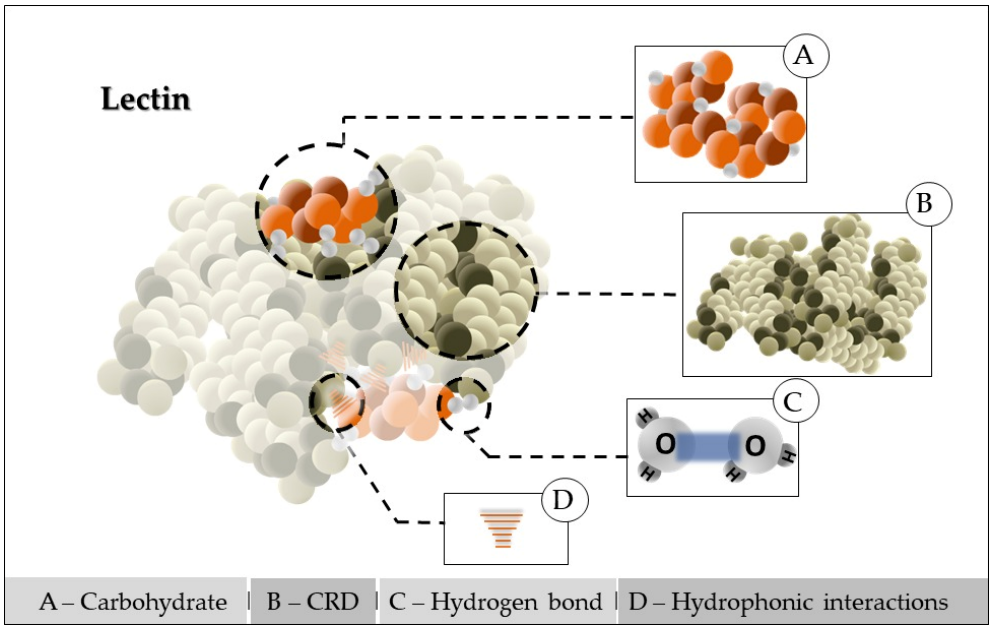

Figure 1. Scheme illustrating the binding of lectin to the carbohydrate through the carbohydrate recognition domain. The carbohydrate–lectin interaction involves, among other non-covalent forces, the formation of hydrogen bonds and hydrophobic interactions.

The structure of proteins and their activity are strongly influenced by environmental factors such as pH and temperature, in addition to chemical factors such as the presence of ions [24]. Temperature is a considerable factor in the maintenance of the native activity of lectin hemagglutination. Singh (2013) working with lectin of *Momordica charantia* (MCJ) observed that at 30 °C their activity was maintained, however the increase in temperature to 55 °C reduced their hemagglutinating activity by 50%, and this percentage of activity was maintained for 12 min [25]. The total loss of lectin activity occurred at 65 °C, with no recovery, even after a temperature reduction, and this showed that the kinetics of inactivation of lectin hemagglutination activity at this temperature is an irreversible process.

Similar to temperature, pH is a significant influencing factor in lectin activity. Hemagglutination tests with lectin MCJ showed that it is active at pH values between 3 and 11. The hemagglutinating activity of MCJ increases with an increase in pH, and the activity maximizes in the pH range of 5 to 8. With the increase in pH, the hemagglutinating activity of MCJ decreased, maintaining less than 50% of pH agglutination activity between 10 and 11 [25].

Most lectins require metal ions to present agglutinating activity, as is the case with lectins of legumes that require Mn^{2+} and Ca^{2+}. Studies conducted by Sharon and Lis [26] with concanavalin A (ConA) indicated that, in an acid iced medium, there was a removal of metal ions from the ConA molecule, eliminating its ability to bind to carbohydrates. However, such a reaction can be circumvented with the addition of Mn^{2+} and Ca^{2+} ions in this order, considering that metals confer a high degree of structural stability to ConA, protecting lectins against heat inactivation [26,27].

On the other hand, these same ions at high concentrations are capable of agglutinating cells, as observed by Silva et al. [15] in their study on the influence of Ca^{2+} and Mn^{2+} ions for their hemagglutinating activity of cassava leaf lectin. Thus, to ensure that the agglutination is being mediated by lectin and not by excess ions, it is necessary to inhibit this activity with the addition of carbohydrates [15].

3. Lectin-Carbohydrate Interaction

The recognition of the specificity of lectin–carbohydrate interactions involve different techniques, such as affinity chromatography, electrophoresis, spectroscopy in the ultraviolet-visible region, and calorimetry, among others [28]. Usually, the simplest method of identifying the presence of lectins in a medium is through hemagglutination inhibition (HI) tests, and despite its low accuracy, it is the most widely used method. IH classifies the amount of carbohydrate needed to inhibit the agglutination of erythrocytes caused by lectin and can help determine the specific carbohydrate for a lectin of interest. Specificity is given regarding carbohydrates that present a greater inhibition of the agglutination reaction [29].

In this type of assay, serial dilutions of the sample are performed in a saline solution, followed by incubation with human or animal erythrocytes. Thus, the added lectin binds to the carbohydrates present on the surface of erythrocytes, causing a cross-link between the cells, called the hemagglutination network (Figure 2a). When there is a successful inhibition, the addition of carbohydrates prevents the formation of the hemagglutination network (Figure 2b). In this case, the lack of agglutination is a positive result, proving the employment of the inhibiting activity of the carbohydrate. Using different concentrations of various carbohydrates, a semi-quantitative evaluation of the affinity and specificity of a lectin can be obtained [30].

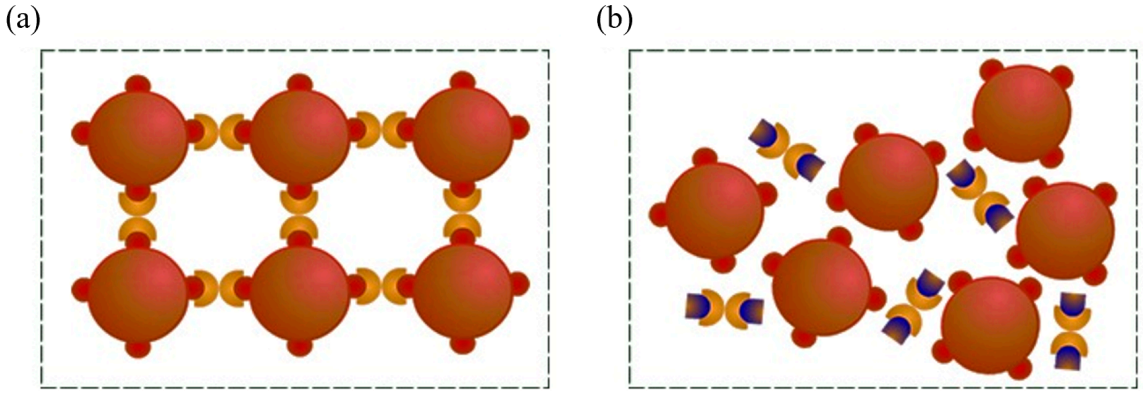

Figure 2. Representation of the behavior of the lectin in the hemagglutination inhibition. (**a**). Before interactions and (**b**). After interactions. Here, round ball shape means erythrocytes, moon shape means lectins and bullet shape is carbohydrates.

The most common classification for these proteins is based on their sources, which are animals and plants as well as microorganisms. Lectins have been extracted from sources

such as shimeji mushroom [17] and black beans [21]. Some studies have also been carried out with the extraction of Jambo seed lectins [31], okra seeds [32], and wheat germ [33]. Table 1 shows some sources of lectins as well as their specific binders.

Table 1. Lectins and their specific binders.

Lectins	Source	Extract	Specific Binders	References
Con A	*Canavalia ensiformis*	Pork bean	α-D-mannosil and α-D-glycosil	[34]
PNA	*Arachis hypogaea*	Peanut	N-acetylgalactosamine	[35]
WGA	*Triticum vulgare*	Wheat germ	N-acetylglycosamine	[36]
Jacalina	*Artocarpus integrifólia*	Jackfruit	α-galactopyranosids and methyl-α-D-galactopyranoside	[37]
Ricin	*Ricinus communis*	Castor bean	β-D-galactosyl	[38]
Emal	*Eugenia malaccensis*	Red Jambo	Glucose	[31]
PHA	*Phaseolus vulgaris*	Bean	N-acetyl-D-galactosamine	[39]
BanLec	*Musa paradisiaca*	Banana	Mannose-binding	[40]
PeCL	*Penicillium chrysogenum*	Fungus	Mannose-binding	[41]
FSL	*Fusarium sambucinum*	Fungus	D-xylose, L-fucose D-mannose, N-acetyl-D-glucosamine	[42]
PTA e LRA	*Pinellia ternata Lycoris radiata*	Plant	Mannose ligand	[43]

Source: Adapted from refs. [22,31].

The lectin–carbohydrate relationship and the specificity of this interaction are widely studied, and despite the vast amount of these glycoproteins in nature, not all processes involving this mechanism are fully understood. Interestingly, despite the high similarity between plant lectins, many presented remarkably distinct biological profiles, with a wide range of biological activities that include anti-inflammatory, antidepressant, and vasodilatory activities. Some lectins have been identified as promising antitumor agents, e.g., ConA and the ML-I lectin of the Bird's herb plant, which are in preclinical and clinical trials for treatment of human liver cancer and malignant melanoma [16].

In addition, lectins can be used in the production of biosensors for the food industry, in verifying the existence of certain groups of analytes and/or microorganisms, and in ensuring the quality of raw materials and industrialized products [44]. The technology of biorecognition of lectins by sugars has been applied in clinical diagnosis and the removal of blood viruses [45]. This group of glycoproteins is found in many organisms, from bacteria to plants and animals [3].

4. Purification of Lectins

Lectins can be purified using conventional methods such as salt or acid precipitation, aqueous system extraction, and chromatographic separation, such as ion exchange and molecular or affinity exclusion [21]. Considering the ability of lectins to bind to carbohydrates and other glycoproteins, the most used technique for the purification of this group of proteins is affinity chromatography [7,8]. This capacity is explored and being studied for the interaction with N-acetylglycoamine [9], derived from galactose [7], mannose [10], and proteins [11], among others.

Among the most used procedures for protein purification are chromatographic techniques, such as ion exchange and affinity, that have different separation principles, such as load difference and binding affinity, respectively. They are considered the most powerful methods for purification and are used to isolate and purify the biomolecule of interest to the other, leading to the appropriate purity for the intended use [46]. The principle of separation for this physical–chemical method is based on the difference in the interaction of the components of the mixture with the stationary phase, resulting in differential migrations of the compounds, thus allowing them to be separated. During the procedure, proteins are bound in a porous, solid material with appropriate chemical characteristics, called a stationary phase, and maintained in a column by which a buffer solution is percolated, characterized as a mobile phase [14]. Table 2 presents the various techniques for the purification of lectins.

Table 2. Lectin purification techniques.

Technique	Lectin	Food	References
Hydrophobic interaction chromatography	*Tethya sp.*	Marine sponge	[47]
Ion exchange chromatography	*Jacalina (Artocarpus integrifólia)*	Jackfruit	[15]
Affinity chromatography	*Con A*	Pork beans	[26]
Gel filtration chromatography	PeCL	Fungus (*Penicillium chrysogenum*)	[41]
Affinity chromatography followed by ion exchange	*Gymnopilus spectabilis (GSL)*	Mushroom	[48]
Ion exchange chromatography	BanLec	Banana	[40]
Exclusion chromatography in gel	FSL (*Fusarium sambucinum*)	Fungus	[42]
Ion exchange chromatography	PTA e LRA	*Pinellia ternata* and *Lycoris radiata*	[43]
Molecular exclusion (PD-10 Desalting)	*Swartzia laevicarpa*	Legume	[49]

Among the modes of operation of the various chromatographic processes are the fixed bed and the expanded bed. The fixed bed has high efficiency, being a technique of easy implementation in production processes. However, the presence of large biomolecules and highly concentrated materials challenges its capacity and productivity. Expanded bed adsorption allows for the use of feeding that contains particulate matter, eliminating the need for initial clarification of the medium, reducing the number of process steps, and avoiding the loss of biomolecule activity. On the other hand, this chromatographic method requires special types of columns and accessories that cannot be installed in traditional packaged bed chromatography systems [50].

To overcome these problems, especially mass-transfer problems, researchers have been more interested in the study of super-macroporous polymeric monolithic columns, as these allow the process to occur at a superficial speed comparable to traditional high-efficiency liquid chromatography and higher than that used in an expanded bed, using low pressure [14]. In this sense, affinity chromatography is the most-used technique for lectin purification, which consists of perceiving a sample on a stationary adsorbent phase containing the specific pairs of binding previously balanced by the mobile phase to achieve the best interaction conditions (Figure 3).

The molecules of the present solute interact selectively with the immobilized pairs, being retained in the stationary phase. Subsequently, they are washed for the removal of unbound or weakly bound compounds and then passed to the mobile phase under desorption conditions, reversing the specific bond that had been formed. Because this is a widely used technique for the purification of lectins which bind to carbohydrates or glycoproteins coupled in the matrix, according to specificity, these molecules are eaten with carbohydrates or glycoproteins in a solution; for example, the Sephadex® G-50 matrix retains lectins with specificity to glucose, D-fructose, D-mannose, sucrose, and methyl-β-D-glycopyranoside, while the chitin matrix is specific to N-acetyl-D-glycosamine and oligosaccharides [51,52].

Affinity chromatography employs different types of binding agents (binders) and stationary phase supports (matrices). By improving various properties, such as specificity, selectivity, reproducibility, conjugation chemistry, and cost-benefit of these components, this method can be applied for large-scale purification to achieve the desired yield and purity of the product [12]. It is worth mentioning that an important factor for ensuring good efficiency of affinity chromatography is the choice of the appropriate mobile phase in which to perform the elution. This step requires the complete dissociation of the adsorbent–adsorbate complex, obtaining high purity and using the lowest possible volume of solution. The elution methods used may be selective or non-selective [13].

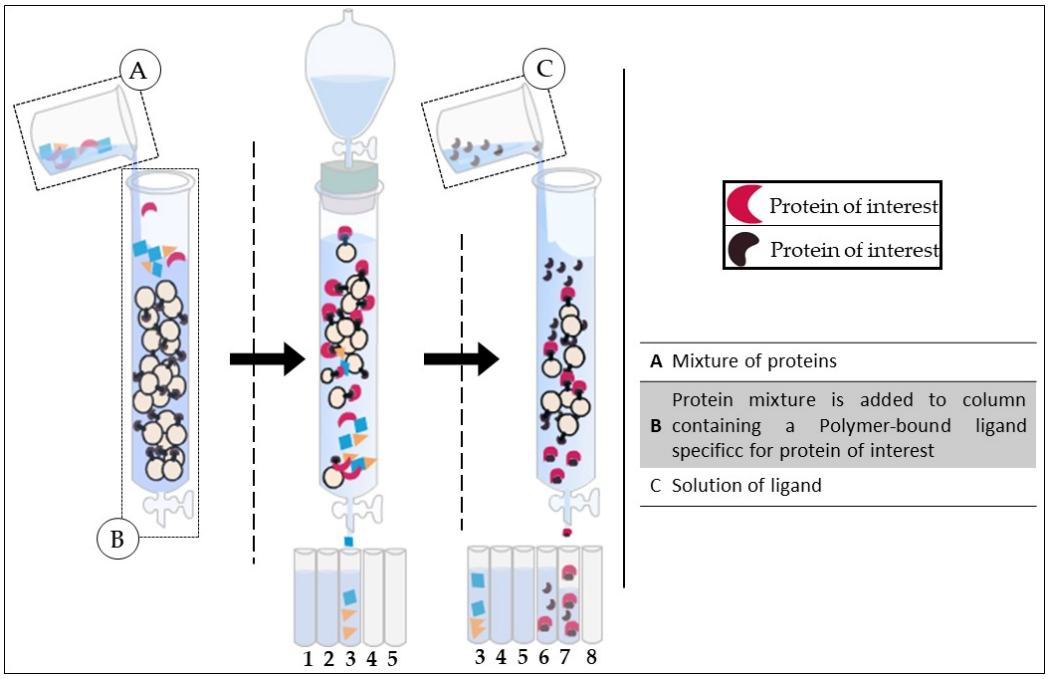

Figure 3. Affinity chromatography for separating biomolecules.

The selective methods take advantage of the natural properties of the biospecific interactions of proteins, using a solution containing a high concentration of free binder, and may be the same as those bonded on the surface of the matrix, thus generating a competition between soluble ligands and immobilized ligands, where the soluble must be in excess, and, in this way, dissociation will occur and adsorbate will move to the soluble phase. Non-selective methods alter the physical properties of the adsorbent by varying pH, temperature effect, or use of denaturing proteins, reducing the intensity of the binding protein and thus promoting dissociation of the complex.

Another factor to be considered is the type of ligand to be immobilized in the stationary phase. Several types of binders can be immobilized. Such binders can be grouped into two categories: high-specificity binders and general binders. High-specificity ligands bind to a narrower group of molecules such as antigens for antibodies and enzyme substrates. These types of binders are used when the goal is to analyze or purify a specific solute. General binders bind to a family of solutes without distinction between individual compounds. This is the case with quality metals, triazine dye, proteins A and G, and lectins. These are aimed toward separating a class of compounds and have weaker bonds with the target molecule than high-specificity ligands [13,14]. Table 3 shows some examples of ligands that are used in affinity chromatography.

Table 3. Main binders in affinity chromatography.

Technique	Biomolecule	Ligand	References
Chromatography by Affinity	Concanavalin A (Con A); Peanut (PNA)	Immobilized glucose	[53]
	Blackberry lectins	Concanavalin A	[54]
	Lizosima	Tris (hydroxyethyl)aminomethane	[51]
		N-acetyl-D-glycosamine (D-GlcNAc)	[55]
	Gymnopilus spectabilis (GSL)	Manosil-Sepharose	[48]
	BanLec		[40]
	Arisaema jacquemontii Blume (AJL)	Syapituin	[56]
	lectin ('Millectin')	Mannose	[57]

5. Monolithic Polymeric Cryogels

Although the strategies for developing a chromatographic purification process of biomolecules on an industrial scale are known, large biomolecular structures and very concentrated materials challenge the limits of conventional chromatographic methods regarding their purification capacity. For this reason, there is interest in the use of macroporous monoliths in chromatography [14].

The introduction of fixed-bed columns, such as macroporous monoliths, for chromatographic separation of biomolecules has aroused a lot of interest. These polymeric monolithic columns are supports with large and interconnected pore structures used for the separation and purification of bioproducts from unclarified media or solutions containing particles or cell mixture [3,32].

Macroporous monoliths, called cryogels, are produced from a solution of monomers and hydrophilic polymers using cryogeleification techniques, previously used for biomedical applications. Cryogeleification is a specific type of synthesis of polymeric gels in which formation begins in the cryogenic treatment of systems capable of forming gels [2]. The difference in the concentration of the monomer solution and polymers provides a higher degree of physical–chemical interlacing between the chains and, consequently, a decrease in pore size and porosity (connectivity between pores).

Monolithic cryogels exhibit multiple pores that can range from 1 µm to 100 µm in diameter, and these dimensions can be controlled by changing synthesis parameters including the nature and type of the polymer, the synthesis temperature, and the composition of the cross-linking agent [58]. In this context, several polymeric compounds can be combined in the synthesis of cryogels, as well as the use of different freezing temperatures, as can be observed in Table 4.

Table 4. Examples of systems for the preparation of polymeric cryogels.

Monomers and Reticulates	Solvent	Catalyst	T (°C)	References
Methacrylic acid + oligo ethylene glycol Diacrylate	Water	APS+ TEMED	−20	[59]
2-hydroxymethylmethacrylate + N-vinyl imidazole + ethylene glycolDimethacrylae	Water	APS + TEMED	−16	[60]
N-isopropilacrilamide + itaconic acid + N, N′-methylene-bis-acrylamide	Water	APS + TEMED	−22	[61]
Acrylamide + N, N′-methylene-bis-acrylamide + Alil- glycidyl ether	Water	APS + TEMED	−12	[9,51]
N-isopropilacrilamide + acrylic acid + N, N′-methylene-bis-acrylamide	Water	APS + TEMED	−22	[62]
Poly- (L) -histidine + glycidil methacrylate (PGMA) + N′-methylene-bis-acrylamide + HEMA	Water	APS + TEMED	16	[63]
Acrylamide + lauryl acrylate + ethylene glycol dimethacrylate (EGDMA)	Water	PDMS + PBPO	−18	[64]
Poly (2-hydroxyethyl methacrylate) (PHEMA)	Water	APS + TEMED	−12	[65]

Here, APS: ammonium persulfate; TEMED: N, N, N, N tetramethylethylenediamine; HEMA: 2-hydroxyethyl methacrylate; Phenyl (2,4,6-trimethyl benzoyl)- phosphine oxide (PBPO); Poly (dimethyl siloxane) (PDMS). Source: adapted from [66].

Among the possible polymers used in the synthesis of these compounds stands out the macroporous monoliths of polyacrylamide, obtained from the polymerization of acrylamide molecules (Aam) with the cross-linking forming agent N, N′-methylene-bis-acrylamide (BAam), added or not from other monomers (such as allyl glycidyl ether, AGE), under conditions of moderate freezing (−10 °C to −20 °C), using the technique known as cryogeleification [67]. The monoliths thus synthesized are called polyacrimide cryogels (pAAm), and their use has been reported by several authors such as Carvalho et al. [32], Machado et al. [50], and Mól et al. [55].

During the cryogeleification process, growing ice crystals are responsible for the development of the macroporous structure. More than 90% of the monolith is composed of water and only 10% is the gel phase, i.e., polymer with strongly joined water, so ice crystals play the role of porogenic models, that is, the shape and size of the crystals determine the shape

and size of pores that develop during cryogen synthesis [68–70]. Vainerman [71] studied the formation dynamics of cryogels using freezing temperatures of −10 °C, −20 °C, and −30 °C and realized that at the temperature of −10 °C, the pores formed were higher [71]. This characteristic occurs because when higher temperatures are used in the cryogeleification process, freezing occurs more slowly, resulting in the development of larger ice crystals, and larger pores were formed when the higher temperature was used. However, temperatures well above freezing gels do not allow for the formation of a macroporous gel, as observed by Plieva et al. [68] when they studied cryogels with the same concentrations but submitted to temperatures of −20 °C and 20 °C. It is known that the structure of the gel formed at 20 °C is compact and has smaller pores, while cryogel formed at −20 °C presents large and interconnected pores ranging from 10 µm to 100 µm, which reinforces the importance of cryogeleification in the synthesis of these compounds.

Despite the advantages presented by polymeric cryogels, the large pores and the high porosity of their structure make their surface area smaller than that of traditional chromatographic columns, reducing the efficiency of the purification process. On the other hand, the properties of cryogels can be transformed post-fabrication with processes called functionalization or activation, which consists of chemically or physically modifying the surface of such columns [72]. A viable alternative is to transform polymeric cryogels into adsorbents of specific affinity, through grafting of the same in solutions containing binder groups of interest, thus providing a good alternative to traditional affinity-based chromatography matrices [73–75].

Since there is a wide variety of monolithic supports that are available for purifying the most diverse existing biomolecules, there is not one universal solution. To improve the process of using monolithic beds as support for the immobilization and purification of biomolecules, several approaches to the immobilization of affinity ligands have been reported [76].

In adsorption processes, the conformation and apparent activity of the immobilized or purified biomolecule are influenced by the properties of the monolithic column used with support. The reactive groups in most cases are inserted on the surface of the polymer matrix to provide the formation of a covalent bond between the biomolecule and the support. Each immobilization method presents a different chemical reaction to achieve maximum adsorption of percolated biomolecules. Cryogel functionalization techniques mean immobilization via covalent binding, biospecific adsorption, and cation activation, among others [77,78]. In these techniques, functionalization is performed through the circulation of solutions containing reticulating agents through the monolithic column or through immersion of the support in the solution containing the ligand groups. Reactive groups, such as epoxy groups, are introduced to the surface of the support to allow the formation of a covalent bond between the biomolecule and the support in the subsequent step. The insertion of new chemical structures on the surface allows us to obtain stationary phases that have a more or less specific relationship with a particular protein. Table 5 shows the main binders used in the functionalization of cryogels as well as the techniques used.

Table 5. Cryogel functionalization techniques.

Technique	Binders	Application	References
Glutaraldehyde method	β-D-Galactosidase immobilized in Chitosan	Continuous lactose hydrolysis and synthesis of galactooligosaccharides (GOS)	[79]
Cellular immobilization	poly (vinyl alcohol) PVA	Continuous synthesis of alkyl galactosides by transgli cosiled	[68]
Covalent ligation	Theiminodiatic cido (IDA)	Direct capture of unclarified crude oil enzymes	[80]

Table 5. *Cont.*

Technique	Binders	Application	References
Covalent ligation	Ligands of anionic exchange [group 2-(dimethylamino)ethyl]	Purification of *Escherichia coli* cells	
Immobilization technique enzymatic, epoxy method	Human serum albumin (HSA)	Purification of biomolecules	[81]
Chemical coping	α-chymotrypsin	enantioselective hydrolysis from a D, L-Phe-OEt Schiff base (D, L-SBPH)	[82]
Immobilization technique by epoxy method	Polietyleneimine immobilized (PEI), polymyxin B (PMB) and lysozyme	Capture of bacterial endotoxins (BEs)	[83]

In this context, among the various matrices, there are notable differences concerning the chemical properties of monomers used in the column synthesis process. This fact should be considered, especially with the presence of specific functional groups. The functional group of the monomer, such as the epoxy groups provided by the AGE monomer, can be used in a future stage of the reaction as an anchoring group to immobilize specific ligands or to improve specific properties such as hydrophilicity, hydrophobicity, pH, or activation temperature [84]. For proper application of a monomer, monoliths with various performances can be designed, as observed by Ingavle et al. [78], in the use of functionalized cryogels with epoxy groups departing from glycidyl methacrylate (GMA) for the removal of biotoxins using the protective antigen of anthrax toxin.

In the functionalization stage, some aspects should be observed to improve the properties and avoid certain problems in the use of these columns, such as steric impediment. Thus, the formation of spacer arms minimizes or solves the related problem by expanding the possibilities of interaction, since it increases accessibility to all the binding centers available in a protein. In contrast, spacer arms can promote the non-specific interactions of various proteins on the matrix [51,76].

It is known that supports with epoxy groups on their surfaces are among the most used because the epoxy groups can easily react with amine (NH_2), thiol (SH), or hydroxyl (OH) groups of proteins and form very stable covalent bonds. In addition, epoxy groups are very stable at pH values close to neutral, which makes the support containing these groups suitable for long storage periods. One method that supports a macroporous matrix containing reactive epoxy radicals is the glutaraldehyde method [13,85]. In this method, a monolith containing reactive epoxy groups (epoxy-activated monolith) is initially converted to the amine form (amine-activated monolith) by a reaction of epoxy groups with reagents containing amine grouping, such as ethylenediamine. The amine-activated monolith then reacts with glutaraldehyde, forming an aldehyde-activated monolith capable of reacting with an amine grouping of molecules [13,51]. Lysozyme, also known as N-acetylmuramide glycanohydrolase, is an enzyme that aids digestion. It is present in most cells, such as spleen, and secretory fluids, such as tears and milk. Lyases are members of a family of enzymes known as hydrolases, which break covalent bonds through water. These digestive enzymes have high market value due to their vast potential applications, including in the pharmaceutical and food industries. They are used in the food industry as additives to dairy products, as cell disruptors to release intracellular products in bacteria, as medicinal agents for ulcers and infections, and in various other applications [86,87]. There is a diverse selection of cryogels, each with distinct physicochemical and porosity properties that make them potentially suitable carriers for cell immobilization. These features include, similar to biopolymer immobilization, cell immobilization using cryogel-type surfaces, which can be accomplished using either attachment (covalent or adsorption) or entrapment techniques [88–90]. This is because the immobilization of cells is comparable to the immobilization of biopolymers. To entrap cells in the pore walls of a spongy cryogel, the organism (such as bacteria, spores, or organelles) is first suspended in a gel solution (such as monomers or reactive polymers and cross-linking agents for polymerization gelation).

The mixture is then subjected to cryogenic treatment to achieve the desired result of cell adhesion within the tear pore walls.

As a result, a super-macroporous matrix is produced. This matrix encapsulates immobilized cells within a cross-linked gel phase of the pore walls. It is essential to emphasize that organic materials can be made from organic polymers. An inorganic polymer, such as a cryo-silica gel, should not ignore this possibility. This is an example of something that is possible (freeze-melted silica gel) [91,92]. The main advantage lies in the ability to form a long spacer arm between the cryogel and the switcher, avoiding possible effects of steric impediment, which can lead to a greater capacity of immobilization of the ligand and purification of the target molecule [9,93]. The method presents better results when compared to others, as evaluated by Gonçalves et al. [9], which obtained data on carbohydrate immobilization capacity in monolithic columns using the methods of Schiff base, epoxy, ethylenediamine, and glutaraldehyde. The latter presented the highest immobilization capacity, with about 147 mg of carbohydrate per gram of cryogel; thus, it is being used to evaluate the immobilization of different sugars in cryogel, aiming at an application for the purification of lectins with different specificities. Based on this evaluation, further research in this area is of fundamental importance to expand knowledge and contribute to the optimization of such a process.

6. Conclusions

Studies on unconventional adsorbents, such as chromatographic supports, can substantiate, enrich, and assist projects in various areas of knowledge. Polyacrylamide cryogens are emerging and efficient, and can be synthesized and have their matrices modified for multiple purposes and chromatographic techniques. They are also functional and have low costs compared to conventional chromatographic adsorbents. In this context, lectins can mainly be used in the prevention of autoimmune diseases and in studies with biosensors. Thus, the use of polymeric macroporous monolithic adsorbents applied in the purification of lectins using affinity chromatography is an alternative to obtaining functional biomolecules with a high degree of purity, has low synthesis cost, is versatile in terms of application when compared to commercial columns, and has the potential to act as a biosensor. It also can be used on an industrial scale to increase people's quality of life.

Author Contributions: Conceptualization, J.F.d.S. and R.d.C.I.F.; methodology, J.F.d.S.; software, R.F.S.; investigation, C.M.G.L., D.L.d.S., I.S.d.N., S.d.O.R., I.R.A.d.M., S.V., T.B.E. and R.d.C.I.F.; data curation, L.A.G., M.R.I.F. and R.F.S.; writing—original draft preparation, C.M.G.L., D.L.d.S., I.S.d.N., S.d.O.R., L.A.G., T.B.E. and R.d.C.I.F.; writing—review and editing, S.d.O.R., W.K., S.V., M.R.I.F., I.R.A.d.M. and H.D.M.C.; supervision, M.U.K. and R.d.C.I.F.; project administration, R.d.C.I.F.; funding acquisition, M.U.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been supported with scholarship to Josiane Ferreira da Silva by CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (finance code 001).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Janson, J.C. Principles, High Resolution Methods, and Applications. In *Protein Purification*; John Wiley & Sons: Hoboken, NJ, USA, 2011; pp. 3–50. ISBN 978-0-471-74661-4.
2. Dainiak, M.B.; Allan, I.U.; Savina, I.N.; Cornelio, L.; James, E.S.; James, S.L.; Mikhalovsky, S.v.; Jungvid, H.; Galaev, I.Y. Gelatin-Fibrinogen Cryogel Dermal Matrices for Wound Repair: Preparation, Optimisation and in Vitro Study. *Biomaterials* **2010**, *31*, 67–76. [CrossRef] [PubMed]
3. Perçin, I.; Khalaf, R.; Brand, B.; Morbidelli, M.; Gezici, O. Strong Cation-Exchange Chromatography of Proteins on a Sulfoalkylated Monolithic Cryogel. *J. Chromatogr. A* **2015**, *1386*, 13–21. [CrossRef] [PubMed]
4. Tao, S.P.; Wang, C.; Sun, Y. Coating of Nanoparticles on Cryogel Surface and Subsequent Double-Modification for Enhanced Ion-Exchange Capacity of Protein. *J. Chromatogr. A* **2014**, *1359*, 76–83. [CrossRef] [PubMed]
5. Ünlüer, Ö.B.; Ersöz, A.; Denizli, A.; Demirel, R.; Say, R. Separation and Purification of Hyaluronic Acid by Embedded Glucuronic Acid Imprinted Polymers into Cryogel. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2013**, *934*, 46–52. [CrossRef] [PubMed]

6. Banerjee, S.; Chaki, S.; Bhowal, J.; Chatterjee, B.P. Mucin Binding Mitogenic Lectin from Freshwater Indian Gastropod Belamyia Bengalensis: Purification and Molecular Characterization. *Arch. Biochem. Biophys.* **2004**, *421*, 125–134. [CrossRef]
7. Oliveira, J.T.A.; Melo, V.M.M.; Câmara, M.F.L.; Vasconcelos, I.M.; Beltramini, L.M.; Machado, O.L.T.; Gomes, V.M.; Pereira, S.P.; Fernandes, C.F.; Nunes, E.P.; et al. Purification and Physicochemical Characterization of a Cotyledonary Lectin from Luetzelburgia Auriculata. *Phytochemistry* **2002**, *61*, 301–310. [CrossRef]
8. Roy, I.; Sardar, M.; Gupta, M.N. Cross-Linked Alginate-Guar Gum Beads as Fluidized Bed Affinity Media for Purification of Jacalin. *Biochem. Eng. J.* **2005**, *23*, 193–198. [CrossRef]
9. Gonçalves, G.R.F.; Gandolfi, O.R.R.; Santos, C.M.S.; Bonomo, R.C.F.; Veloso, C.M.; Fontan, R.d.C.I. Development of Supermacroporous Monolithic Adsorbents for Purifying Lectins by Affinity with Sugars. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2016**, *1033–1034*, 406–412. [CrossRef]
10. Ourth, D.D.; Rose, W.M. Purification, Characterization and Seasonal Variation of Mannose-Binding C-Type Lectin in Ictalurid Catfish. *Aquaculture* **2011**, *321*, 191–196. [CrossRef]
11. Jung, W.K.; Park, P.J.; Kim, S.K. Purification and Characterization of a New Lectin from the Hard Roe of Skipjack Tuna, Katsuwonus Pelamis. *Int. J. Biochem. Cell Biol.* **2003**, *35*, 255–265. [CrossRef]
12. Arora, S.; Saxena, V.; Ayyar, B.V. Affinity Chromatography: A Versatile Technique for Antibody Purification. *Methods* **2017**, *116*, 84–94. [CrossRef]
13. Mallik, R.; Hage, D.S. Affinity Monolith Chromatography. *J. Sep. Sci.* **2006**, *29*, 1686–1704. [CrossRef]
14. Pfaunmiller, E.L.; Paulemond, M.L.; Dupper, C.M.; Hage, D.S. Affinity Monolith Chromatography: A Review of Principles and Recent Analytical Applications. *Anal. Bioanal. Chem.* **2013**, *405*, 2133–2145. [CrossRef]
15. Ferreira da Silva, J.; Lemos da Silva, D.; Gomes Nascimento, R.; Ayra Alcântara Veríssimo, L.; Martins Veloso, C.; Ferreira Bonomo, R.C.; da Costa Ilhéu Fontan, R. Enhancements in Sugar Immobilization in Polymeric Macroporous Matrices for Affinity Capture. *J. Appl. Polym. Sci.* **2019**, *136*, 47956. [CrossRef]
16. Gondim, A.C.S.; Romero-Canelón, I.; Sousa, E.H.S.; Blindauer, C.A.; Butler, J.S.; Romero, M.J.; Sanchez-Cano, C.; Sousa, B.L.; Chaves, R.P.; Nagano, C.S.; et al. The Potent Anti-Cancer Activity of Dioclea Lasiocarpa Lectin. *J. Inorg. Biochem.* **2017**, *175*, 179–189. [CrossRef] [PubMed]
17. Suzuki, T.; Abe, T.; Umehara, K.; Choi, J.H.; Hirai, H.; Dohra, H.; Kawagishi, H. Purification and Characterization of a Lectin from the Mushroom Hypsizigus Marmoreus. *Mycoscience* **2015**, *56*, 359–363. [CrossRef]
18. Selvaprakash, K.; Chen, Y.C. Functionalized Gold Nanoparticles as Affinity Nanoprobes for Multiple Lectins. *Colloids Surf. B Biointerfaces* **2018**, *162*, 60–68. [CrossRef] [PubMed]
19. Matoba, Y.; Sato, Y.; Oda, K.; Hatori, Y.; Morimoto, K. Lectins Engineered to Favor a Glycan-Binding Conformation Have Enhanced Antiviral Activity. *J. Biol. Chem.* **2021**, *296*. [CrossRef]
20. Gajbhiye, V.; Gong, S. Lectin Functionalized Nanocarriers for Gene Delivery. *Biotechnol. Adv.* **2013**, *31*, 552–562. [CrossRef]
21. He, S.; Shi, J.; Walid, E.; Zhang, H.; Ma, Y.; Xue, S.J. Reverse Micellar Extraction of Lectin from Black Turtle Bean (Phaseolus Vulgaris): Optimisation of Extraction Conditions by Response Surface Methodology. *Food Chem.* **2015**, *166*, 93–100. [CrossRef]
22. Lavín de Juan, L.; García Recio, V.; Jiménez López, P.; Girbés Juan, T.; Cordoba-Diaz, M.; Cordoba-Diaz, D. Pharmaceutical Applications of Lectins. *J. Drug Deliv. Sci. Technol.* **2017**, *42*, 126–133. [CrossRef]
23. Santos, A.L.E.; Júnior, C.P.S.; Neto, R.N.M.; Santos, M.H.C.; Santos, V.F.; Rocha, B.A.M.; Sousa, E.M.; Carvalho, R.C.; Menezes, I.R.A.; Oliveira, M.R.C.; et al. Machaerium Acutifolium Lectin Inhibits Inflammatory Responses through Cytokine Modulation. *Proc. Biochem.* **2020**, *97*, 149–157. [CrossRef]
24. Carrillo, C.; Cordoba-Diaz, D.; Cordoba-Diaz, M.; Girbés, T.; Jiménez, P. Effects of Temperature, PH and Sugar Binding on the Structures of Lectins Ebulin f and SELfd. *Food Chem.* **2017**, *220*, 324–330. [CrossRef]
25. Singh, A.; Trans, K.S.-C.S. Effect of Temperature, PH and Denaturing Agents on Biological Activity of MCJ Lectin. *Chem. Sci. Trans.* **2013**, *2*. [CrossRef]
26. Sharon, N.; Lis, H. Legume Lectins—A Large Family of Homologous Proteins. *FASEB J.* **1990**, *4*, 3198–3208. [CrossRef]
27. Kanellopoulos, P.N.; Tucker, P.A.; Pavlou, K.; Agianian, B.; Hamodrakas, S.J. A Triclinic Crystal Form of the Lectin Concanavalin A. *J. Struct. Biol.* **1996**, *117*, 16–23. [CrossRef]
28. de Lima, L.R.A.; da Silva, L.P.B.G.; de Almeida, S.M.V.; Cahú, T.B.; Beltrão, E.I.C.; de Carvalho Júnior, L.B. Lectin-Carbohydrate Complex Evaluation by Chemiluminescence. *Anal. Biochem.* **2018**, *548*, 91–95. [CrossRef]
29. Morokutti, A.; Redlberger-Fritz, M.; Nakowitsch, S.; Krenn, B.M.; Wressnigg, N.; Jungbauer, A.; Romanova, J.; Muster, T.; Popow-Kraupp, T.; Ferko, B. Validation of the Modified Hemagglutination Inhibition Assay (MHAI), a Robust and Sensitive Serological Test for Analysis of Influenza Virus-Specific Immune Response. *J. Clin. Virol.* **2013**, *56*, 323–330. [CrossRef]
30. Adamová, L.; Malinovská, L.; Wimmerová, M. New Sensitive Detection Method for Lectin Hemagglutination Using Microscopy. *Microsc Res. Tech.* **2014**, *77*, 841–849. [CrossRef]
31. Brustein, V.P.; Souza-Araújo, F.v.; Vaz, A.F.M.; Araújo, R.V.S.; Paiva, P.M.G.; Coelho, L.C.B.B.; Carneiro-Leão, A.M.A.; Teixeira, J.A.; Carneiro-Da-Cunha, M.G.; Correia, M.T.S. A Novel Antimicrobial Lectin from Eugenia Malaccensis That Stimulates Cutaneous Healing in Mice Model. *Inflammopharmacology* **2012**, *20*, 315–322. [CrossRef]
32. Carvalho, B.M.A.; Carvalho, L.M.; Silva, W.F.; Minim, L.A.; Soares, A.M.; Carvalho, G.G.P.; da Silva, S.L. Direct Capture of Lactoferrin from Cheese Whey on Supermacroporous Column of Polyacrylamide Cryogel with Copper Ions. *Food Chem.* **2014**, *154*, 308–314. [CrossRef] [PubMed]

33. Baieli, M.F.; Urtasun, N.; Miranda, M.v.; Cascone, O.; Wolman, F.J. Efficient Wheat Germ Agglutinin Purification with a Chitosan-Based Affinity Chromatographic Matrix. *J. Sep. Sci.* **2012**, *35*, 231–238. [CrossRef] [PubMed]
34. Rüdiger, H.; Gabius, H.J. Plant Lectins: Occurrence, Biochemistry, Functions and Applications. *Glycoconj. J.* **2002**, *18*, 589–613. [CrossRef] [PubMed]
35. Chacko, B.K.; Appukuttan, P.S. Peanut (Arachis Hypogaea) Lectin Recognizes α-Linked Galactose, but Not N-Acetyl Lactosamine in N-Linked Oligosaccharide Terminals. *Int. J. Biol. Macromol.* **2001**, *28*, 365–371. [CrossRef] [PubMed]
36. Liu, J.; Gao, F.; Huang, H.; Zeng, L.; Huang, X.; Zhu, G.; Li, Z. Determination of Trace Alkaline Phosphatase by Solid-Substrate Room-Temperature Phosphorimetry Based on Triticum Vulgare Lectin Labeled with Fullerenol. *Chem. Biodivers.* **2008**, *5*, 606–616. [CrossRef]
37. Kabir, S. The Isolation and Characterisation of Jacalin [Artocarpus Heterophyllus (Jackfruit) Lectin] Based on Its Charge Properties. *Int. J. Biochem. Cell Biol.* **1995**, *27*, 147–156. [CrossRef]
38. Simmons, B.M.; Russell, J.H. A Single Affinity Column Step Method for the Purification of Ricin Toxin from Castor Beans (Ricinus Communis). *Anal. Biochem.* **1985**, *146*, 206–210. [CrossRef]
39. Habibi, J.; Backus, E.A.; Huesing, J.E. Effects of Phytohemagglutinin (PHA) on the Structure of Midgut Epithelial Cells and Localization of Its Binding Sites in Western Tarnished Plant Bug, Lygus Hesperus Knight. *J. Insect Physiol.* **2000**, *46*, 611–619. [CrossRef]
40. Koshte, V.L.; van Dijk, W.; van der Stelt, M.E.; Allbers, R.C. Isolation and Characterization of BanLec-I, a Mannoside-Binding Lectin from Musa Paradisiac (Banana). *Biochem. J.* **1990**, *272*, 721–726. [CrossRef]
41. Francis, F.; Jaber, K.; Colinet, F.; Portetelle, D.; Haubruge, E. Purification of a New Fungal Mannose-Specific Lectin from Penicillium Chrysogenum and Its Aphicidal Properties. *Fungal Biol.* **2011**, *115*, 1093–1099. [CrossRef]
42. Singh, R.S.; Thakur, S.R.; Kennedy, J.F. Purification and Characterisation of a Xylose-Specific Mitogenic Lectin from Fusarium Sambucinum. *Int. J. Biol. Macromol.* **2020**, *152*, 393–402. [CrossRef]
43. Gaofu, Q.; Shiqing, M.; Fayin, Z.; Zhiniu, Y.; Xiuyun, Z. In Vitro Assessment of Plant Lectins with Anti-Pinwood Nematode Activity. *J. Invertebr. Pathol.* **2008**, *98*, 40–45. [CrossRef]
44. Rahaie, M.; Kazemi, S.S. Lectin-Based Biosensors: As Powerful Tools in Bioanalytical Applications. *Biotechnology* **2011**, *10*, 438–453. [CrossRef]
45. Tullis, R.H.; Duffin, R.P.; Handley, H.H.; Sodhi, P.; Menon, J.; Joyce, J.A.; Kher, V. Reduction of Hepatitis C Virus Using Lectin Affinity Plasmapheresis in Dialysis Patients. *Blood Purif.* **2009**, *27*, 64–69. [CrossRef]
46. Boyle, J. Lehninger Principles of Biochemistry: Nelson, D., and Cox, M. *Biochem. Mol. Biol. Educ.* **2005**, *33*, 74–75. [CrossRef]
47. Duarte, P.L. *Purificação e Caracterização Bioquímica de Uma Lectina Extraída Do Fluido Celomático Do Ouriço-Do-Mar, Echinometra Lucunter, Exposto a Indução Bacteriana*; Universidade Federal do Ceará: Fortaleza, Brazil, 2018.
48. Alborés, S.; Mora, P.; Bustamante, M.J.; Cerdeiras, M.P.; Franco Fraguas, L. Purification and Applications of a Lectin from the Mushroom Gymnopilus Spectabilis. *Appl. Biochem. Biotechnol.* **2014**, *172*, 2081–2090. [CrossRef]
49. Maranhão, P.A.C.; Teixeira, C.S.; Sousa, B.L.; Barroso-Neto, I.L.; Monteiro-Júnior, J.E.; Fernandes, A.V.; Ramos, M.V.; Vasconcelos, I.M.; Gonçalves, J.F.C.; Rocha, B.A.M.; et al. CDNA Cloning, Molecular Modeling and Docking Calculations of L-Type Lectins from Swartzia Simplex Var. Grandiflora (Leguminosae, Papilionoideae), a Member of the Tribe Swartzieae. *Phytochemistry* **2017**, *139*, 60–71. [CrossRef]
50. Machado, A.P.d.F.; Minim, L.A.; Fontan, R.d.C.I.; Minim, V.P.R.; Gonçalves, G.R.F.; Mól, P.C.G. Adsorptive Behavior of α-Lactalbumin on Cation-Exchange Supermacroporous Monolithic Column. *Fluid Phase Equilib.* **2015**, *401*, 64–69. [CrossRef]
51. Gonçalves, G.R.F.; Gandolfi, O.R.R.; Santos, L.S.; Bonomo, R.C.F.; Veloso, C.M.; Veríssimo, L.A.A.; Fontan, R. da Costa Ilhéu Fontan, R. Immobilization of Sugars in Supermacroporous Cryogels for the Purification of Lectins by Affinity Chromatography. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2017**, *1068–1069*, 71–77. [CrossRef]
52. Zhou, W.; Gao, Y.; Xu, S.; Yang, Z.; Xu, T. Purification of a Mannose-Binding Lectin Pinellia Ternata Agglutinin and Its Induction of Apoptosis in Bel-7404 Cells. *Protein Expr. Purif.* **2014**, *93*, 11–17. [CrossRef]
53. Che, A.F.; Huang, X.J.; Xu, Z.K. Polyacrylonitrile-Based Nanofibrous Membrane with Glycosylated Surface for Lectin Affinity Adsorption. *J. Memb. Sci.* **2011**, *366*, 272–277. [CrossRef]
54. Absar, N.; Yeasmin, T.; Raza, M.S.; Sarkar, S.K.; Arisaka, F. Single Step Purification, Characterization and N-Terminal Sequences of a Mannose Specific Lectin from Mulberry Seeds. *Protein J.* **2005**, *24*, 369–377. [CrossRef] [PubMed]
55. Mól, P.C.G.; Veríssimo, L.A.A.; Eller, M.R.; Minim, V.P.R.; Minim, L.A. Development of an Affinity Cryogel for One Step Purification of Lysozyme from Chicken Egg White. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2017**, *1044–1045*, 17–23. [CrossRef] [PubMed]
56. Kaur, M.; Singh, K.; Rup, P.J.; Kamboj, S.S.; Saxena, A.K.; Sharma, M.; Bhagat, M.; Sood, S.K.; Singh, J. A Tuber Lectin from Arisaema Jacquemontii Blume with Anti-Insect and Anti-Proliferative Properties. *J. Biochem. Mol. Biol.* **2006**, *39*, 432–440. [CrossRef]
57. Kvennefors, E.C.E.; Leggat, W.; Hoegh-Guldberg, O.; Degnan, B.M.; Barnes, A.C. An Ancient and Variable Mannose-Binding Lectin from the Coral Acropora Millepora Binds Both Pathogens and Symbionts. *Dev. Comp. Immunol.* **2008**, *32*, 1582–1592. [CrossRef]
58. Savina, I.N.; Gun'Ko, V.M.; Turov, V.V.; Dainiak, M.; Phillips, G.J.; Galaev, I.Y.; Mikhalovsky, S.V. Porous Structure and Water State in Cross-Linked Polymer and Protein Cryo-Hydrogels. *Soft Matter* **2011**, *7*, 4276–4283. [CrossRef]

59. Chen, Z.; Xu, L.; Liang, Y.; Wang, J.; Zhao, M.; Li, Y. Polyethylene Glycol Diacrylate-Based Supermacroporous Monolithic Cryogel as High-Performance Liquid Chromatography Stationary Phase for Protein and Polymeric Nanoparticle Separation. *J. Chromatogr. A* **2008**, *1182*, 128–131. [CrossRef]
60. Tekin, K.; Uzun, L.; Şahin, Ç.A.; Bektaş, S.; Denizli, A. Preparation and Characterization of Composite Cryogels Containing Imidazole Group and Use in Heavy Metal Removal. *React. Funct. Polym.* **2011**, *71*, 985–993. [CrossRef]
61. Vakifli, A.; Demirel, G.B.; Caykara, T. Swelling Characteristics of Poly(Nisopropylmethacrylamide-Co-Itaconic Acid) Gels Prepared in Various Conditions. *J. Appl. Polym. Sci.* **2010**, *117*, 817–823. [CrossRef]
62. Akkaya, B.; Akkaya, R. A Crosslinked Carboxylic Acid Containing Cation Exchange Monolithic Cryogel for Human Serum Albumin Separation. *J. Macromol. Sci. Part A Pure Appl. Chem.* **2012**, *49*, 736–743. [CrossRef]
63. Çimen, D.; Türkmen, D.; Denizli, A. Poly-(L)-Histidine Immobilized Cryogels for Lysozyme Purification. *Ad Sci. Tech.* **2016**, *34*, 469–487. [CrossRef]
64. Haider, Z.; Haleem, A.; Ahmad, R.U.S.; Farooq, U.; Shi, L.; Claver, U.P.; Memon, K.; Fareed, A.; Khan, I.; Mbogba, M.K.; et al. Highly Porous Polymer Cryogel Based Tribopositive Material for High Performance Triboelectric Nanogenerators. *Nano Energy* **2020**, *68*, 104294. [CrossRef]
65. Bereli, N.; Şener, G.; Yavuz, H.; Denizli, A. Oriented Immobilized Anti-LDL Antibody Carrying Poly(Hydroxyethyl Methacrylate) Cryogel for Cholesterol Removal from Human Plasma. *Mater. Sci. Eng. C* **2011**, *31*, 1078–1083. [CrossRef]
66. Okay, O. Polymeric Cryogels Macroporous Gels with Remarkable Properties. *Advs Pol. Sci.* **2014**, *263*, iii–iv. [CrossRef]
67. Plieva, F.M.; Savina, I.N.; Deraz, S.; Andersson, J.; Galaev, I.Y.; Mattiasson, B. Characterization of Supermacroporous Monolithic Polyacrylamide Based Matrices Designed for Chromatography of Bioparticles. *J. Chromatogr. B Analyt. Tech. Biomed. Life Sci.* **2004**, *807*, 129–137. [CrossRef]
68. Plieva, F.M.; Galaev, I.Y.; Noppe, W.; Mattiasson, B. Cryogel Applications in Microbiology. *Trends Microbiol.* **2008**, *16*, 543–551. [CrossRef]
69. Privar, Y.; Malakhova, I.; Pestov, A.; Fedorets, A.; Azarova, Y.; Schwarz, S.; Bratskaya, S. Polyethyleneimine Cryogels for Metal Ions Sorption. *Chem. Eng. J.* **2018**, *334*, 1392–1398. [CrossRef]
70. Dragan, E.S.; Lazar, M.M.; Dinu, M.V.; Doroftei, F. Macroporous Composite IPN Hydrogels Based on Poly(Acrylamide) and Chitosan with Tuned Swelling and Sorption of Cationic Dyes. *Chem. Eng. J.* **2012**, *204–205*, 198–209. [CrossRef]
71. Amin, R.; Alam, F.; Dey, B.K.; Mandhadi, J.R.; Emran, T.B.; Khandaker, M.U.; Safi, S.Z. Multidimensional Chromatography and Its Applications in Food Products, Biological Samples and Toxin Products: A Comprehensive Review. *Separations* **2022**, *9*, 326. [CrossRef]
72. Kumar, A. *Supermacroporous Cryogels: Biomedical and Biotechnological Applications*; CRC Press: Boca Raton, FL, USA, 2016; ISBN 9781482228823.
73. Bibi, N.S.; Singh, N.K.; Dsouza, R.N.; Aasim, M.; Fernández-Lahore, M. Synthesis and Performance of Megaporous Immobilized Metal-Ion Affinity Cryogels for Recombinant Protein Capture and Purification. *J. Chromatogr. A* **2013**, *1272*, 145–149. [CrossRef]
74. Yun, J.; Kirsebom, H.; Galaev, I.Y.; Mattiasson, B. Modeling of Protein Breakthrough Performance in Cryogel Columns by Taking into Account the Overall Axial Dispersion. *J. Sep. Sci.* **2009**, *32*, 2601–2607. [CrossRef] [PubMed]
75. Wang, L.; Wang, L.; Zhang, D.; Li, F.; Wang, M.; Huang, M.; Zhang, H.; Song, L. A Novel C-Type Lectin from Crab Eriocheir Sinensis Functions as Pattern Recognition Receptor Enhancing Cellular Encapsulation. *Fish. Shellfish Immunol.* **2013**, *34*, 832–842. [CrossRef] [PubMed]
76. Andaç, M.; Galaev, I.Y.; Denizli, A. Affinity Based and Molecularly Imprinted Cryogels: Applications in Biomacromolecule Purification. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2016**, *1021*, 69–80. [CrossRef] [PubMed]
77. Talbert, J.N.; Goddard, J.M. Enzymes on Material Surfaces. *Colloids Surf. B Biointerfaces* **2012**, *93*, 8–19. [CrossRef] [PubMed]
78. Ingavle, G.C.; Baillie, L.W.J.; Zheng, Y.; Lis, E.K.; Savina, I.N.; Howell, C.A.; Mikhalovsky, S.V.; Sandeman, S.R. Affinity Binding of Antibodies to Supermacroporous Cryogel Adsorbents with Immobilized Protein A for Removal of Anthrax Toxin Protective Antigen. *Biomaterials* **2015**, *50*, 140–153. [CrossRef]
79. Klein, M.P.; Fallavena, L.P.; Schöffer, J.D.N.; Ayub, M.A.Z.; Rodrigues, R.C.; Ninow, J.L.; Hertz, P.F. High Stability of Immobilized β-d-Galactosidase for Lactose Hydrolysis and Galactooligosaccharides Synthesis. *Carbohydr. Polym.* **2013**, *95*, 465–470. [CrossRef]
80. Arvidsson, P.; Plieva, F.M.; Savina, I.N.; Lozinsky, V.I.; Fexby, S.; Bülow, L.; Yu Galaev, I.; Mattiasson, B. Chromatography of Microbial Cells Using Continuous Supermacroporous Affinity and Ion-Exchange Columns. *J. Chromatogr. A* **2002**, *977*, 27–38. [CrossRef]
81. Mallik, R.; Jiang, T.; Hage, D.S. High-Performance Affinity Monolith Chromatography: Development and Evaluation of Human Serum Albumin Columns. *Anal. Chem.* **2005**, *76*, 7013–7022. [CrossRef]
82. Belokon, Y.N.; Kochetkov, K.A.; Plieva, F.M.; Ikonnikov, N.S.; Maleev, V.I.; Parmar, V.S.; Kumar, R.; Lozinsky, V.I. Enantioselective Hydrolysis of a Schiff's Base of D, L-Phenylalanine Ethyl Ester in Water-Poor Media via the Reaction Catalyzed with α-Chymotrypsin Immobilized on Hydrophilic Macroporous Gel Support. *Appl. Biochem. Biotech.—Part. A Enzym. Eng. Biotechnol.* **2000**, *88*, 97–106. [CrossRef]
83. Hanora, A.; Plieva, F.M.; Hedström, M.; Galaev, I.Y.; Mattiasson, B. Capture of Bacterial Endotoxins Using a Supermacroporous Monolithic Matrix with Immobilized Polyethyleneimine, Lysozyme or Polymyxin B. *J. Biotechnol.* **2005**, *118*, 421–433. [CrossRef]
84. Arrua, R.D.; Strumia, M.C.; Igarzabal, C.I.A. Macroporous Monolithic Polymers: Preparation and Applications. *Materials* **2009**, *2*, 2429–2466. [CrossRef]

85. Uygun, M.; Akduman, B.; Ergönül, B.; Aktaş Uygun, D.; Akgöl, S.; Denizli, A. Immobilization of Amyloglucosidase onto Macroporous Cryogels for Continuous Glucose Production from Starch. *J. Biomater. Sci. Polym. Ed.* **2015**, *26*, 1112–1125. [CrossRef]
86. Baydemir, G.; Türkoğlu, E.A.; Andaç, M.; Perçin, I.; Denizli, A. Composite cryogels for lysozyme purification. *Biotechnol. Appl. Biochem.* **2015**, *62*, 200–207. [CrossRef]
87. 20Bulut, E.; Sargin, I.; Arslan, O.; Odabasi, M.; Akyuz, B.; Kaya, M. In situ chitin isolation from body parts of a centipede and lysozyme adsorption studies. *Mater. Sci. Eng. C* **2017**, *70*, 552–563. [CrossRef]
88. Kaetsu, I. *Radiation Synthesis of Polymeric Materials for Biomedical and Biochemical Applications. Recent Trends in Radiation Polymer Chemistry*; Springer: Berlin/Heidelberg, Germany, 1993; pp. 81–97. [CrossRef]
89. Kumakura, M. Preparation method of porous polymer materials by radiation technique and its application. *Polym. Adv. Technol.* **2001**, *12*, 415–421. [CrossRef]
90. Lusta, K.; Chung, I.; Sul, I.; Park, H.-S.; Shin, D.-I. Immobilization of fungus Aspergillus sp. by a novel cryogel technique for production of extracellular hydrolytic enzymes. *Process Biochem.* **2000**, *35*, 1177–1182. [CrossRef]
91. Cocquempot, M.F.; Thomas, D.; Champigny, M.L.; Moyse, A. Immobilization of thylakoids in porous particles and stabilization of the photochemical processes by glutaraldehyde action at subzero temperature. *Eur. J. Appl. Microbiol. Biotechnol.* **1979**, *8*, 37–41. [CrossRef]
92. Scardi, V. Immobilization of enzymes and microbial cells in gelatine. *Methods Enzymol.* **1987**, *135B*, 293–299. [CrossRef]
93. Luo, Q.; Zou, H.; Zhang, Q.; Xiao, X.; Ni, J. High-Performance Affinity Chromatography with Immobilization of Protein A and L-Histidine on Molded Monolith. *Biotechnol. Bioeng.* **2002**, *80*, 481–489. [CrossRef]