

Chapter 2

Applications of Cyclic β -Glucans

Abstract Cyclic glucans have larger inner cavity diameter (~ 0.88 nm) when compared to those found in cyclodextrins (~ 0.85 nm). This property is valuable in pharmaceutical and food industries. They are used as wound dressing material and for the synthesis of nanowire. Cyclic β -glucans are used as inclusion agents for drugs, including indomethacin, ergosterol, vitamin D3, vitamin E, vitamin K1, propericiazine, reserpine, fluorescein, and flavones. β -(1,3)-(1,6) cyclic glucans have antitumorigenic and immunostimulating activity. They bind to the dectin-1 receptor of macrophages and stimulate immunogenicity. They are used as solubility enhancers for drugs, including zearalenone, paclitaxel, and naproxen. Linear β -(1,3)-glucan is used as one-dimensional host for the synthesis of water-soluble single-walled carbon nanotubes.

Keywords Chiral separation • Inclusion complexation • Enantiomers • Wound dressing

Many polysaccharides from mushrooms, including those derived from *Lentinus edodes* (lentinan), *Grifola frondosa* (grifolan), *Sclerotinia sclerotiorum* (scleroglucan), and *Schizophyllum commune* (schizophyllan) exhibit anticarcinogenic properties in both animals and humans (Harnack et al. 2011; Ewart et al. 2007; Newman et al. 1992; Okada et al. 1985; Kofuji et al. 2010; Miller et al. 1994; Spolaore et al. 2006; Chihara et al. 1987). They possibly mediate via the immune system of the host, through the activation of leukocytes and production of inflammatory cytokines. Cyclic β -(1,2) glucans form inclusion complexes with a variety of hydrophobic guest molecules, including amphotericin B, fluorescein, flurbiprofen, indomethacin, mefenamic acid, phenylbutazone, steroids, and vitamins (Koizumi et al. 1984b). They can adopt conformational structures with relatively small polar cavities and hence can be used as drug carriers. They have also been used as inclusion agents for non-steroidal, anti-inflammatory agents, and immunostimulators (York 1995). The antitumorigenic effects of cyclic β -(1,3)-(1,6) glucans are known (Xiao et al. 2004). The algae *Chlorella*, contains

β -1,3-glucan which is an immunostimulator, a free radical scavenger and a reducer of blood lipids (Spolaore et al. 2006). Cyclic β -(1,6)-(1,3)-glucans produced by free-living cells and bacteroids of *Bradyrhizobium japonicum* USDA 110, elicit isoflavonoid production in soybean (*Glycine max*) host (Miller et al. 1994). These flavonoids act as inducers of nodulation genes in several *Rhizobium* and *Bradyrhizobium* strains (Phillips 1992; Gottfert 1993).

Complexation of a family of cyclic β -(1,2) glucans with poorly soluble compounds, including ergosterol, indomethacin, paclitaxel, and luteolin have been reported (Kwon et al. 2000; Lee et al. 2001a, b; 2003). A family of cyclic glucans with DP ranging from 17 to 27 has been tested as host for the formation of inclusion complexes with poorly soluble ergosterol. The purified cyclic glucans from *Rhizobium meliloti* 2011 show better complexing behavior with ergosterol than cyclodextrin. Whereas, cyclic glucan-17mer do not form any complex. A mixture of cyclic glucans is seen to complex better with poorly water-soluble molecules better than a single glucan. Although their exact 3D structures are not clearly known, NMR and conformational analysis indicate that cyclic glucans have relatively flexible backbone structures, with a narrower hole which is formed due to the stretching of β -(1,2) linkage bonds (Mimura et al. 1996; Choi et al. 2000).

Oligosaccharides show great potential for improving intestinal microflora in humans and livestock. Generally, branched oligosaccharides are less readily digested than the linear polymers (Gross and Scholz 2001). Desirable oligosaccharides are those that are utilized primarily by *Bifidobacteria*, a specific class of Gram-positive bacteria (Fishbein et al. 1998). Maintenance of stable intestinal populations of *Bifidobacteria* lowers the pH of the digestive tract and prevents overgrowth of undesirable species and pathogens, including *Escherichia*, *Salmonella*, *Clostridia*, etc.

The cyclic β -(1,3), β -(1,3)-(1,6), and cyclic β -(1,6)-(1,3) glucans are not exploited fully for their industrial application which may be due to their lower DP value (10–13) and their lesser yield from microbial sources. In contrast the cyclic β -(1,2) with DP 17–24 is well studied for its pharmaceutical applications.

2.1 In Food

The β -(1,3)-(1,6)-glucan-enriched materials from the mushroom *Lentinus edodes* are high-fiber and low-calorie substitute for wheat flour. When they are incorporated in cakes instead of wheat they increase the batter viscosity, shear thinning effects, and elasticity. The cakes appear with decreased volume with increased hardness (Kim et al. 2011). There is no report on cyclic glucan as food supplement.

2.2 Medical Technology

Application of yeast glucan on wounds rapidly induces healing when compared to treatment with carrageenan, levan, inulin, dextran, starch, and inorganic talcum powder. This effect is attributed by reticuloendothelial system, including lympho reticular cells of macrophages, endothelial and reticulum cells. The proliferation of macrophage increases the resistance to infection, tumor growth, and quickens the wound healing process. Lentinan (1–3)- β -D-glucan with (1–6)- β -D-glucopyranoside branch possess antitumour activity in allogenic, syngenic, and autochthonous primary hosts. It suppresses the chemical and viral oncogenesis and prevents cancer recurrence or metastasis after an operation (Leibovich and Danon 1980; Lloyd et al. 1998).

2.3 As Wound Dressing Material

Wound dressings are prepared with different polymeric materials, including polyurethane, polymethacrylate, polyvinylpyrrolidone, carboxymethylcellulose, collagen, alginates, chitin, chitosan, or hyaluronic acid. The physical form of the dressing includes hydrocolloid, ointment, fiber, film, foam, and gel (Purna and Babu 2000). Synthetic polymers incorporated into granulation tissue remain in the body for a long time after use. To avoid such problems the dressing must facilitate the quick and successful healing, it should be safe, biocompatible, and curative to enhance the healing process. β -glucans from yeast are reported for their anticancer, blood cholesterol lowering, and anti-inflammatory activities (Ross et al. 1999; Ikewaki et al. 2007).

A novel wound dressing material is prepared from a complex of β -glucan [(1,3),(1,6)-linked] and chitosan (CS), which is biocompatible, bioabsorbable, biodegradable, and exhibits therapeutic value (Kofuji et al. 2010). Traditional wound dressing from pigskin are biocompatible but induce immune reactions in humans. Figure 2.1 shows the preparation of a wound dressing sheet from β -glucan-chitosan complex (adapted Kofuji et al. 2010). The characteristic of the complex is analyzed with X-ray diffraction, rheology, biodegradability, therapeutic efficacy.

2.4 Microparticulate Form of β -Glucan for Pharmaceutical Application

Immunologically active, homogeneous, nonaggregated, and microparticulate β -glucan is prepared from *Saccharomyces cerevisiae*. Product of 1–2 μ in diameter is prepared after Sonication and spray drying. This microparticulate β -glucan suspension enhances the phagocytosis of mouse peritoneal macrophages than

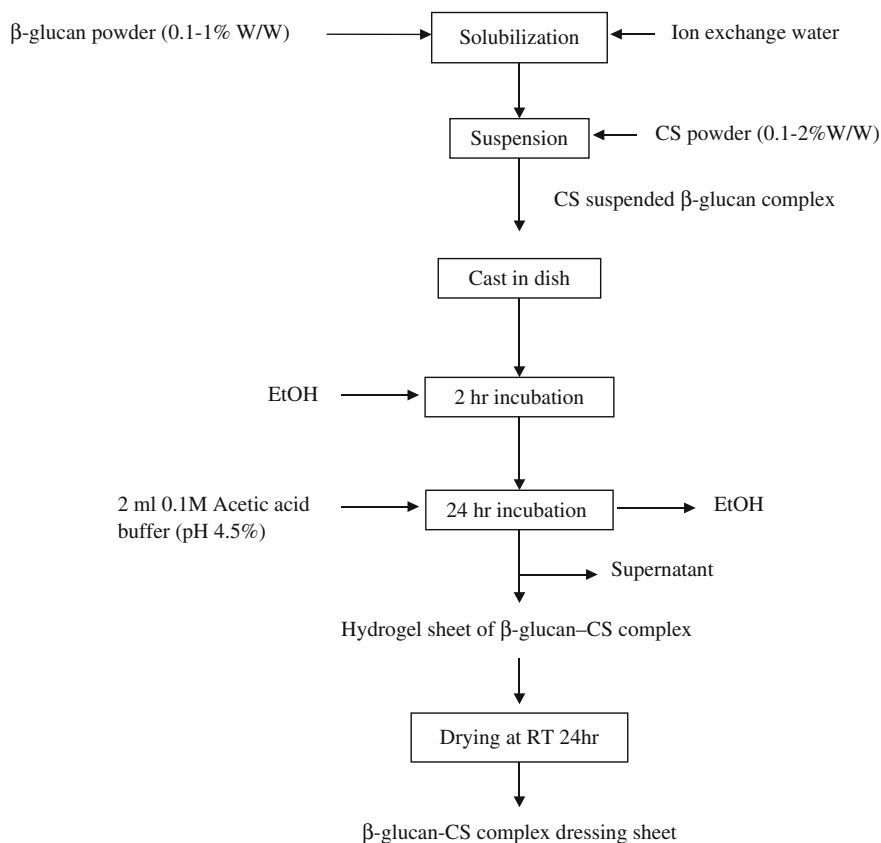


Fig. 2.1 Procedure for the preparation of β -glucan-CS complex sheet

aggregated β -glucan particles (Hunter et al. 2004). These glucans are polymers of β -(1,3)-D-glucose [with or without β -(1,6)-D-glucose side chains]. Generally they are aggregate of 5 to 100 μ in diameter in saline. For effective pharmaceutical application the size should be reduced to 1–2 μ by the method suggested by Hunter et al. 2004 (Fig. 2.2).

Polymeric microspheres are used for numerous applications in drug delivery, including vaccination, personal care, and medical diagnostics (LaVan et al. 2002; Sinha and Trehan 2003). For biomedical applications drugs can be protected and released at target sites by these microspheres (Champion et al. 2008). Drug carrying microspheres administered via intravascular (Song et al. 1998), inhalation (Masahiro and Byron 2005), nasal (Li et al. 2005), subcutaneous (Yamaguchi et al. 2002), and other routes in animals and humans are reported in literature. Macrophages are one of the phagocytic cells in the body which bind particles through a receptor-mediated process and internalize them by engulfment thereby clearing out from the body. This mechanism is one of the defense mechanisms of the body against pathogens and nonindigenous particulate matter (Djaldetti et al. 2002).

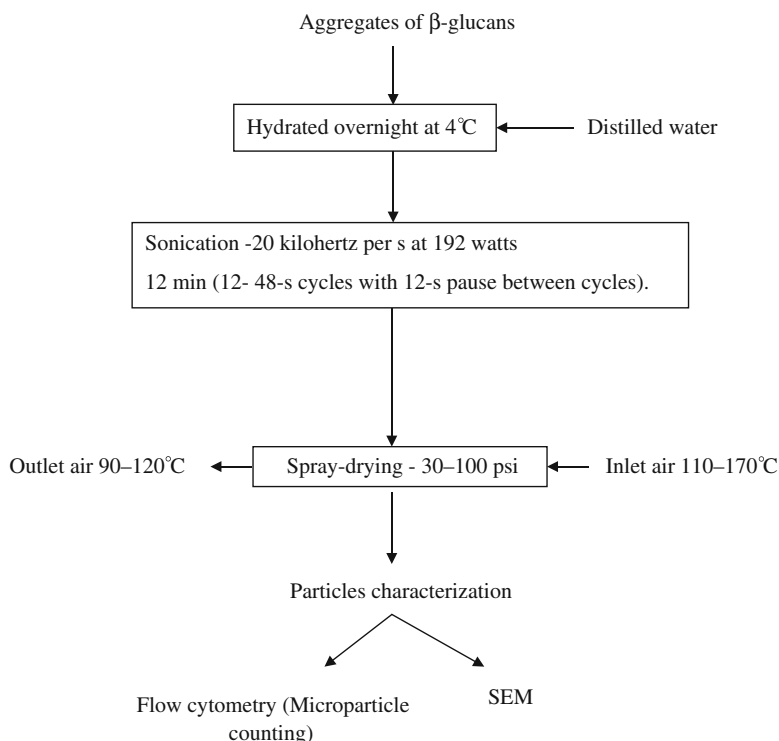


Fig. 2.2 Procedure for microparticulate β -glucan preparation

The glucan particles are hollow and porous and are 2–4 μm microspheres. Their outer shell provides β -glucan receptors (dectin-1 (D1) and complement receptor 3 (CR3) (Brown and Gordon 2001) for uptake by phagocytic cells. Glucan particles can be used for macrophage-targeted delivery of soluble biomolecules or drugs, including DNA, siRNA, protein, and small molecules through encapsulation inside their hollow core (Ernesto et al. 2011). These microspheres can also be used for encapsulation of nanoparticle which combines both the drug encapsulation properties of the nanoparticle and macrophage-targeting property of the glucan. The incorporation can be done by nanoparticles as core inside a glucan particle (glucan-nanoparticle) or by chemically derivatized glucan particle (nanoparticle-glucan).

2.5 Synthesis of Selenium Nanowires

Selenium nanowires of 34–120 nm in size is prepared using glucan produced by *Shinorhizobium* (Lee et al. 2009). The synthesis is carried out in water at room temperature. Amorphous Se in the form of spherical colloids is converted to

nanostructures by the assistance of the *Shinorhizobial* glucan as described in Fig. 2.3. The product can be characterized by X-ray diffraction (2θ h range of $10\text{--}88^\circ$), Raman Scattering spectrometer (514 nm), SEM, and TEM. These single-crystalline nanostructures may find applications in nanoelectronics, nanooptoelectronics, nanosensors, and nanobiotechnology (Patolsky et al. 2007; Lieber and Wang 2007). Microbial carbohydrate-doped multiwall carbon nanotube (MWNT)-modified electrodes are prepared by using cyclic β -(1,2) glucan and α -cyclodextrin (cyclodextrin). This MWNT is used for determining the presence of 4-(2-aminoethyl)benzene-1,2-diol (3,4 dihydroxyphenylalanine; dopamine) in a solution of 0.5 mM ascorbic acid, a representative interfering agent in neurotransmitter detection (Jin et al. 2010).

2.6 Drug Delivery

The complex-forming ability of cyclic β -(1,2) glucan with slightly water-soluble guest molecules is exploited in drug delivery (Koizumi et al. 1984a). A study describes this potential with glucan (of DP = 17) obtained from a mutant strain, *R. phaseoli* RA-12. Liquid chromatography with charcoal column is used for purification of this cyclic β -(1,2) glucan for this study. Three different methods are used for enhancing the solubility of different drugs with glucan (Figs. 2.4, 2.5, 2.6). Only three drugs are solubilized in method 1 (Indomethacin, Fluorescein, and Propericiazine). Whereas in method 2 faster equilibration is achieved leading to solubilization of several drugs (Indomethacin, Ergosterol, Vitamin D3, Vitamin E, Vitamin K1, Propericiazine, Reserpine, Fluorescein). Method 3 also led to solubilization of all the drugs except Vitamin K3.

It is observed that cyclic β -(1,2) with acetone forms a binary inclusion complex, while it forms a ternary complex with acetone and a guest molecule. Acetone is then evaporated. There is no enhancement of solubility of Vitamin K3 by glucan.

2.7 Enantiomeric Separator

Chirality is a property desired in pharmaceutical, food additive, and agrochemical industries (Lee and Jung 2002; Lee and Jung 2003). Cyclic β -(1,2) glucans isolated from *Rhizobium meliloti* are used as Nuclear Magnetic resonance spectroscopy (NMR) chiral shift agent to separate the signals from enantiomers of propranolol (Lee and Jung 2002; Lee et al. 2004). They are also used as chiral stationary phase for enantiomeric separation (Lee et al. 2004). A stationary phase prepared with cyclic glucan (*Rhizobium meliloti* 2011) are chemically immobilized onto a porous silica support via aminopropyltrimethoxysilane as a linker is able to separate a racemate of bupivacaine, propranolol, and fenoprofen (Jung et al. 2004).

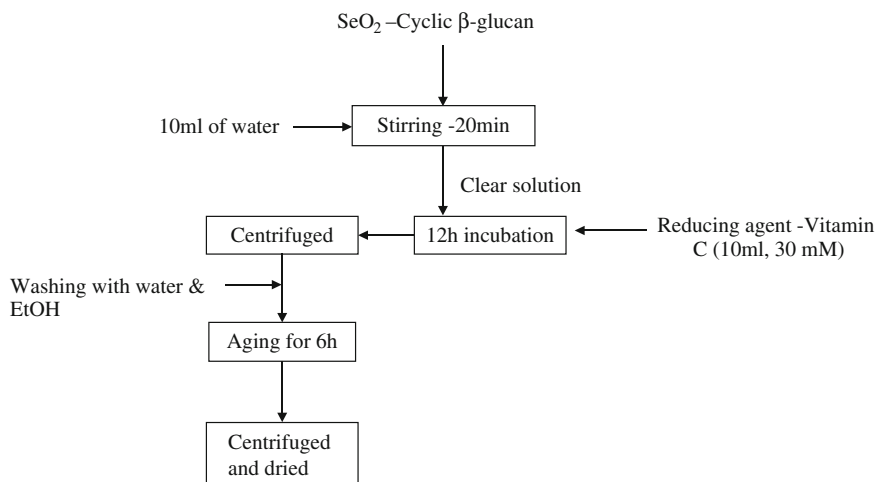


Fig. 2.3 Procedure for selenium nanowire synthesis by cyclic β -glucan

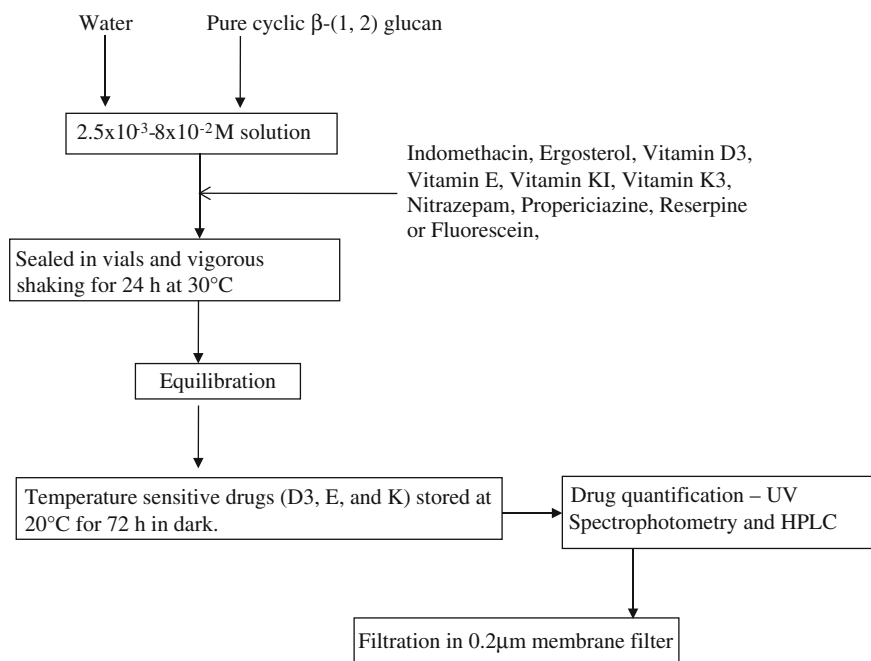


Fig. 2.4 Solubility enhancement method for different drugs with glucan (method 1)

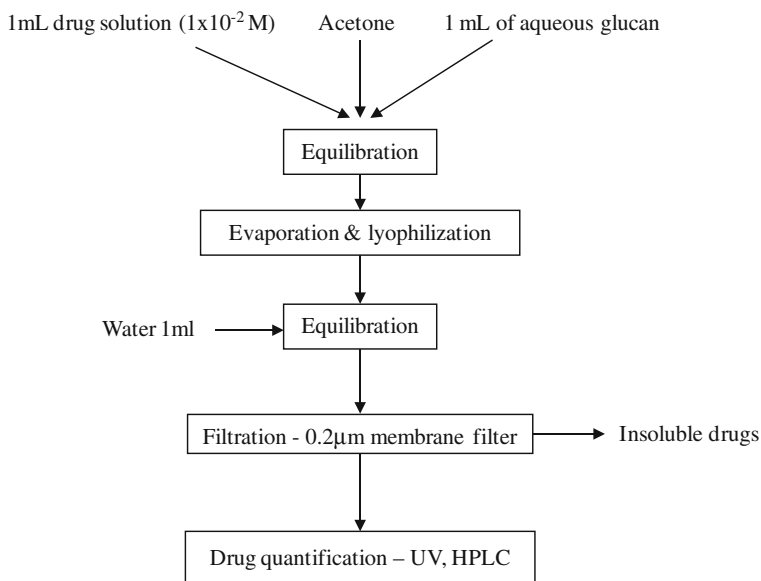


Fig. 2.5 Solubility enhancement method for different drugs with glucan (method 2)

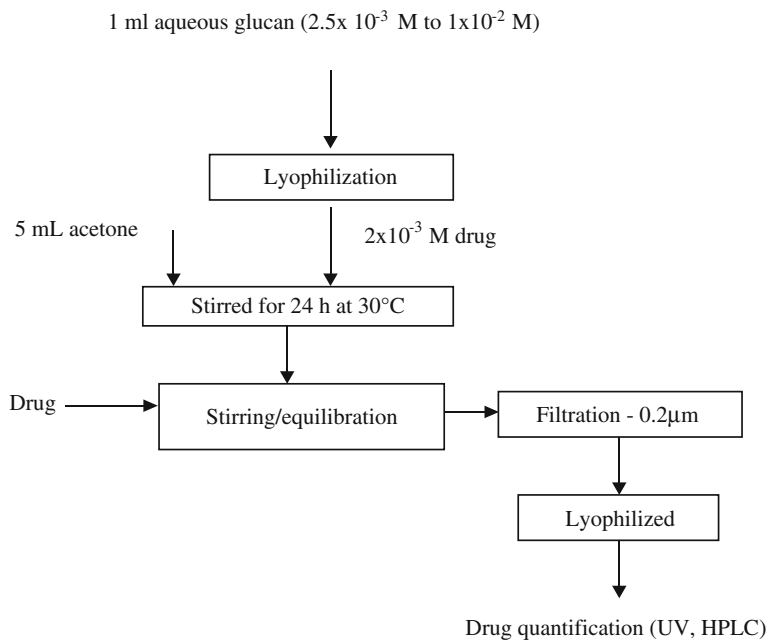


Fig. 2.6 Enhancing solubility of different drugs with glucan (method-3)

A plant flavonoid, luteolin, exuded from alfalfa is a well-known inducer of the Nod factor produced by *R. meliloti* (Peters et al. 1986). Luteolin has very poor solubility in water which can be improved by complexing it with cyclic glucans synthesized by *R. meliloti*. NMR spectroscopic analysis shows that the chemical shifts of the aromatic ring moieties of luteolin are altered due to its complexation with cyclic glucans. Rhizobial cyclic glucans enhance the solubility of hydrophobic legume-derived flavonoids by forming a complex (Lee and Jung 2003).

Cyclic glucans produced by *Rhizobium meliloti* 2011, are used as a chiral additive for the separation of enantiomers of terbutaline, amethopterin, thyroxine, and N-acetylphenylalanine in aqueous capillary electrophoresis (CE) (Lee and Jung 2003; Choi et al. 2000). Enantiomeric separation takes place in the normal- or reverse-polarity mode when a high concentration of neutral (60 mM) or anionic (40 mM) cyclic glucan is added to the background electrolyte. This glucan provides the required difference in both the binding of the enantiomers and their mobility.

2.8 In Chiral Technology

Separation of enantiomers of physiologically active chiral compounds and their characterization is of prime application in pharmaceutical, clinical, and toxicological studies (Lee and Jung 2002). The demand from chemical and pharmaceutical industries for enantiomerically pure compounds necessitates the researchers to use different chiral separators. Cyclodextrins, macro cyclic antibiotics, and some proteins are used for separation of enantiomers. The neutral cyclic- β (1,2)-glucans with DP ranging from 17–27 (Molecular Weight 3568.6) from *R. meliloti* 2011 are used as chiral NMR solvating agent (Kwon et al. 2000). Enantiomeric separations of compounds, such as N-acetylphenylalanine, propranolol, and catechin have been reported (Glasoe and Long 1960; Kitae et al. 1998).

Rhizobium trifolii TA-1 cyclic- β (1,2)-glucans (Cys) are chemically modified into carboxymethylated cyclic- β (1,2)-glucans (CM-Cys) which act as chiral selector for separation of flavonoids (Jeon et al. 2010). Cyclic- β (1,2)-glucans isolated from *Rhizobium* species is used for enantioseparation of various chemicals, including antiasthmatic drug terbutaline, antineoplastic agent amthopterin, thyroxine hormone, and α -amino acid derivative, N-acetylphenylalanine (Lee and Jung 2003). Chiral catechin is separated with Cys by micellar electrokinetic chromatography (MEKC) (Park and Jung 2005). The highly sulfated cyclic- β (1,2)-glucans from cyclic- β (1,2)-glucans are used as chiral additives for the separation of flavonoids, including arterenol, atenolol, isoproterenol, propranolol, metoprolol, and isosakuranetin (Park et al. 2004). These derivatives are more effective than the parent cyclic- β (1,2)-glucan in the stereoisomeric separation of catechin, isosakuranetin, and neohesperidin. Using chemical derivatization, cyclic- β (1,2)-glucan is modified into carboxymethylated cyclic- β (1,2)-glucan and then used as a chiral additive for the separation of ten flavonoids, including six

flavanones, one flavanol, and three flavanone-7-O-glycosides in CE (Wistuba et al. 2006).

2.9 Chiral Stationary Phase

Analysis of chiral compounds by gas chromatography and HPLC using chiral stationary phase is a practical analytical method. Chiral stationary phase can also be used in large-scale industrial separations. Glucans enhance solubility of poorly soluble guest molecules (Lee et al. 2001a, b). Cyclic- β (1,2)-glucan is chemically immobilized onto porous silica by the linker, aminopropyltrimethoxysilane (Felix et al. 1996). Chiral drugs, including bupivacaine, propranolol, and fenpropfen are separated by cyclic- β (1,2)-glucan bonded stationary phase. The characteristic cyclic scaffold and its inclusive complex-forming ability provide the differential binding capacity for enantiomers (Jung et al. 2004).

2.10 Carboxymethylated Cyclic β -(1,2)-Glucans

Carboxy methylated cyclic glucans may find application in separation of different biomolecules. The procedure involves the addition of 1.5 g of cyclic- β (1,2)-glucan, 24.3 mL of 16.3 % monochloroacetic acid solution, and 22.2 mL of 8.4 g NaOH in water, then stirred for 4 h at 50° C and neutralized with 6 M HCl. This mixture is precipitated by adding 7 vol of MeOH and kept overnight at 4 °C. Then it is centrifuged and the precipitate is desalted on Bio-Gel P-4 column. The thin-layer chromatography (5:5:4 BuOH–EtOH–H₂O), NMR spectroscopy, MALDI-TOFMS, and IR spectroscopy can confirm this modification reaction.

The carboxymethylated cyclic- β (1,2)-glucan derivatives are synthesized (85 % yield) by a one step process. The hydroxyl groups in the cyclic- β (1,2)-glucan are modified with monochloroacetic acid. Modification could be confirmed with FTIR and NMR. The flavonoids, including eriodictyol, hesperidin, hesperetin, 3,5,7,3',4pentahydroxyflavanone (taxifolin-pentahydroxyflavanone (taxifolin), isosakuranetin, catechin, naringin, and naringenin are successfully separated using carboxymethylated cyclic- β (1,2)-glucans. Enantiomeric separation is not achieved in the case of neohesperidin and homoeriodictyol. The most effective chiral resolution occurs at glucan concentration of 75 mM with six flavonoids except catechin and hesperidin which are successfully separated only at 100 mM concentration (Jeon et al. 2010).

2.11 Inclusion Complexes

A family of purified neutral cyclic- β (1,2)-glucans with DP ranging from 17–27 is used as inclusion agent for poorly soluble compound such as ergosterol. Enhanced solubility of ergosterol is obtained with neutral cyclic- β (1,2)-glucans when compared to another polysaccharide such as β -cyclodextrin. A Monte Carlo (MC) docking-minimization method was used to understand the molecular basis of host–guest complex formation of these two polysaccharides with ergosterol. The study indicated a ‘hand-shake’ mechanism for complexation of cyclic- β -(1,2)-glucan with ergosterol (Kwon et al. 2000).

In aqueous solution the complex with ergosterol is not observed (Koizumi et al. 1984a). So chloroform and acetone are tested as organic solvents. The former is found to be more efficient than the latter. The preparation of this complex involves several steps. First, ergosterol is dissolved in chloroform to obtain a 15×10^{-3} M solution. Then 1 mL of this solution is added to 1 mL of aqueous neutral cyclic- β (1,2)-glucan and the mixture is shaken for 24 h at 30 °C in the dark. After equilibration, the mixture is evaporated, lyophilized, and dissolved in 1 mL of water. The insoluble ergosterol is removed by filtration using a 0.2 μ m membrane filter (Whatman) and the filtrate is lyophilized and the ergosterol concentration in the filtrate is determined with a HPLC using a bonded silica gel packed column with mobile phase containing a mixture of 55 % ethanol and 45 % water at 35 °C.

The SAXS (small angle X-ray scattering) analysis of cyclic- β (1,2)-glucan indicates that its molecular conformation is in irregular doughnut-like ring with a thick cylindrical shape (Andre et al. 1995; York et al. 1993; Mimura et al. 1996). The structure is interpreted as the time average snapshot during dynamic docking mechanism. Monte Carlo simulation also indicates that glucan rearranges itself to form a pocket which leads to the formation of thermodynamically stable inclusion complex with ergosterol. This complex is more stable than the one formed between β -CD and ergosterol.

2.12 β -D-Glucans Complexation with Zearalenone

Zearalenone (ZEN), a 6-(10-hydroxy-6-oxo-trans-1-undecenyl)- β -resorcylic acid lactone, is a nonsteroid estrogenic toxin produced by several *Fusarium* species. This toxin along with other mycotoxins contaminate grains, including wheat, barley, grain sorghum, corn, and grain products including flour, bread, and processed commercial feed, milk and edible animal tissues (Mycotoxins 2003). Estrogenic property of ZEN causes negative effects on reproduction. The control of mycotoxins in agricultural and in the harvested products is very difficult and there are no practical solutions. β -D-glucans from cell wall of *Saccharomyces cerevisiae* is found to be a good adsorbent of ZEN, because it has great invitro

affinity toward Zearalenone. β -D-glucans as dietary adsorbent reduces the bio-availability of ZEN (Yiannikouris et al. 2004).

Inorganic materials, including clays, bentonites, and aluminosilicates are also used to eliminate mycotoxins through adsorption (Lemke et al. 1998, 2001). But they may impart dioxin and heavy metals contamination to the food products. This problem can be overcome by using organic compounds from yeast (Freimund et al. 2003) and bacterial cell walls (El-Nezami et al. 2000, 2002). They have the ability to form complexes with several mycotoxins and prevent their harmful effects on the environment.

2.13 Inclusion Complex with Paclitaxel

Paclitaxel is a complex diterpenoid drug which is used to treat advanced ovarian, breast, and non-small cell lung cancers. The drug binds to β -tubulin on microtubules and interferes the depolymerization process (Rowinsky et al. 1990). Its poor water solubility makes its usage difficult (Lee et al. 2001a, b). It is observed that cyclic- β (1,2)-glucan allows favorable accessibility to either the B or A-ring of paclitaxel. HPLC analysis reveals the improved solubility of drug with glucan. NMR data shows noticeable changes in the chemical shifts or peak shapes in the aromatic regions of paclitaxel upon interaction with the glucan molecule.

The preparation of this complex involves several steps as listed below. Paclitaxel is dissolved in ethanol to prepare a 2.0 mM of solution. To this solution different concentrations of aqueous neutral cyclic- β (1,2)-glucan is added and kept in shaker for 24 h at 30 °C and equilibrated in the dark condition. The mixture is evaporated, lyophilized, and dissolved in water. The insoluble matter is filtered with a 0.2 μ m membrane filter.

2.14 Inclusion Complexation with a Plant Flavonoid Luteolin

The plant flavonoid luteolin is the inducer of *nod* (nodulation) factor in *Rhizobium meliloti* (Peters et al. 1986). It is released from alfalfa plant and it has poor water solubility. Cyclic- β (1,2)-glucan from *Rhizobium meliloti* with 17–27 DP is used as a host molecule for the complexation of luteolin in aqueous media. The procedure for the preparation of this complex involves the following steps. 10 mM of stock solution of luteolin is prepared in acetone. One ml of this stock is added to 1 ml of aqueous solution of neutral cyclic- β (1,2)-glucan. The mixture is kept under shaking for 24 h at 30 °C, then partially evaporated, lyophilized, and mixed with 1 ml of distilled water. The insoluble luteolin is removed by 0.4 μ m membrane filter. The product could be analyzed and quantified with a HPLC.

Changes in the NMR peak shape and chemical shift is observed after complexation. Chemical shifts of the aromatic ring moieties in luteolin is changed

upon complexation with glucan. FTIR analysis shows restricted vibrations in the carbonyl stretching regions (at 1649 and 1607 cm^{-1}) of luteolin which on complexation broadens and shifts toward higher frequency (Lee et al. 2003). In plants, the complexation process potentially enhances the nodulation process where the luteolin act as inducer (Abe et al. 1982).

2.15 Inclusion Complexation with Naproxen

A non-steroidal anti-inflammatory drug, Naproxen (NAP; D-2-(6-methoxy-2-naphthyl)-propionic acid), is used for the treatment of rheumatic diseases. It has analgesic and antipyretic properties (Mahler et al. 1976; Calvo et al. 1987; Sevelius et al. 1980). When administered orally, NAP shows very low solubility in water and causes the gastrointestinal side effects, drowsiness, and dizziness (Valero et al. 2003). The complexation of NAP with sulfated cyclic- β (1,2)-glucan to improve its aqueous solubility has been reported (Kwon et al. 2012).

500 mg of glucan (0.16 mM) is dissolved in 5 ml of anhydrous pyridine. One gram of succinic anhydride (10 mM) is solubilized in 3 ml of pyridine. These two solutions are mixed and heated to 100 °C and 5 mg of DMAP [4-(dimethylamino) pyridine] (0.041 mM) is then added. The mixture is incubated for 24 h, the solvents are evaporated and mixed with 10 mL of water. The succinylated glucan is precipitated by adding 50 ml of isopropyl alcohol. The precipitate is washed three times with 10 ml of isopropyl alcohol, dried, and purified in DEAE Sephadex and Bio-gel P2 columns. The product can be characterized with MALDI-TOF mass spectrometer and NMR spectroscopy. A differential charge distribution occurs in the carboxyl groups of NAP which indicates the formation of inclusion complex of glucan and NAP. The interaction and inclusion complexation of NAP and cyclic- β (1,2)-glucan is confirmed by the disappearance of endothermic peak in the DSC (Differential scanning calorimetry) (Connors and Mollica 1966).

2.16 Functionalized β -(1,3)-Glucan in Carbon Nanotubes

Carbonaceous capsules or biomaterial have great potential in the fields of nano-reaction templates, catalyst supports, adsorbents, biomedical devices, and lithium ion batteries (Douglas and Young 2006; Tang et al. 2002). Single-walled or multiwalled carbon nanotubes are used as components for carbonaceous capsules and they act as electron-conductive functional materials (Tans and Dekker 2000). Layer-by-Layer is the established method to create controlled two or three-dimensional carbon nanotubes (Rouse et al. 2004). β -(1,3)-glucan if used as one-dimensional host for single-walled nanotube leads to the formation of water-soluble one-dimensional nanocomplexes (Numata et al. 2004). A layer-by-layer method is used to synthesize thin and multi-carbon nanotube based hollow

capsules from carbon nanotube complexes with cationic or anionic complementary functionalized β -(1,3)-glucan as building-block. Ionic β -(1,3)-glucan wraps around a single-walled carbon nanotube and double-walled carbon nanotube and forms water-soluble complexes with ionic groups on their exterior surface (Sugikawa et al. 2008).

2.17 Application of Cyclic β -(1,3),(1,6)-Glucans in Chiral Technology

The bradyrhizobial cyclic β -(1,3),(1,6)-glucans is used for the chiral discrimination of catechin, which is a polyphenolic compound isolated from tea leaves, grape seeds, and the wood and bark of trees acacia and mahogany. They exhibit antioxidant, anticarcinogenic, antiallergic, antiatherogenic activity (Kwon et al. 2007a). The (+) and (−)-catechin show opposite effects on glycogen metabolism in rat hepatocytes (Nyfeler et al. 1983). (−)-catechin without (+) isomer show allochemical activity, whereas the (+)-catechin without (−) isomer show antibacterial activity (Bais et al. 2002). Therefore, separation of this chiral mixture is very important. Separation of enantiomers of catechin and epicatechin can be performed on a HPLC and CE with cyclodextrins (Worth et al. 2000; Bonoli et al. 2003; Larger et al. 1998) and cyclic β -(1,2)-glucan as chiral additives (Park and Jung 2005; Lee and Jung 2003). The chiral separation of catechin is performed when the cyclic β -(1,3),(1,6)-glucans were added to the background electrolyte in CE. The successful separation of catechin, may be due to the chiral recognition induced by the stereochemical factors associated with the β -glycosidic linkage and the ring structure of glucans (Kwon et al. 2007a).

Bradyrhizobial glucans are also used for separation of flavanones, including eriodictyol, homoeriodictyol, hesperetin, naringenin, and isosakuranetin using CE (Kwon et al. 2007b).

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