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Review

Analysis of sugars in traditional Chinese drugs

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Abstract

This review is presented of chromatography and electromigration methods currently in use to determine sugars in traditional Chinese drugs: gas chromatography (GC), high-performance liquid chromatography (HPLC), ion-exchange chromatography, gel column chromatography (GCC), paper chromatography (PC) and thin layer chromatography (TLC), capillary electrophoresis (CE) and gel electrophoresis (GEP). The detection methods combined with above separation methods including ultra-violet, mass spectra, fluorescent light, refractive index (RI), electrochemical detection are also described. For the complicacy of structural analysis of polysaccharides in traditional Chinese drugs, the hyphenation procedures concerned with this analysis are introduced in this article too.

Keywords: Sugars; Traditional Chinese drugs; Chromatography; Review

Contents

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1. Introduction

1.1. Pharmacological applications of sugars in traditional Chinese herbs

Sugars are extensively existed in traditional Chinese herbs and their dry weight can reach 80–90% of the whole plants. As in other plants, sugars in traditional Chinese herbs are existed in three forms: monosaccharides, oligosaccharides and polysaccharides. Monosaccharides are also the basic constituent units of oligosaccharides and polysaccharides. There are more than 200 kinds of natural monosaccharides founded in plants, but most monosaccharides are existed in combinative forms except a few monosaccharides, such as glucose and fructose, are in their dissociated forms. Oligosaccharides usually consist of 2–9 monosaccharide units which are connected in straight chains or branched chains by glucoside bonds. Polysaccharides usually include more than 100 even several thousands of monosaccharide constituent units and perform very different physical and chemical properties. Until today, there are more than 300 kinds of polysaccharides extracted from the natural plants, in which the water-soluble polysaccharides from traditional Chinese herbs are most important for their significant pharmaceutical applications [1–4].

In the past decades, it has been found that the polysaccharides in plants are not only the energy resources but play key biological roles involving in many life processes as well. The mechanisms of pharmaceutical effects of bioactive polysaccharides on diseases have been extensively studied and more and more kinds of natural polysaccharides with different curative effects have been tested and even applied in therapies [5–7]. The major pharmaceutical applications of the polysaccharides in traditional Chinese drugs are reflected in following aspects:

Anti-cancers: Many kinds of polysaccharides in traditional Chinese drugs, such as the polysaccharides in *Lycium barbarum* [8], *Ganoderma lucidum* [9], *Saposhnikoria divaricata* [10], *Achyranthes bidentata* [11], *Klebsiella pneumoniae* [12], *Pseudostellaria heterophylla* [13] etc., have been reported to have anti-tumor activities through improving patients' immunity ability or killing tumor cells. Besides restraining the propagations of initiative tumor cells in patients, polysaccharides can effectively prohibit the propagations of tumor cells induced by chemical or viral reasons.

Immunity developments: More than 100 kinds of polysaccharides in Chinese traditional herbs have been found to have activations on improving human beings' immune activity [14,15]. The reason is that the administration of eutherapeutic natural polysaccharides could stimulate the production of interleukins that further improve the regeneration of T cells and B lymphocyte cells. Some examples of traditional Chinese drugs containing these functional polysaccharides are *Radix angellicae sinensis* [16], *Panax ginseng* [17], *Achyranthes bidentata* [18], *Arthur tzianabos* [6], *Chinese lacquer* [19,20], *Cordyceps sinensis* [21], etc. Some polysaccharides are even assumed to inhibit the binding of HIV to T cell in the first stage of HIV infection and have been proved to use as anti-HIV drugs [22–24].

Blood sugar reduction: The obvious effect of natural polysaccharides on reducing blood sugar has more and more become interesting and many experiments have proved that some polysaccharides could enhance the secretion of insulin and further adjust the carbohydrate metabolism. The polysaccharides extracted from *Radix ginseng*, *Ganoderma lucidum*, *Atractylodes macrocephala*, *Lithospermum officinale*, *Chinese yam*, *Chinese ephedra*, *Pumpkin*, etc. have been proved to have the activities of reducing blood sugar and some related polysaccharides medicines have been entered Chinese market [25–30]. *Momordica charantia* polysaccharide is even regarded as plant insulin for its special ability on reducing blood sugar [31].

Anti-inflammation: The anti-inflammation effect of natural polysaccharides is carried out by directly killing bacteria and viruses, or by improving patients' immune abilities. It is proved that sulfate *Radix achyranthis* polysaccharide and *Acanthopancis trifoliati* polysaccharide have a strong inhibiting activation on B type hepatitis viruses: HBsAg and HBeAg [32]. Other polysaccharides in Chinese traditional medicines, such as *Lycium barbarum* [33], *Achyranthes bidentata* [34], *Fructus lycii* and *Prunella vulgaris* [35,36] have been reported to perform strong activations on different inflammations therapies too.

Other pharmaceutical values of the polysaccharides in Chinese traditional drugs include anti-complementary, alleviating hyperthermia-induced damage, anti-ulcers, sedation, etc. [35,37–39].

1.2. Analytical methods of sugars

Because many kinds of proteins, dyes, volatilizable oils, lipids, biological bases and other ingredients are usually existed together with sugars in plants, the analysis of sugars in traditional Chinese drugs is very difficult. The analysis of sugars in traditional Chinese drugs, especially polysaccharides, usually consists of three procedures: extraction, purification and analysis. For the sugar extraction procedure, water, dilute basic solutions and organic solvents at different temperatures could be selected as dissolvent according to the aim ingredients. For the sugar purification procedure, multi-precipitation, salting out and column chromatography (including cellulose, ion-exchange resin, gel, zone capillary, HPLC, etc.) are usually used. For the third procedure of sugar analysis, the most used methods are chromatographic separations combining with suitable detectors and other structural analytical techniques including MS, IR, NMR, etc.

Compared to monosaccharides and oligosaccharides, the analysis of polysaccharides is more difficult for their large molecular weights, complex structures and inert chemical activations. Colourimetry with different chromogenic systems, such as carbazole–sulfuric acid, anthracenone–sulfuric acid, phenol–sulfuric acid, etc. could be applied to the total content analysis of polysaccharides. However, as the most strong

separation techniques, chromatographic methods are extensively applied to the compositional and structural analysis of polysaccharides when combined with other detection methods including UV, LIF, ED, IR, MS, NMR, etc. Electromigration methods have very high separation efficiency and are also extensively used for analysis of sugars.

There are some papers reviewing the analysis of sugars in different matrixes [40–44]. In this paper, only the chromatography and electromigration methods for analysis of sugars in traditional Chinese drugs, especially the pharmaceutical polysaccharides, are reviewed.

2. Chromatographic methods

2.1. Gas chromatography (GC)

In analysis of sugars in traditional Chinese drugs, GC is mainly applied to determine dissociated monosaccharides, and constituent monosaccharides of both oligosaccharides and polysaccharides. GC separation has the advantages of simple instrumentation, high selectivity, quickness and high accuracy. Table 1 lists the applications of GC in analysis of sugars in some traditional Chinese drugs.

Prior to constituent monosaccharides identification of oligosaccharides and polysaccharides by GC, the samples are usually hydrolyzed into monosaccharide units with acidic reagents such as sulfuric acid, hydrochloric acid or trifluoroacetic acid at a seal container or under the protection of N_2. Because of the limited volatility of sugars, another important procedure for GC of sugars is to transfer them into volatilizable and stable derivatives. There are two species of derivatives of sugars in GC analysis, one species are trimethylsilyl ether derivatives, which are formed by sugars with hexamethyldisilane (HMDS), trimethylchlorosilane (TMCS), trimethylsilyl (TMS) etc. in nonaqueous organic solvents such as pyridine or dimethyl sulfoxide. These species of derivatives are easily made and strongly volatilizable [45,47]. Another species of sugar derivatives are acetate derivatives, including trifluoroacetate alditol derivatives, trifluoroacetic acid alditol derivatives, acetic acid oxime derivatives and acetic acid nitrile derivatives, which are usually formed in pyridine, butylenes oxide or methyl imidazole solvents. Trimethylsilyl derivatives are separated perfectly in GC with moderate polar stationary liquids, and tailing peaks appear if strong polar stationary liquids are used. However, for acetate derivatives, strong polar stationary liquids are the best choice [31,51,53,66,71].

The columns used in GC analysis of sugars include packing columns and capillary columns. Capillary columns, such as OV-1, OV-225, SP-2100, SE-30, etc. perform much higher separation efficiency than packing columns and very suitable to constituent monosaccharide identification of heteropolysaccharides in traditional Chinese drugs.

It is found that the most used detection method coupled with GC analysis of sugars is flame ionization detection (FID), because FID has better selectivity and higher sensitivity. The GC–MS combination has extended the application scope of GC analysis of polysaccharides in traditional Chinese herbs [45,60,65–67]. The conjunction mode of monosaccharide units in Chinese herb polysaccharides can be identified by analyzing the relative retain times and the fragments of methylated derivatives of monosaccharides with GC–MS. This method is much more accurate than the indirect Smith degeneration method.

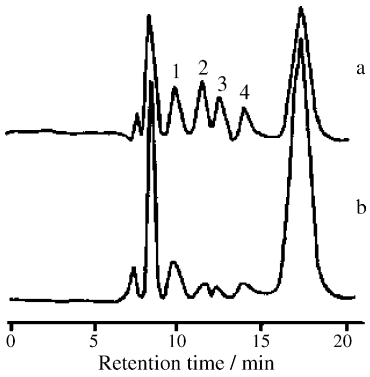

Fig. 1. HPLC chromatograms of mixed standard sample of (a) monosaccharides; and (b) hydrolysate of Maoba Damu Lacquer polysaccharide (LPS). (1) Glucurone; (2) rhamnose; (3) xylose; and (4) arabinose [85].

2.2. High-performance liquid chromatography (HPLC)

HPLC is also extensively applied in the analysis of sugars in traditional Chinese drugs for its high separation efficiency and simple operations. Compared to GC separation, HPLC does not need derivation if the goal only concentrates on separation of sugars. Besides qualitative and quantitative determination of dissociated monosaccharides, oligosaccharides and constituent monosaccharides of polysaccharides in traditional Chinese drugs, HPLC is very accurate to determine molecular weights of polysaccharides. Fig. 1 is a typical HPLC chromatogram of standard monosaccharides and hydrolyzed monosaccharides from *Maoba Damu Lacquer* polysaccharide [85]. The applications of HPLC for analysis of sugars in some traditional Chinese drugs are listed in Table 2.

The columns used in HPLC of sugars in traditional Chinese drugs include chemical-bonded alkyl column and amino-bonded silica-gel column. Water can be used as eluent for chemical bonded alkyl column, but the separation efficiency is not satisfactory [72]. The separation effect could be improved by reducing temperature, reducing the linear rate of mobile phase or adding sodium chlorate. The amino bonded silica-gel column can be used to separate general monosaccharides and oligosaccharides, in which monosaccharides flow out firstly, followed by disaccharides and then other oligosaccharides with more molecular weights. For this column, the mixture of acetonitrile and water is usually used as mobile phase and the retain times of sugars are reduced with the decrease of water ration. Since reductive sugars can

Table 1
GC of sugars in traditional Chinese drugs

Matrix	Sample preparation	Column	Eluent	Detection	Reference
Soluble sugars in *Fagopyrum tataricum* (Linn) Gaertn	Extract: 80 and 50% alcohol; derive: trimethylsilyl imidazole, pyridine	SE-30 capillary column (30 m × 0.25 mm × 0.10 μm)	He, 18 ml/min	MS	[45]
Seven monosaccharides in Aloe	Leach: water, 60 °C, 1 h	SE-30 column (30 m × 0.23 mm × 0.11 μm)	N_2, 30 ml/min	FID	[46]
Monosaccharides in *Chrysanthemum* tea	Leach: water, 60 °C, 1 h; extract: hexane; derive: hexamethyldisilane (HMDS), trimethylchlorosilane (TMCS) (2:1, v/v)	Flexible quartz capillary column (35 m × 0.53 mm × 0.2 μm)	N_2, 30 ml/min	FID	[47]
Soluble sugars in *Acanthopanax senticosus* tea	Leach: hot water, 60 °C, 1 h	Flexible quartz capillary column (35 m × 0.53 mm × 0.2 μm)	N_2, 30 ml/min	FID	[48]
Constituent monosaccharides in Safflower polysaccharide	Hydrolyze: 2 mol/l trifluoroacetic acid, 96 °C, 6 h; derive: 0.5 ml acetic anhydride, 90 °C, 30 min	Glass capillary column (0.29 mm × 24 cm)	ND[a]	FID	[49]
Constituent monosaccharides in *Osmunda japonica* Thunb. polysaccharide	Hydrolyze:1 mol/l H_2SO_4, seal, 100 °C, 6 h; neutralize: $BaCO_3$; derive: hydroxylamine, pyridine, 0 °C, 30 min	HP-1 cross-linking flexible quartz capillary column (12 m × 0.2 mm × 0. 33 μm)	H_2, 30 ml/min; N_2, 20 ml/min	FID	[50]
Constituent monosaccharides in *Momordica charantia* polysaccharide	Leach: boiling water,4 h; lysis: 48 h; precipitate: alcohol; derive: hydroxylamine, pyridine,90 °C, 30 min acetic anhydride 90 °C, 30 min	OV-225 flexible quartz capillary column (21 m × 0.2 mm)	He, 0.195 MPa	FID	[31]
Constituent monosaccharides in *Fructus corni* polysaccharide	Leach: hot water; precipitate: alcohol; filter: butyl-toyopearl 650 °C column, Sephadex G-200 column; hydrolyze: 2 mol/l trifluoroacetic acid, 120 °C, 1 h; derive: acetic anhydride, pyridine	OV-225 quartz capillary column (0.32 mm × 25 m)	H_2, 48 cm/s (linear rate)	FID	[51]
Constituent monosaccharides in Zadi-5 polysaccharide	Leach: water, 70–80 °C, 2 h; precipitate: alcohol; remove dyes: acetone; hydrolyze: 1 mol/l H_2SO_4, 100 °C, 2 h	HPX-70 capillary column	ND	ND	[52]
Constituent monosaccharides in Chinese chestnut polysaccharide	Leach: hot water; remove dyes: 20% H_2O_2; precipitate: alcohol; remove proteins: Sevag method; hydrolyze: 1 mol/l H_2SO_4, 100 °C, 8 h	OV-225 quartz capillary column (25 m × 0.32 mm)	He, 60 ml/min	FID	[53]
Constituent monosaccharides in *Jiruhen agaru*-8 polysaccharide	Leach: didistilled water, 70–80 °C, 2 h; precipitate: alcohol; hydrolyze: 2 mol/l H_2SO_4, 100 °C, 2 h; acetylation: 1-methyl imidazole, acetic anhydride, glacial acetic acid	HPX-70 capillary column	ND	ND	[54]
Constituent monosaccharides in *Saponikovia divaricata* polysaccharide	Hydrolyze: 1 mol/l H_2SO_4, 100 °C, 4 h	OV-1, flexible quartz capillary column (12 m × 0.2 mm × 0.33 mm)	ND	ND	[55]
Constituent monosaccharides in four polysaccharides from *Poria cocos*	Leach: PC1 by water, PC2 an PC2a by hot water (30 min), PC3 by alcohol, PC4 by methyl acid	Open chain crown ether column (0.2 mm × 28 m)	H_2, 24 ml/min	FID	[56]
Constituent monosaccharides in *Dioscorea esculenta* (Lour.) Burkill polysaccharide	Leach: hot water; precipitate: alcohol; purify: DEAE-52 celloluse column, Sephadex G-100 column	OV-225 glass capillary column (0.29 mm × 25 m)	N_2, 46 ml/min	ND	[57]

Constituent monosaccharides in *Rhodila sachalinensis* A. Bor. polysaccharide	Leach: water; precipitate: alcohol;	SE-30 column (50 m × 0.22 mm)	N_2	ND	[58]
Constituent monosaccharides in *Choerospondias axillaries* (Roxb) Hill polysaccharide	Leach: water, 2 h, 70–80 °C; precipitate: alcohol; wash: acetone; hydrolyze: 1 mol/l H_2SO_4, 2 h	HPX-70 capillary column	ND	ND	[59]
Constituent monosaccharides in *Rhodiola crenulata* polysaccharide	Hydrolyze: 0.5 mol/l H_2SO_4, 5 h; neutralize: $BaCO_3$	OV-21 flexible quartz capillary column (0.2 mm × 15 m)	He, 15 ml/min	MS	[60]
Constituent monosaccharides in *Monascus purpureus* polysaccharide	Hydrolyze: 2 mol/l H_2SO_4, 100 °C, 9 h; neutralize: $BaCO_3$	Flexible quartz capillary column (5 m × 0.2 mm)	N_2, 2 ml/min	ND	[61]
Constituent monosaccharides in *Exocarpium citri grandis* polysaccharide	Leach: hot water, 4 h; precipitate: 95% alcohol; remove proteins: Sevag method; hydrolyze: 2 mol/l H_2SO_4, 100 °C, 10 h	DB-1 cross-linking quartz capillary column (30 m × 0.25 mm)	He, 19.5 ml/min	FID	[62]
Constituent monosaccharides in *Ganoderma lucidum* polysaccharide	Hydrolyze: 2 mol/l H_2SO_4, 6 h; neutralize: $BaCO_3$	Ultra-1 glass capillary column (25 m × 0.32 mm)	N_2, 40 ml/min	FID	[63]
Constituent monosaccharides in five water soluble polysaccharides from *Ganoderma lucidum*	Leach: GL1 and GL2 by phosphate, GL3 by hot water, GL4 by basic solution, GL5 by hot basic solution	Open chain crown ether column (0.25 mm × 28 m)	ND	ND	[64]
Constituent monosaccharides in *Aloe barbadensis* polysaccharide	Leach: hot water; purify: Sephadex G-100 gel column; hydrolyze: H_2SO_4, N_2 protection	HP-6890 GC	ND	MS	[65]
Constituent monosaccharides in Aloe polysaccharide	Leach: deionized water; precipitate: absolute alcohol; hydrolyze: 2 mol/l TFA	HP-6890 GC, column (30 m × 0.25 mm × 0.25 μm)	He (20 ml/min)	MS	[66]
Constituent monosaccharides in *Hippophae rhamnoides* L. polysaccharide	Leach: 0.1 mol/l NaOH; purify: DEAE Sephadex A-25 column; methylate: NaOH-DMSO, methyl iodide, seal, 7 min	OV-101 glass column (25 m × 012 μm)	ND	MS	[67]
Constituent monosaccharides in Aloe polysaccharide	Leach: deionized water, 90 °C,1 h; precipitate: alcohol; oximation and acetylation: xylosic alcohol, pyridine, hydroxylamine	OV-225 Chromosorb WAW DMCS stainless steel column (1 m × 3.2 mm)	N_2, 50 ml/min	FID	[68]
Constituent monosaccharides in*Orobanche caerulescens* Steph. polysaccharide	Leach: water; precipitate: 80% alcohol; derive: pyridine, mannitol	SE-30 column (50 m × 0.2 mm × 0.25 μm)	N_2	FID	[69]
Constituent monosaccharides in *Auricularia polytricha* polysaccharide	Hydrolyze: 2 mol/l H_2SO_4, seal, 8 h; derive: pyridine, anhydride acetic acid, 100 °C, 2 h	OV-225 Chromosorb W AW DMCS stainless steel column (3 m × 3 mm)	N_2, 40 ml/min	FID	[70]
Constituent monosaccharides in *Rosa laevigata* michx polysaccharide	Leach: water, 0.15% ammonium oxalate, 0.15 mol/l NaOH, respectively, 96 °C, 1.5 h; precipitate: 95% alcohol; hydrolyze: 1.5 mol/l H_2SO_4, 5 h	OV-101 cross-linking quartz capillary column (12 m × 0.25 mm)	ND	ND	[71]

[a] ND: no description.

Table 2
HPLC of sugars in traditional Chinese drugs

Matrix	Sample preparation	Column	Eluent	Detection	Reference
Sucrose, glucose and fructose in *Prunus salicina* Lind Var.	Leach: 80% alcohol, 70 °C, 1 h	μ-Spherogel carbohydrate column (46 mm i.d. × 300 mm)	Water, 1.3 ml/min	RI (RID-2AS)	[80]
Constituent monosaccharides in polysaccharides from six kinds of *Herba dendrobii*	Leach: hot water; precipitate: alcohol; purify: CTAB; hydrolyze: 1 mol/l H_2SO_4	μ-Bondapak column	Acetonitrile–water (80:20), 2.0 ml/min	ND[a]	[81]
Molecular weight of polysaccharides from eight strains of *Coriolus versicolor*	Leach: hot water; precipitate: 95% alcohol	TSK G4000SW column (7.6 mm × 300 mm)	0.02 mol/l NaAc, 0.6 ml/min,	RI (Gilson-132)	[82]
Molecular weight of *Zizyphus jujuba* Mill. polysaccharide	Leach: water, 2 h; precipitate: 95% alcohol	μ-Bondagel E-1000 column	0.02 mol/l NaAc, 0.5 ml/min	RI (HP-1047A)	[83]
Constituent monosaccharides in *Melastoma dodecandrum* Lour polysaccharide	Leach: water; precipitate: 95% alcohol, 24 h; removing proteins: Sevag method; hydrolyze: 2 mol/l trifluoroacetic acid, 100 °C, 8 h	Shim-Pack VP-ODS column (150 mm × 4.6 mm)	Methanol–acetonitrile, 1 ml/min	Aminobenzone–phthalic acid color reaction	[84]
Constituent monosaccharides in four polysaccharides from *Poria cocos*	Leach: PC1 by water, PC2 and PC2a by hot water (30 min), PC3 by alcohol, PC4 by methyl acid	Micrapak NH_2-10 column (4 mm × 300 mm)	Acetonitrile–water–methanol (70:10:5) 1.6 ml/min	RI	[56]
Constituent monosaccharides in *Chinese lacquer* polysaccharide	ND	μ-Bondapak NH_2 column (300 mm × 7.18 mm i.d.)	Acetonitrile–water–methanol (70:25:5) 1.6 ml/min	RI (RI-23)	[85]
Soluble sugars in *Fagopyrum tataricum* (Linn) Gaertn	Leach: 80, 50% alcohol	Lichrosphere NH_2 column (150 mm × 416 mm × 5 μm)	85% Acetonitrile, 1.8 ml/min	RI	[45]
Molecular weight of *Dioscorea opposita* Thunb polysaccharide	Leach: hot water, 2 h; precipitate: 95% alcohol; purify: Sephadex G-100 column	Biosep SEC-S3000 column (300 mm × 718 mm)	0.2 mol/l NaCl, 1 ml/min	ND	[86]
Constituent monosaccharides in *Coriolus versicolor* (L.) Fr. polysaccharide	Hydrolyze: 2 mol/l trifluoroacetic acid, 100 °C, 6 h	Licrospher 100 NH_2 column	(A) Distilled water; (B) acetonitrile (from 15 to 30% within 30 min)	ELSD (SEDEX 55)	[87]

[a] ND: no description.

Table 3
IC of sugars in traditional Chinese drugs

Matrix	Sample preparation	Column	Eluent	Reference
Isolation of four polysaccharides from *Achyranthes bidentata*	Extract: boiling water, 3% trichloroacetic acid; precipitate: pre-cooled 95% ethanol	DEAE-Sepharose fast-flow column (6 mm × 20 cm)	0.01 mol/l phosphate (pH 6.8) containing 0–1.0 mol/l NaCl, 25 ml/h	[18]
Isolation of *Angelica sinensis (Oliv) diels* polysaccharides	Dissolve: pure water	DEAE-5PW column (7.5 mm × 75 mm)	0–1.0 mol/l NaCl, 26 min; 1.0 mol/l NaCl, 2 min, 0.5 ml/min	[91]
Isolation of *Porphyra yezoensis Ueda* polysaccharides	Leach: distilled water, 3 h; remove proteins: Sevag method	DE-23 column (2.6 cm × 20 cm)	Water, 0.4 mol/l NaCl, 20 ml/h	[92]
Constituent monosaccharides in *Asian lacquer* polysaccharides	Hydrolyze: 2 mol/l trifluoroacetic acid, 8 h	Dionex CarboPac PA-1 column	Acetonitrile–water (8:2), 1 ml/min	[73]

form Schiff base with NH_2 and further destroy the amino bonded silica-gel column, pre-column derivatization is necessary for analysis of reductive sugars.

A paper recently reports analyzing Lacquer polysaccharides by RP-HPLC, in which the mixture of the hydrolyzed sample was applied at 80 °C in an Amido-80 column eluted with acetonitrile–water (8:2) solution at a flow rate of 1 ml/min [73].

This separation method is usually combined with differential refractive index detection (RI), which has some advantages of high stability, simple operation and no damage to samples. But following disadvantages of RI limit its applications in sugars analysis: (a) the results are influenced by temperature and composition of mobile phase; (b) this method is not suitable to gradient elution; and (c) the sensitivity is not enough high for analysis of trace sugars in samples.

UV is also combined with HPLC for sugars detection, but the effective absorption wavelength is nearby 190 nm because of no chromophoric group in most sugars. For UV detection, the samples and mobile phases must be pretreated to pure or super pure degree to avoid disturbing. The detection sensitivity of UV is similar with RI and it could be greatly improved by forming strong absorptive derivatives of sugars through pre-column or post-column derivatizations.

One extensively studied and applied way to greatly improve sensitivity of analysis of sugars by HPLC is fluorescence light (FL) detection based on fluorescent derivatization. There are two kinds of fluorescent derivatization methods: pre- and post-column derivatization. For sugars analysis, the general derivants include: 2-aminopyridine (2-AP) [74], 9-fluorenylmethyl hydrazine (FMOC) [75], 2-aminobenzoic acid (2-AA) [76], 2-aminobenzamide (2-AB) [77], 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) [78], 1-phenyl-3-methyl-5-pyrazolone (PMP) [79], etc. Despite fluorescence detection is very sensitive and high selective, it suffers from the complicated procedure and the disturbance caused by unavoidable side reactions.

Evaporative light-scattering detection (ELSD), pulsed amperometric detection (PAD) and conductive detection are also applied to combination with HPLC for sugars analysis. For example, HPLC-ELSD is used to determine the constituent monosaccharides of *Coriolus versicolor* polysaccharide, the detection limit is similar with RI method but not affected by gradient elution [87].

2.3. Ion chromatography (IC)

Ion chromatography (IC), which is sometimes regarded as one kind of HPLC separations, is also applied to analyze sugars in traditional Chinese drugs (Table 3). Most water-soluble sugars in traditional Chinese drugs are weak acids with pK_a more than 11, so they are charged by ionic complexes formations in aqueous media. In a NaOH eluent solution, sugars are totally or partly changed into anions and can be further separated when flowing through anion-exchange columns. Both anion- and cation-exchange columns can be used for sugars analysis. For anion-exchange column, NaOH, borate and other salts can be used as eluent, while distilled water, Ca-EDTA, dilute phosphoric acid, dilute sulfuric acid, etc. are used for cation-exchange column. Dionex Company has developed three kinds of ion exchange columns: Carbopac PA 1, Carbopac MA 1 and Carbopac PA 100. Because these columns are made of thin shell anion exchange resin as packing and styrene-diethylene polymers with large particle size as basic core, they exhibit the advantages of good mobility and small reverse pressure [88].

PAD is proved superior to RI and ELSD, in respect of selectivity, sensitivity and the possibility of gradient elution. This method is very suitable to detect sugars when combined with IC. Because the current responses of sugar oxidizations on the surface of noble metals in basic media are very strong, the detection sensitivity of PAD is very high, which has been improved from the initial 50 nmol to 10 pmol [73,89,90].

2.4. Gel column chromatography (GCC)

For the analysis of sugars in traditional Chinese drugs, GCC (including gel permeation (GPC) and gel filter chromatography (GFC)) is mainly applied to purification and molecular weight determination of polysaccharides, which is listed in Table 4. This method is of quickness, high selectivity and good repeatability when applied to molecular determination. The polysaccharides flow out the column in the order of molecular weights, which are finally measured via a calibra-

Table 4
GCC of sugars in traditional Chinese drugs

Matrix	Sample preparation	Column	Eluent	Reference
Purification of *Bupleurum chinense* DC	Leach: boiling water; remove proteins: glacial acetic acid; precipitate: 95% alcohol	Sephadex G-75 column	Water	[93]
Purification of *Plantagin* polysaccharides	Leach: water, 2 h; precipitate: 95% alcohol; removing proteins: Sevag method; lysis: 2 day	DEAE Sephadex A-225 column (2.5 cm × 60 cm)	0.1 mol/l NaCl, 20 ml/h	[94]
Purification of *Zizyphus jujuba* mill. polysaccharide	Leach: water, 2 h; precipitate: 95% alcohol	DEAE-cellulose column, Sephadex S-300 column (3 cm × 60 cm)	0.2 mol/l NaAc	[83]
Molecular weight of *Angelica sinensis (Oliv) Diels* polysaccharide	Dissolve: pure water	Bio, sep SEC-S3000 column (7.8 mm × 300 mm)	(A) H_2O; (B) 0.15 mol/l NaCl; (C) 0.02 mol/l Na_2HPO_4–NaH_2PO_4, 0.5 ml/min	[91]
Purity of *Dioscorea esculenta* polysaccharide	Leach: water; precipitate: alcohol; purify: DEAE-52 cellulose column, Sephadex G-100 column	Sepharose CL-6B column (15 mm × 900 mm)	0.1 mol/l NaCl, 8 ml/h	[59]
Purity of *Lentinus edodes* polysaccharide	Leach: water, 2 h; precipitate: 95% alcohol; removing proteins: Sevag method	Sephadex G-100 (1.0 cm × 70 cm)	0.1 mol/l NaCl, 10 ml/h	[95]
Molecular weight of carboxymethyl/sulfated/debranching Lacquer polysaccharides	Carboxymethylation: monochloroacetic acid; sulfation: $ClSO_3H$, pyridine; $NaIO_4$ oxidation: 0.075 mol/l $NaIO_4$	TSK G5000-PW column, TSK G3000-PW column	0.01 mol/l phosphate, 0.2 mol/l Na_2SO_4	[20]

tion curve from a series of standard samples. In GPC/GFC, the general packing materials are rigid or half-rigid water-soluble gels, such as Sephadex, TSK, Macrosphere, Bio-Gel, Bondagel, etc. and the eluents usually are distilled water, NaCl, Phosphate, etc.

2.5. Paper chromatography and thin layer chromatography (PC and TLC)

In the analysis of sugars in traditional Chinese drugs, PC and TLC are usually applied to purity measurement and constituent monosaccharides identification of oligosacchrides and polysaccharides (Table 5). The most used plate in TLC is silica gel plate, in which the activity is determined by the water content in silica gel. Silica gel G, H and F are common used in TLC. During the fabrication of silica gel G, $CaSO_4$ is added as adhesion agent, but sometimes it is replaced by carboxymethyl cellulose (CMC), or hyroxymethyl cellulose for better mechanic strength [55]. Silica gel F_{254} is fabricated by adding fluorescent zinc silicate into silica gel, which is very stable in water and organic solvents [84]. From Table 5, it is found that the usual detection method for TLC of sugars is phenylamine–phthalic acid colour reaction.

PC and TLC of sugars are of high speed and simple instrumentation, but the shortages is unsatisfactory repeatability and the accuracy of experimental results is seriously affected by the researcher's experience.

3. Electromigration methods

3.1. Capillary electrophoresis (CE)

As a new separation technique, CE has been quickly developed during past two decades and its separation efficiency is much higher than the common GC and HPLC methods. The instrument of CE is relatively simple and the sample consuming is very small. In sugars analysis, CE is used to directly determine monosaccharides and part of oligosaccharides, and indirectly determine the constituent monosaccharides of oligosaccharides and polysaccharides. The applications of CE for analysis of sugars in traditional Chinese drugs are listed in Table 6. Because the pK_a of monosaccharides are more than 11, monosaccharides are negatively charged in strong basic running buffer and can be further separated under a fixed electric field. For improving the separation efficiency, some surface-active agents, such as SDS, tetrahydrofuran (THF), hexadecyltrimethylammonium bromide (CTAB), etc. are added into the running buffer.

UV and FL detections, which are described in GC and HPLC sections, are also suitable to combine with CE separation for sugars analysis. Electrochemical detection (ED) is very fit to CE separation for its high sensitivity, cheap instrumentation and simple operation. Amperometric detection (AD), one kind of electrochemical detection methods, has been applied to determine the constituent monosaccharides

Table 5
PC and TLC of sugars in traditional Chinese drugs

Matrix	Sample preparation	Plate	Developing agent	Detection	Reference
Constituent monosaccharides in *Melastoma dodecandrum* Lour polysaccharide	Leach: hot water; precipitate: 95% alcohol, 24 h; remove proteins: Sevag method; hydrolyze: 2 mol/l trifluoroacetic acid, 100 °C 8 h	Silica gel F_{254} (0.15 mol/l Na_2HPO_4–0.5% CMC)	Acetone–water (96:4)	Phenylamine–phthalic acid	[84]
Constituent monosaccharides in *Saponikovia divaricata* polysaccharide	Hydrolyze: 1 mol/l H_2SO_4, 100 °C, 4 h	Silica gel (0.5% sodium hyroxymethyl cellulose)	Acetone–water (94:6)	Phenylamine–phthalic acid	[55]
Constituent monosaccharides in *Rhodila sachalinensis* A. Bor. polysaccharide	Leach: water; precipitate: alcohol	Xinhua-1 filter paper (7 cm ×40 cm)	*n*-Butyl alcohol–acetic acid–water (4:1:5)	*n*-Butyl alcohol–phenylamine–phthalic acid	[58]
Monosaccharides and polysaccharides in *Actinostemma lobatum*	Leach: monosaccharides by 80% alcohol, polysaccharides by water; precipitate: 95% alcohol; hydrolyze: 1 mol/l H_2SO_4, 6 h	ND[a]	(A) *n*-Butyl alcohol–acetic acid–water (4:1:5), 24 h; (B) acetic ether–pyridine–water (2:1:2), 18 h	*n*-Butyl alcohol–phenylamine–phthalic acid	[96]
Constituent monosaccharides in *Monascus purpureus* polysaccharide	Hydrolyze: 2 mol/l H_2SO_4, seal, 9 h; Neutralize: $BaCO_3$	Silica gel (10 cm × 20 cm)	*n*-Butyl alcohol–ammonia–water (60:40:5)	Phenylamine–phthalic acid	[61]
Constituent monosaccharides in *Abelmoschus manihot* (L.) polysaccharide	Leach: water, 70–80 °C, 1 h; precipitate: 95% alcohol; remove proteins: digallic acid; remove dyes: active carbon; hydrolyze: 2 mol/l trifluoroacetic acid, 100 °C, 6 h	Silica gel G	Isopropanol–acetone–acetic acid (2:2:1)	Phenylamine–phthalic acid	[97]
Constituent monosaccharides in *Tremella aurantialba* polysaccharide	Hydrolyze: 1 mol/l H_2SO_4, 100 °C, 2 h; neutralize: $BaCO_3$	ND	*n*-Butyl alcohol–acetic acid–water (4:1:5)	Phenylamine–phthalic acid	[98]
Constituent monosaccharides in *Pirulina platensis* polysaccharide	Hydrolyze: 2 mol/l H_2SO_4, 110 °C, 6 h; neutralize: $BaCO_3$	Whatman 1 filter paper (10 cm × 35 cm)	*n*-Butyl alcohol–acetic acid–water (4:1:5)	*n*-Butyl alcohol–phenylamine–phthalic acid	[99]
Constituent monosaccharides in *Bupleurum marginatum* Wall. polysaccharide	Hydrolyze: 1 mol/l H_2SO_4; neutralize: $Ba(OH)_2$	Silica gel H (10 cm × 10 cm)	*n*-Butyl alcohol–methanol–water (50:15:35)	Phenylamine–phthalic acid	[100]

[a] ND: no description.

Table 6
CE of sugars in traditional Chinese drugs

Matrix	Sample preparation	Capillary	Running buffer	Detection	Reference
Sucrose, glucose, fructose in *Angelica, Astragalus membranceus, Codonopsis pilosula*	Extract: methanol, 15 h	Polyimide-coated fused silica capillary (45 cm 360 μm o.d. × 25 μm i.d.), 5 kV	0.05 mol/l NaOH buffer solution (pH 12.7)	AD (copper electrode, 0.65 V)	[101]
Constituent monosaccharides in *Radix glycyrrhizae* polysaccharide	Leach: water; precipitate: alcohol; purify: Sephedex G-200 column; hydrolyze: 1 mol/l H_2SO_4	Silica capillary (50 cm × 50 μm i.d.), 26 μA	50 mmol/l boric acid, pH 10.2, 20 kV	UV (254 nm, 1-aminobenzene derivation)	[102]
Constituent monosaccharides in *Panax quinquefolium* polysaccharide	Leach: boiling water, 4 h; precipitate: 85% alcohol; remove dyes: active carbon; wash: chloroform–orthobutanol (20:4)	Fused silica capillary (75 μm × 57 cm), 20 kV	0.05 mol/l borate–21 mg/ml KH_2PO_4	UV (200 nm)	[103]
Constituent monosaccharides in *Angelica sinensis* and *Flax* polysaccharide	Leach: water, 90–100 °C 6 h; precipitate: nonaqueous ethanol; remove proteins: chloroform–isopentanol; hydrolyze: 2.0 mol/l H_2SO_4, 5.0 mol/l HCl acid, N_2 protection, 2.5 h	Fused silica capillary (25 μm i.d. × 360 μm o.d. × 50 cm), 12 kV	0.090 mol/l NaOH	AD (copper electrode, 0.60 V)	[104]
Constituent monosaccharides in *Ligustrum lucidum* polysaccharide	Leach: water, 90–100 °C, 6 h; precipitate: non-aqueous ethanol; remove proteins: chloroform–isopentanol; hydrolyze: 2.0 mol/l H_2SO_4, N_2 protection, 10 h	Fused silica capillary (25 μm i.d. × 360 μm o.d. × 50 cm), 12 kV	0.045 mol/l NaOH	AD (copper electrode, 0.60 V)	[105]
Constituent monosaccharides in *Cordyceps sinensis* polysaccharide	Hydrolyze: 2 mol/l trifluoroacetic acid, nitrogen protection, 12 h, 100 °C	Beckman untreated fused silica capillary (75 μm i.d. × 50 cm)	0.1 mol/l NaOH–5 mmol/l tryptophan	UV (280 nm, tryptophan as background)	[106]

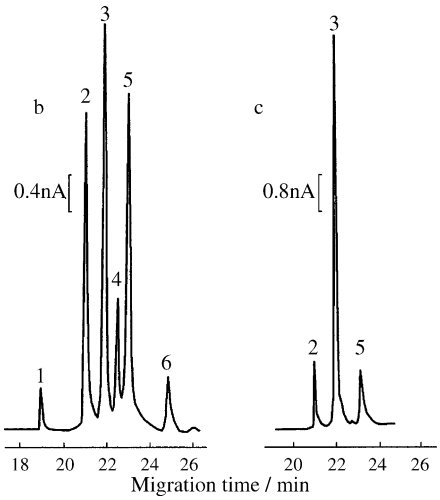

Fig. 2. Capillary electropherograms of hydrolyzed monosaccharides from angelica sinensis polysaccharides (b) and hydrolyzed monosaccharides from flax polysaccharides (c) under the optimum conditions of CZE-AD. (1) Fucose; (2) galactose; (3) glucose; (4) rhamnose; (5) arabinose; and (6) xylose [105].

in some traditional Chinese herbs. The used working electrodes are copper or gold electrodes and the detection sensitivity reaches μmol/l [101,104,105]. Fig. 2 is capillary electropherograms of hydrolyzed monosaccharides from *Angelica sinensis* polysaccharides and *flax* polysaccharides under the optimum conditions of CZE-AD [105].

3.2. Chip capillary electrophoresis (CCE)

Chip capillary electrophoresis (CCE) is a new μ-TAS (micro-total analysis system) technique and developed rapidly in recent years, in which separation channel, reaction container and detector are collected on a chip with only several square centimeters by micro-fabrication techniques and all procedures of sampling, separation, detection can be fulfilled together. CCE is successfully applied to DNA sequencing for its predominance in high efficiency, high speed and low consuming. Some reports on analysis of glucose and cyclodextrin by CCE combining with ED have appeared, but still not for sugars in traditional Chinese drugs [107–109].

3.3. Gel electrophoresis (GEP)

As a traditional separation technique, GEP is of easy operation and simple instrumentation. For the analysis of sugars in traditional Chinese drugs, it is mainly used to purification and purity measurement of polysaccharides (Table 7). The common used propathene gel electrophoresis (PAGE) has much higher separation efficiency and quantitative accuracy than other GEP methods for the stability and meshy structure of propathene molecules. The running buffers are usually Tris–HCl and Tris–H_3PO_4 systems, and the separation voltage applied is as high as 400 V. For detection of sugars with GEP, the suitable dyes include Bromophenol blue, thymol blue [49], Schiff's reagent [84], Alcian blue [70,110,112], *p*-aminoanisole [111], etc. There are still some reports on analyzing sugars in traditional Chinese drugs by paper fiber electrophoresis, but it is only used as reference for its lower accuracy. Compared to GC, HPLC and CE, GEP is time-consuming and not enough sensitive, which limit its applications in sugars analysis.

4. Hyphenation procedures

For the analysis of dissociated monosaccharides and some oligosaccharides in traditional Chinese drugs, the most difficult points are effective separation and sensitive detection,

Table 7
GEP of sugars in traditional Chinese drugs

Matrix	Sample preparation	Column	Running buffer	Indicator	Reference
Purity of safflower polysaccharide	Leach: hot water; precipitate: alcohol; remove proteins: Sevag method	Polypropylene	0.5 mol/l Tris–HCl (pH 9.1)	Bromophenol blue–0.2% thymol blue	[49]
Purity of *Melastoma dodecandrum* Lour. polysaccharide	Leach: hot water; precipitate: 95% alcohol, 24 h; remove proteins: Sevag method; lysis	Polypropylene	Tris–HCl (pH 9.1)	Schiff's reagent–periodic acid	[84]
Purity of *Auricularia polytricha* (Mont) Sacc. polysaccharide	Hydrolyze: 2 mol/l H_2SO_4, 8 h; acetylation: pyridine, anhydride acetic acid, 100 °C, 2 h	Polypropylene	0.5 mol/l borate (pH 9.0)	Alcian blue	[70]
Purify of *Akebia quinala* polysaccharide	Purify: Sepharose CD-4B gel column	Polypropylene		5% Alcian blue–acetic acid, 0.1% Coomassie blue	[110]
Purity of *Cucurbita moschata* (Duch.) Poiret polysaccharide	Leach: distilled water, 75–80 °C; precipitate: alcohol; purify: Sepharose CL-4B column, Sephadex A-200 column	Waterman glass fiber paper (2 cm × 20 cm)	0. 025 mol/l borate (pH 9.3), 400 V, 20 min	*p*-Aminoanisole–sulfuric acid	[111]
Purity of *Cucurbita moschata* polysaccharide	Leach: hot water; remove proteins: Sevag method; lysis: 48 h; precipitate: alcohol	Polypropylene, 110 V	Tris–glycine (pH 8.3)	Alcian blue-8GX	[112]

because of the isomeric forms and no chromophoric group in their molecules. Some advanced chromatographic separation techniques, such as GC, HPLC and CE, can complete the analysis of monosaccharides when combining with derived UV, FL and ED detections. However, the analysis of polysaccharides in traditional Chinese drugs is much more difficult for their numerous types, complicated structures and inert chemical properties. The general procedures for analysis of polysaccharides include pretreatment, extraction, purification, purity measurement, molecular weight measurement, constituent monosaccharides identification, structural analysis, etc. which could be finished only by the hyphenation of different analytical techniques. Some united applications of multiple analytical methods on analysis of polysaccharides in traditional Chinese drugs are listed in Table 8.

The preparation of polysaccharides in Chinese drugs usually consists of following procedures: (a) the powdered materials are treated in order with acetone, diethyl ether, alocohol/methyl alcohol for 2 h under heating in water bath to remove surface fats; (b) the pretreated materials are leached with hot water, dilute acid, or dilute base at 90∼100 °C for 4∼6 h of stirring and usually repeated for three times; (c) the filter liquids from above procedure are collected and precipitated with two to five times (v/v) of 95% or absolute alcohol, followed washing with acetone and diethyl ether to obtain crude polysaccharides; (d) the proteins in crude polysaccharides are removed by Sevag method or trichloroacetic acid; (e) the dyes are taken off by weak basic resin such as DEAE cellulose column or Duolite A-7 column; (f) the crude polysaccharides are finally purified by precipitation, salting out or column chromatography, etc.

The purity of polysaccharides can be determined by chromatography methods including PC, GPC/GFC, GE and HPLC. One polysaccharide is proved as pure if only one fleck or chromatographic peak appears. The PC method is not very credible, but the result of HPLC is very accurate. Polarimetry, UV and ultra-centrifugation are also useful for determination of polysaccharide purity. For assuring a correct result, at least two kinds of methods should be compared in this procedure [115,117,119,122].

GPC/GFC and HPLC are suitable to measure the molecular weights of polysaccharides in traditional Chinese drugs. For HPLC method, it needs molecular weight-known polysaccharides as standards and special gel as packing [113,116]. For GPC/GFC method, it also needs standard polysaccharides to make a calibration curve, and the molecular weight of the goal polysaccharide is calculated depending on its elution volume [114,117,120–122]. The results can be compared with osmotic pressure method, vapor pressure method, light dispersion method and dynamic viscosity method, especially the light dispersion method does not need standard polysaccharides.

The types and molar ratio of constituent monosaccharides of hetero-polysaccharides in traditional Chinese drugs are usually identified with PC, TLC, GC, HPLC and CE methods. Prior to chromatographic separation, the pure polysaccharides are hydrolyzed at a strong acidic condition under heating in water bath, followed by neutralization with NaOH, $Ba(OH)_2$, or $BaCO_3$. The results of PC and TLC are limited in accuracy and can only be used as references, but GC, HPLC and CE can provide very accurate results for their strong selectivity. Certainly, the best analytical conditions should be selected to ensure separating all monosaccharides in a polysaccharide sample.

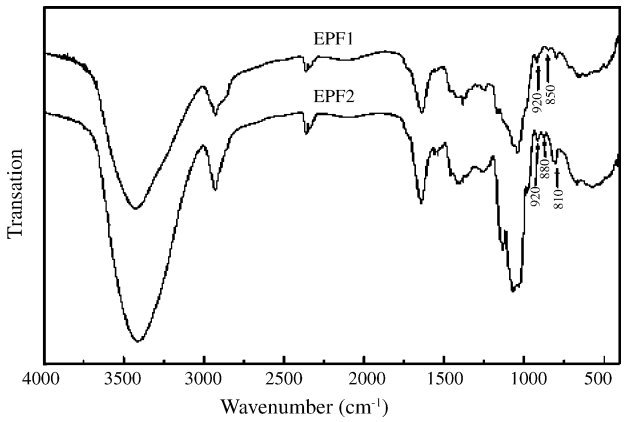

Fig. 3. The FT-IR spectra of extracellular polysaccharide fractions from the *G. tsugae* mycelium [121].

Other structural analysis of polysaccharides includes linkage style of neighbour monosaccharose, structure of end carbon-glucoside bond, ratio of glucosidic bonds, straight strain or cyclic structure, etc.

The linkage style of neighbour monosaccharides can be determined by GC–MS. Polysaccharide sample is hydrolyzed into methylated monosaccharides, then transferred into volatile derivatives for GC–MS analysis, such as *o*-methyl glucoside and *o*-trimethylsilyl ether derivatives. The linkage style of neighbour monsaccharides is deduced by comparing the relative retention time and the major fragments of MS with reference data [67,73,113].

There are two types of end carbon-glucoside bonds: α and β styles, which can be judged by IR and NMR techniques. In IR spectra, the C–H bond in α-style has an absorption peak nearby 844 cm^{-1}, while that of the C–H bond is in β-style nearby 891 cm^{-1}. Fig. 3 shows the IR spectra of extracellular polysaccharide fractions (EPF) of *Ganoderma tsugae* [121]. In the anomeric region (950–700 cm^{-1}), both fractions exhibited the obvious characteristic absorption at 810 cm^{-1} corresponding to the existence of mannose. EPF 1 also exhibited the absorption at 920 and 850 cm^{-1}, typical for α-configuration. While the obvious absorption peaks at 910 and 880 cm^{-1} in EPF 2, revealing the co-existence of α and β configurations. In ^1H NMR, the chemical shift (δ) of α-C–H bond is more than 5.00 ppm, but that of β-C–H bond is less than 5.00 ppm, In ^{13}C NMR spectra, the δ value of α-C–H bond is 97–101 ppm, but that of β-style is 103–105 ppm [116,117,119].

The ratio of different glucosidic bonds can be calculated by their relative peak areas in ^{13}C NMR spectra, because in the NMR spectra of a polysaccharide with more than one kind of

Table 8
Hyphenation techniques of sugars in traditional Chinese drugs

Sample	Purification	Purity analysis	Molecular weight determination	Constituent monosaccharide identification	Structural identification	Reference
Asian lacquer polysaccharide	GCC (CM-Sephadex G-100 column)	ND[a]	GPC (TSK-gel PWXL column)	RP-HPLC, IC-PAD	GC–MS, IR, NMR	[73]
Dioscorea opposita Thunb polysaccharide	GCC (DEAE-cellulose and Sephadex G-100 column)	HPLC	HPLC	PC, GC	GC–MS, H-NMR, IR, C-NMR	[113]
Hippophae rhamnoides L. polysaccharide	GCC (DEAE-Sephadex A-25 column)	GCC (glass fiber PE, Sepharose CL-4B column)	ND	GC	GC–MS, IR, C-NMR	[67]
Ginkgo biloba polysaccharide	ND	ND	GFC (Sepharose 4B column)	GCC (Sephadex G2200 column)	HPEC	[114]
Ganoderma lucidum polysaccharide containing selenium	GCC (DEAE-cellulose column, Sepharose CL-4B column)	GCC (Sephadex G-100 column), polyacrylamide GEP, UV	ND	GC and TLC	IR	[115]
Morchella esculenta L. polysaccharide	IC (DEAE-cellulose), GC (Sepharose CL-6B gel column)	ND	HPLC	GC	IR, NMR	[116]
Lentinus edodes (Berk.) polysaccharide	GCC (DEAE-cellulose and cellulose gel columns)	GEP, GCC (Sepharose CL-6B column)	GPC	GC	IR	[117]
Lentinus edodes polysaccharide	GCC (Sephadex G-100)	PC, GFC	ND	PC, GC	ND	[95]
Dendrobium moniliforme (L.) polysaccharide	GCC (DEAE-cellulose and Sephacryl columns)	GPC	TLC, GC		IR	[118]
Lentinus edodes polysaccharide	GCC (Sephadex G-150 column)	GCC (Sephadex G-200 column), polyacrylamide GE, UV	ND	TLC	IR	[119]
Polysaccharides from *Radix ginseng* rubra, *Radix ophiopogonis* and *Fructus Schisandrae chinensis*	GCC (Sephadex G-75 column)	Agarose GE, UV	GCC (Sephadex G-200 column)	TLC, HPLC,	IR, IO_4^- oxidation and Smith degradation	[120]
Ganoderma tsugae polysaccharide	GCC (DEAE-Sepharose CL-6B column)	ND	GCC (TSK-GEL column)	GC	IR, C-NMR	[121]
Porphyra haitanensis polysaccharide	GCC (DEAE-cellulose column)	UV, PC, GFC	GCC (Sepharose 6B column)	GC	IR, Smith degradation	[122]

[a] ND: no description.

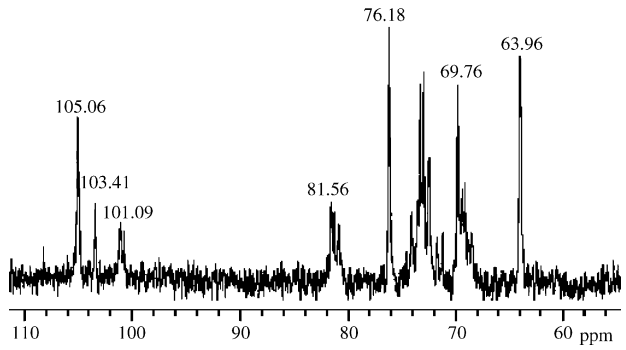

Fig. 4. ^{13}C spectra of extracellular polysaccharide fraction 2 from the *G. tsugae* mycelium in D_2O at 25 °C [121].

glucosidic bonds, the peak area of carbon is corresponding with the conjunctive style of glucosidic bond [67,113].

Whether the structure of a polysaccharide including α (1 → 6) glucosidic bond is straight or cyclic can be determined by the *δ*-value in ^{13}C NMR spectra, since both *δ*-values of C_1 and C_4 are different corresponding with the polysaccharides structures and the experimental results can be compared with the standard data in references. Fig. 4 is the ^{13}C spectra of extracellular polysaccharide fraction 2 from *G. tsugae* mycelium in D_2O at 25 °C [121]. From these ^{13}C spectra, it is found that this polysaccharide has the (1 → 6)-linked α-D-mannopyranosyl mainchain and (1 → 2) linked α-D-mannopyranosyl sidechains with terminal β-D-galactopyranosyl units.

For a cyclic structural sugar, whether it is pyran ring or furan ring can be judged by its typical absorption in IR spectra. Generally, there are three strong absorption peaks in 1100–100 cm^{-1} for glucosidic bond in pyran ring, but only two for that in furan ring [116,120–122].

5. Future development

Because of the assured significance of polysaccharides in traditional Chinese drugs in disease therapies, the studies on their structural analysis and pharmaceutical applications become more and more interesting. Undoubtedly, chromatographic methods act as core means in this field. From our personal opinions, the further work may be concentrated on following aspects:

Establishing more accurate extraction and purification methods: The number of polysaccharides in traditional Chinese drugs is numerous, and even one kind of herb maybe contain many different kinds of polysaccharides, which contents are unfixed according to different producing areas. The percentages of extractions are varied depending on extraction conditions and purification methods too. However, most of current studies on the extractions of polysaccharides in traditional Chinese drugs are relatively simple and there is not a complete method to ensure the 100% of extraction.

Applying innovative analytical techniques to the structural analysis of polysaccharides in traditional Chinese drugs: The microstructures of polysaccharides are very complicated and its complete molecular map is very difficult to be obtained. Many new analytical methods, such as pyrolysis gas chromatography (PGC) [123], super flow chromatography (SFC) [124], chip capillary electrophoresis, etc. can be used for structural analysis of polysaccharides when combined with MS, NMR, and IR techniques.

Exploring the pharmaceutical metabolisms of polysaccharides in traditional Chinese drugs: The therapeutic effects of polysaccharides in traditional Chinese drugs on some important diseases including cancers and diabetes are doubtless, but most of the therapeutic mechanisms are not clear till today. The most important task of exploring the pharmaceutical metabolisms of polysaccharides in traditional Chinese drugs is to establish the accurate relationships between the polysaccharide structure and therapeutic effect. Certainly, the numerous number and complicated structures of polysaccharides in traditional Chinese drugs make this task very difficult.

6. Nomenclature

AD	amperometric detection
CE	capillary electrophoresis
CGE	column gel electrophoresis
ED	electrochemical detection
ELSD	evaporative light-scattering detection
FID	flame ionization detection
FL	fluorescence light
GC	gas chromatography
GCC	gel column chromatography
GEP	gel electrophoresis
GFC	gel filter chromatography
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
IR	infra red
LC	liquid chromatography
LLS	laser light scattering
MS	mass spectrometry
NMR	nuclear magnetic resonance
PAD	pulsed amperometric detection
PC	paper chromatography
RI	differential refractive index detection
SEC	size exclusion chromatography
SFC	supercritical flow chromatography
TLC	thin layer chromatography
UV	ultra violet

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