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Review

Macroporous synthetic hydrophilic resin-based packings for the separation of biopolymers

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ABSTRACT

Macroporous synthetic hydrophilic resin-based packings, the TSKgel PW series, are reviewed. The characteristics of the packings and their applications to the separation of biopolymers are briefly described. A wide range of biopolymers such as proteins, peptides, DNA fragments, RNAs, oligonucleotides, polysaccharides and oligosaccharides can be separated successfully by size-exclusion, ion-exchange, reversed-phase, hydrophobic interaction and affinity chromatography on these materials.

CONTENTS

1. INTRODUCTION

Liquid chromatography has progressed greatly during the last two decades and now high-performance liquid chromatography (HPLC) has become one of the most powerful techniques for separating many kinds of substances, including biopolymers. As far as is known, the first HPLC of biopolymers was reported by Chang *et al.* in 1976 [1]. They separated proteins and nucleic acids by using surface-modified spherical silica of particle diameter *ca.* 10 μm. Subsequently, various types of packings based on silicas, synthetic hydrophilic resins, polysaccharides, etc., were developed and it is now possible to separate biopolymers by various modes of HPLC, size-exclusion chromatography (SEC), ion-exchange chromatography (IEC), reversed-phase chromatography (RPC), hydrophobic interaction chromatography (HIC), hydroxyapatite chromatography (HAC) and affinity chromatography (AFC).

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Our group has been developing macroporous synthetic hydrophilic resin-based packings to separate biopolymers for more than 15 years. Since the first development of SEC packings in 1978 [2–4], various packings for IEC [5,6], RPC [7,8], HIC [9,10] and AFC [11–13] have been developed. These packings are now commercially available (TSKgel PW series; Tosoh, Tokyo, Japan) and are widely used in many countries. The characteristics of these packings and applications to the separation of biopolymers are briefly reviewed.

2. CHARACTERISTICS OF MACROPOROUS SYNTHETIC HYDROPHILIC RESIN-BASED PACKINGS, TSKgel PW SERIES

Various grades of PW packings differing in pore size are available for SEC, as listed in Table 1. Therefore, PW can be applied to a wide range of biopolymers, from small molecules such as oligosaccharides, peptides and oligonucleotides up to large molecules such as polysaccharides, proteins and nucleic acids. Separation ranges for polysaccharide and globular protein are roughly estimated by multiplying the ranges for polyethylene glycol in Table 1 by 2 and 10, respectively. These packings are commercially available in prepacked columns. GMPW are mixed-bed columns packed with a mixture of three packings having different pore sizes, G2500PW, G3000PW and G6000PW [14]. The separation range is very wide and the molecular weight calibration graph is almost linear over a wide separation range. Accordingly, GMPW is useful for separating substances such as polysaccharides that have wide molecular weight distributions.

Although the exact chemical composition of PW packings is not available, they are based on synthetic resins containing many primary hydroxyl groups. They are hydrophilic owing to the hydroxyl groups and it is rare that proteins are adsorbed on them by hydrophobic interaction. However, they are not as hydrophilic as poly-

TABLE 1

MACROPOROUS SYNTHETIC HYDROPHILIC RESIN-BASED PACKINGS FOR SEC

Trade name	Particle size (μm)	Molecular weight separation range (polyethylene glycol)
G2000PW	10	100–5000
Oligo-PW	6	100–5000
G2500PW	10	100–5000
G2500PW$_{XL}$	6	100–5000
G3000PW	13	100–50 000
G3000PW$_{XL}$	6	100–50 000
G4000PW	13	2000–300 000
G4000PW$_{XL}$	10	2000–300 000
G5000PW	17	4000–1 000 000
G5000PW$_{XL}$	10	4000–1 000 000
G6000PW	17	40 000–8 000 000
G6000PW$_{XL}$	13	40 000–8 000 000
DNA-PW	10	40 000–8 000 000
GMPW	17	100–8 000 000
GMPW$_{XL}$	13	100–8 000 000

saccharide based packings, and the elution of some small hydrophobic molecules such as benzyl alcohol and *n*-butyl alcohol is slightly retarded when they are separated in water [15]. PW packings except G2000PW and oligo-PW also contain small amounts of carboxyl groups (2–20 μequiv./ml) [14,15]. Consequently, buffers or salt solutions must be used as eluents in the separation of ionic substances in order to eliminate ionic interactions between the packings and the samples. G2000PW and oligo-PW contain positive charges of 0.15–0.20 μequiv./ml [15] and anionic substances tend to be adsorbed on them fairly strongly. The functional group of the positive charges is not known.

PW packings are chemically stable and can be operated at high temperature. It is possible to sterilize them by autoclaving at 120°C. They can be operated in the pH range 2–12, and more extreme pH values are also acceptable for short periods of time. Therefore, various types of ligands can be introduced into PW packings by utilizing hydroxyl groups under severe conditions.

PW packings are also mechanically stable. Accordingly, small particles, *e.g.*, 10 μm in diameter, can be used without problems. Because they hardly swell or shrink, the solvent in the column can be varied widely, using distilled water, salt or buffer solutions even at high concentrations, mixtures of aqueous solutions and water-soluble organic solvents, etc.

Packings for use in IEC, RPC, HIC and AFC listed in Table 2 have been developed and are now commercially available. All these packings except octadecyl-4PW were prepared by introducing ligands into G5000PW. The base material of octadecyl-4PW is G4000PW. All these packings are chemically and mechanically stable just like SEC packings. They can be operated in a wide pH range (2–12) and they can be washed with alkaline or acidic aqueous solutions for regeneration. For example, it has been reported that no change in the characteristics of packings occurred during

TABLE 2

MACROPOROUS SYNTHETIC HYDROPHILIC RESIN-BASED PACKINGS FOR IEC, RPC, HIC AND AFC

Trade name	Mode	Ligand	Ligand content (μmol/ml)
DEAE-5PW	IEC	Diethylaminoethyl	120
SP-5PW	IEC	Sulphopropyl	120
CM-5PW	IEC	Carboxymethyl	120
Phenyl-5PW RP	RPC	Phenyl	N.D.[a]
Octadecyl-4PW	RPC	Octadecyl	N.D.
Phenyl-5PW	HIC	Phenyl	100
Ether-5PW	HIC	Oligoethylene glycol	N.D.
Chelate-5PW	AFC	Iminodiacetic acid	20
Heparin-5PW	AFC	Heparin	5–6[b]
Blue-5PW	AFC	Cibacron Blue F3G-A	3–4
Boronate-5PW	AFC	*m*-Aminophenylboronic acid	40
ABA-5PW	AFC	*p*-Aminobenzamidine	N.D.
Tresyl-5PW	AFC	Tresyl	20

[a] N.D. = not determined.
[b] In mg/ml.

runs with phenyl-5PW RP, phenyl-5PW and chelate-5PW with 0.5 *M* sodium hydroxide solution or 20% acetic acid at 25°C for 2 weeks or 10 days [7,9,11]. Because these packings are based on G5000PW or G4000PW, they have large pores, which are assumed to be *ca.* 1000 or 500 Å in diameter, although they have not been determined exactly. Consequently, they can be applied to large molecules. They have fairly high adsorption capacities even for large molecules. For example, the adsorption capacities of DEAE-5PW are 30–35 mg/ml for ferritin and thryroglobulin with molecular weights of 440 000 and 660 000, respectively [5]. Further, because sample molecules can move quickly in large pores high resolutions are attainable. In addition, the flow-rate dependences of resolution and dynamic adsorption capacity are small owing to the large pores.

DEAE-5PW, SP-5PW and CM-5PW are packings for IEC [5,6]. DEAE-5PW contains only one type of ionic groups with $pK_a \approx 11.5$ and shows little buffering activity at pH < 10. Therefore, little pH fluctuation of the eluent, little change in adsorption strength and rapid re-equilibration can be expected in separations with pH gradient elution. This is advantageous over conventional weak anion exchangers containing a few types of ionic groups with pK_a values of 5–12. Further, owing to the high pK_a value, DEAE-5PW is also applicable to the separation of some basic proteins. The pK_a value of the sulphopropyl group of SP-5PW is *ca.* 2.3. Owing to this low pK_a value SP-5PW can be applied not only to neutral and basic proteins but also to some acidic proteins. This is advantageous compared with carboxymethyl-type cation exchangers such as CM-5PW. Although both SP-5PW and CM-5PW are cation exchangers their selectivities differ.

Phenyl-5PW RP and octadecyl-4PW are packings for RPC [7,8]. The former is suitable for large molecules having molecular weights above 10 000 and the latter for smaller molecules with molecular weights less than 10 000.

Phenyl-5PW and ether-5PW are packings for HIC [9,10]. The hydrophobicities of these packings are adjusted so that proteins are adsorbed on them in the presence of 1–2 *M* antichaotropic ions such as sulphate and phosphate, whereas the proteins are not adsorbed on them in the absence of the ions. Consequently, proteins can be separated under mild elution conditions such as gradient elution with decreasing ammonium sulphate concentration from 1–2 to 0 *M* in common buffer solutions and are usually recovered in their native state. Phenyl-5PW is more hydrophobic than ether-5PW.

Chelate-5PW, heparin-5PW, blue-5PW, boronate-5PW, ABA-5PW and tresyl-5PW are packings for AFC [11–13]. These packings, except tresyl-5PW, are for group-specific AFC separations of samples such as proteins having affinity to heavy metals, coagulation factors, NAD-dependent enzymes, compounds containing carbohydrate and serine proteases. Tresyl-5PW is an activated packing. Many kinds of ligands can be coupled easily under mild conditions via amino, thiol, phenol or imidazole groups. Even large ligands such as antibodies can be coupled in high yields. Although a slightly alkaline pH, 7.5–8.5, is optimum for the coupling of ligands, there is no problem owing to the high chemical stability of tresyl-5PW. In addition, the non-specific adsorption of proteins on these packings is minimal.

3. APPLICATIONS OF TSKgel PW SERIES TO THE SEPARATION OF BIOPOLYMERS

SEC on PW has been applied to various biopolymers. It is particularly useful for polysaccharides because column systems such as GMPW, G6000PW + G3000PW and G5000PW + G3000PW have wide separation ranges and almost linear molecular weight calibration graphs. All non-ionic, anionic and cationic polysaccharides have been separated successfully [14,16]. Derivatives of polysaccharides have also been separated well [14,16]. Oligosaccharides have been separated with high resolution by using PW of smal pore-size such as G2000PW and oligo-PW [15]. It is also possible to separate proteins on PW [4]. SEC on large pore-size PW such as G5000PW and G6000PW has been employed to separate large proteins such as (very) low-density lipoprotein [17], gelatin [14] and sea-worm chlorocruorin [18]. However, SEC on PW has not been applied so often to the separation of common proteins because the resolution on PW is generally lower than that on silica-based packings [19]. Peptides have been separated on PW according to the molecular size with little adsorption by using a special eluent, 0.1% trifluoroacetic acid (TFA) containing 36–45% acetonitrile [15,20]. When peptides are separated in common buffer solutions, some of them elute late by hydrophobic adsorption. Separations of nucleic acids such as DNA fragments, RNA and oligonucleotides have also been reported [21–23]. DNA-PW is a special packing for separating large DNA fragments of 1000–7000 base pairs.

Many proteins have been separated by IEC on DEAE-5PW, SP-5PW and CM-5PW. It has been reported that the resolution is almost comparable to that in gel electrophoresis when the elution conditions are properly selected [24]. Even large proteins such as β-amylase (molecular weight 197 000), catalase (240 000) and fibrinogen (330 000) have been separated with high resolution [5,6]. Separations of basic proteins such as α-chymotrypsin and trypsinogen on DEAE-5PW and separations of acidic proteins such as lipoxidase and β-amylase on SP-5PW have also been reported [5,6]. Further, it has been shown that membrane proteins can be separated successfully [25]. In addition, these ion exchangers are suitable for large-scale separations because they can be regenerated easily by washing with 0.1–0.5 *M* sodium hydroxide solution [26,27]. Large particles 20–30 μm in diameter have been employed in large-scale separations. Separations of peptides have also been reported [6]. It is necessary to include an organic solvent, *e.g.*, 20–40% acetonitrile, in the eluent in the separation of peptides. Some peptides are adsorbed on the packings by hydrophobic interaction in the absence of the organic solvent. IEC on DEAE-5PW has also been applied to the separation of nucleic acids such as RNA and DNA fragments [5,28].

RPC on phenyl-5PW RP and octadecyl-4PW has been applied to proteins and peptides. Phenyl-5PW RP and octadecyl-4PW have been employed mainly for the separation of proteins and peptides, respectively. High resolutions have been obtained not only for common proteins and peptides but also for large proteins such as lactate dehydrogenase (molecular weight 120 000), phosphoglucose isomerase (120 000) and collagen (300 000) [7]. Further, proteins have been recovered in high yield and recoveries as high as 80–90% have been attained even for ovalbumin [7]. Although ghost peaks often appear in successive runs in RPC of proteins, complete elimination of the ghost peaks is easy with phenyl-5PW owing to its chemical stability. Ghost peaks can be eliminated by washing the column with 0.2 *M* sodium hydroxide–acetonitrile (40:60, v/v). The washing is conducted by injecting 100 μl of the mixture five times

successively in the final eluent just before re-equilibration of the column with the initial eluent. It takes only a few minutes [7].

HIC on phenyl-5PW and ether-5PW has been applied to the separation of many proteins. Proteins have been separated with high resolution and yet without denaturation. The recovery of enzymatic activity was high, more than 80% in many instances [9,10,29–31]. When proteins are separated on phenyl-5PW and ether-5PW under the same conditions, they are eluted earlier on the latter because it is less hydrophobic. Because these two packings have different selectivities in the separation of proteins, ether-5PW provides a higher resolution for some samples and phenyl-5PW for others. On average, however, slightly higher resolutions are obtained on phenyl-5PW, because proteins are eluted in a slightly wider range of elution volume when they are separated on phenyl-5PW, although the peak widths are similar on both packings [10]. In separations on phenyl-5PW under typical elution conditions, however, some hydrophobic proteins are eluted late as broad peaks and yet in low yield, and some unstable proteins are partially denatured during separation. On the other hand, a wide range of proteins, including hydrophobic and unstable types, can be separated successfully without the above-mentioned problems on ether-5PW [10]. HIC on phenyl-5PW and ether-5PW are useful for large-scale separations because these packings are chemically stable and can be regenerated easily by washing with 0.1–0.5 *M* sodium hydroxide solution [30,31]. HIC on phenyl-5PW can also be applied to RNA [9]. However, it cannot be applied to DNA fragments because they are not adsorbed on phenyl-5PW even in the presence of ammonium sulphate at a concentration of 2–2.5 *M*.

AFC on chelate-5PW, heparin-5PW, blue-5PW, boronate-5PW and ABA-5PW has been applied to the separation of proteins which interact specifically with these packings. AFC on these packings is useful not only for group separations between two classes of proteins with and without affinity for the packings but also for separations among proteins having affinity for the packings [11,12]. AFC on chelate-5PW, heparin-5PW and ABA-5PW has a resolution comparable to that of IEC, RPC or HIC, whereas AFC on blue-5PW and boronate-5PW has a lower resolution because proteins are eluted as broad peaks. AFC on chelate-5PW has also been applied to the separation of peptides. Peptides containing histidine, tryptophan and cysteine have affinity for chelate-5PW loaded with heavy metals and can be separated with high resolution [32,33]. AFC on tresyl-5PW coupled with various ligands has also been reported [13].

4. CONCLUSION

TSKgel PW series including SEC packings and their derivatives are macroporous synthetic hydrophilic resin-based packings and are useful for the separation of a wide range of biopolymers such as proteins, peptides, DNA fragments, RNAs, oligonucleotides, polysaccharides and oligosaccharides. The base material is sufficiently hydrophilic so that non-specific adsorption of the biopolymers is usually slight. They are chemically stable and can be operated over a wide pH range and at high temperatures. They are also mechanically stable and small particles, *e.g.*, 10 μm in diameter, can be used without any problems. They hardly swell or shrink and therefore are easy to handle. Several grades of different pore sizes are available for SEC, which

can be applied to various samples, with small to large molecules. Further, the derivatives are based on G5000PW with a large pore size and consequently they can be applied to large molecules. Thus, the TSKgel PW series have many advantageous features and have been employed widely not only for analytical- but also for large-scale separations.

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