Sephadex® LH-60

Data File

Chromatography in organic solvents

Sephadex® LH-60 is a versatile separation medium for fractionating lipids and lipid-soluble substances in organic solvents. Its good chemical and physical stabilities have given it a well-deserved reputation for reliability. Sephadex LH-60 has found wide use in the fractionation of lipids, steroids, fatty acids, hormones, vitamins and other small biomolecules. More recent applications have focused on the purification of lipids and lipid-soluble substances of clinical interest.

The key features of Sephadex LH-60 include:

- Fractionates a wide range of lipids and lipid-soluble substances
- Recent applications of clinical interest
- Robust chemical and physical properties
- Separates by gel filtration (size exclusion chromatography), adsorption chromatography or normal phase partition chromatography

Sephadex LH-60 can be used at analytical, preparative or larger scales. In the latter case, it is suitable for either initial purification, e.g. prior to polishing, or for final polishing.

Its companion product, Sephadex LH-20, which fractionates natural products such as steroids, terpenoids, lipids and low molecular weight substances such as peptides of 2 to 35 amino acids, is described in a separate Data File available on request.

Description

Sephadex LH-60 is prepared by the hydroxypropylation of Sephadex G-50, a stable, bead-formed dextran-based medium. The hydroxypropyl groups are attached by ether linkages to the glucose units of the dextran chains in the parent matrix. This method of introducing hydroxypropyl groups does not alter the number of hydroxyl groups on the matrix but does increase the content of alkyl carbon atoms and thus the lipophilic nature of the gel. The resultant medium has, therefore, both hydrophilic and

Fig. 1. Sephadex® LH-60 is a versatile separation medium for fractionating lipids and lipid-soluble substances in organic solvents. Its wide range of applications includes several of clinical interest.

lipophilic properties. As the matrix still contains a number of hydroxyl groups, the polarity of the substances to be fractionated will play an important role in their separation.

Figure 2 shows the partial structure of Sephadex LH-60.

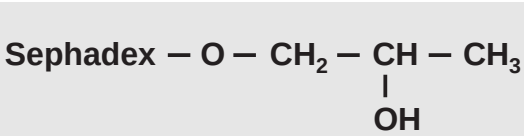

Fig. 2. Partial structure of Sephadex LH-60.

Table 1 summarizes the general physico-chemical and chromatographic properties of Sephadex LH-60.

Sephadex LH-60, which is supplied as a dry powder with a bead diameter of 40–120 µm, swells in polar organic solvents, aqueous solvent mixtures and mixtures of polar and non-polar solvents. Both the wet particle size and the exclusion limit for the gel vary depending on the solvent used for swelling.

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Table 1. General physico-chemical properties and chromatographic performance characteristics of Sephadex LH-60.

Matrix	Hydroxypropylated, cross-linked dextran
Bead form	Spherical, porous
Particle size distribution (dry)	At least 85% of particles have diameters between 40–120 µm
pH stability	
operational	2–11
cleaning	2–13
Chemical stability	Stable in most aqueous and organic eluent systems. Not stable below pH 2 or to strong oxidising agents.
Autoclavable	20 min at 121 °C
Exclusion limit	See "Separation mechanisms"
Operating temperature	4 to 40°C
Delivery conditions	Dry free-running powder
Recommended storage	Maintain dry at +4 °C to +25°C, wet at +4 °C to +8 °C, pH 6-8, with bacteriostat

In mixtures containing both polar and non-polar solvents, the gel takes up predominantly the polar component. Non-polar solvents are therefore of limited interest if used on their own.

Table 2 shows the bed volumes obtained on swelling one gram of dry Sephadex LH-60 in various solvents. Solvents giving a bed volume of less than about 2.5 ml/g dry gel are generally not useful.

Separation mechanisms

Sephadex LH-60 separates by gel filtration (size exclusion chromatography), adsorption chromatography or normal phase partition chromatography.

So long as only size exclusion chromatography effects occur, gel filtration will cause molecules to elute from columns in order of decreasing molecular size. The central part of the sigmoid relation between the distribution coefficient and log Mr can be approximated as a straight line. Sephadex LH-60 gives good fractionation of polyethylene glycols in the molecular weight range 400–10,000 daltons in both methanol and 0.05 M NaCl. In organic solvents, this relationship may deviate from linearity, but true gel filtration is still possible. The fractionation range of polystyrenes in chloroform using Sephadex LH-60 extends up to a molecular weight of 20,000.

The gel-solute interactions of Sephadex LH-60 also enable separation by adsorption chromatography. Because it has a lower matrix content than Sephadex LH-20, the gel is an excellent alternative for separating more strongly adsorbed, lipid-soluble substances.

Table 2. Approximate bed volumes of Sephadex LH-60.

Solvent	Approx. bed vol. ml/g dry gel
Dimethyl sulphoxide	13.4–13.8
Pyridine	13.4–13.8
Dimethylformamide	12.9–13.3
Water	12.4–12.8
Chloroform[1]	12.3–12.6
Ethanol[2]	12.0–12.3
Methanol	11.9–12.3
Ethylene dichloride	11.0–11.3
Propanol	11.0–11.3
Methylene dichloride	11.0–11.3
Butanol	11.0–11.3
Isobutanol	10.8–11.1
Isopropanol	10.0–10.3
Dioxane	9.8–10.1
Tetrahydrofuran	9.6– 9.9
Formamide	8.6– 8.9
Acetone	5.5– 5.8
Ethyl acetate	3.0– 3.2
Acetonitrile	3.1– 3.3
Benzene	2.4– 2.6
Carbon tetrachloride	1.9– 2.1

1 Containing 1% ethanol.
2 Containing 1% benzene.

In addition, Sephadex LH-60 is very suitable for partition chromatography using the "straight phase" technique. It reconstitutes in a wide variety of polar solvents to give a gel with a large, stable stationary phase.

No absolute rules can be given to predict which mechanism will apply. The relative importances of these mechanisms in governing the separation depends on the solvent system chosen and the chemical nature of the substances to be separated, as the following examples show.

An extremely hydrophobic membrane protein (Mr 17,000) was clearly separated on the basis of its molecular weight (gel filtration) rather than partition when run in a mixed solvent system of butanol-methanol-ammonium acetate (1). However, partition effects were evident when polyprenoid menaquinones from *E. coli* were resolved using the mixed solvent system isooctane-methanol-chloroform (2). Adsorption was attributed, at least in part, to the successful purification of a synthetic heptacosapeptide using elution with dimethylformamide (3). This peptide was extensively substituted with aromatic benzyl and benzyloxycarbonyl protecting groups.

More recent applications of Sephadex LH-60 are described later.

Experimental technique

Sephadex LH-60 should be used with columns designed for use in organic solvents. We recommend the Amersham Biosciences SR (solvent resistant) column series. The materials used in these columns have been selected to give trouble-free operation and long term reliability in organic solvents. Each column is supplied with two flow adaptors as standard. A full range of solvent resistant accessories is also available. (See Ordering information for more details). Columns from the XK range will also give excellent results with certain organic solvents.

Columns packed with Sephadex LH-60 run on low to medium pressure chromatography systems such as GradiFrac™ as well as on high performance systems like FPLC®.

Further information can be found in the Amersham Biosciences general products catalogue.

Applications

Recent additions to the extensive list of applications for Sephadex LH-60 include several of biomedical/clinical interest.

Pulmonary surfactant

The investigation of pulmonary surfactant, a complex lipid-protein mixture that lowers surface tension at the air-water interface of the lung is one such example.

Surfactant preparations containing the hydrophobic surfactant proteins SP-B and SP-C have been administered endotracheally to compensate for surfactant deficiency in babies with threatened or manifest respiratory distress syndrome (RDS). The efficacy of this surfactant therapy may depend on the relative proportions of SP-B and SP-C in the preparations. Determining the quantities of these proteins is thus important for assessing the quality of surfactant preparations.

However, due to their very hydrophobic nature and their association with lipids, which comprise approximately 90% of pulmonary surfactant, SP-B and SP-C are very hard to purify and quantify. A simple method to successfully separate and purify SP-B and SP-C using Sephadex LH-60 has been reported.

Both hydrophobic proteins have been characterised following separation on a Sephadex LH-60 column (2.5 × 80 cm) eluted with chloroform/methanol 1:1 (v/v) containing a 5% 0.1 M HCl solution (6, 4). Analysis of the fractions from the chromatogram (Fig. 3) showed that the first two peaks contained SP-B and SP-C respectively and that the last peak contained phospholipids.

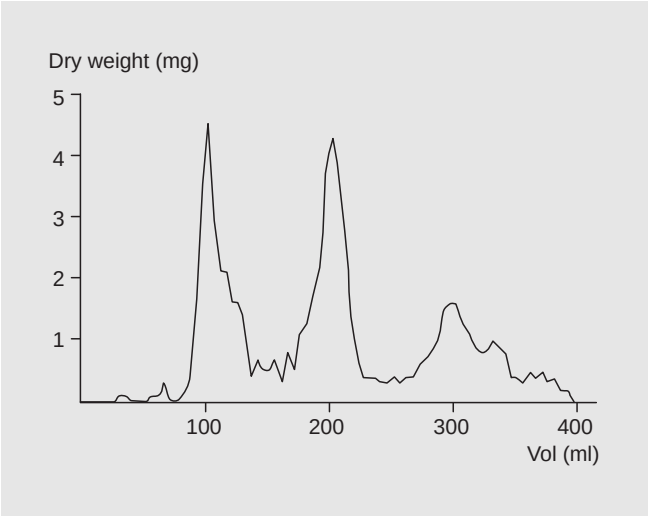

Fig. 3. Elution pattern of a crude extract of hydrophobic surfactant protein on an 80×2.5 cm column of Sephadex LH-60. The first two peaks contained proteins and the last peak phospholipids. (Reproduced with permission.)

Recently it was reported that the amounts of SP-B and SP-C in surfactant preparations can be rapidly quantified on Sephadex LH-60 using 30% dichloromethane, 65% methanol and a 5% 0.1 M HCl solution as a solvent system (5). Figure 4 shows a chromatogram from this work. The 20 cm XK column was run on FPLC System.

Membrane-derived macrophage-activating factor

The potential of *in situ* macrophage activation immunotherapy in combating metastasis is at present limited by the absence of suitable activating agents. With potential therapeutic applications in mind, a highly hydrophobic component derived from the membrane of *Mycoplasma capricolum* was characterised, purified and assessed for its ability to activate macrophages to tumor cytotoxicity (7).

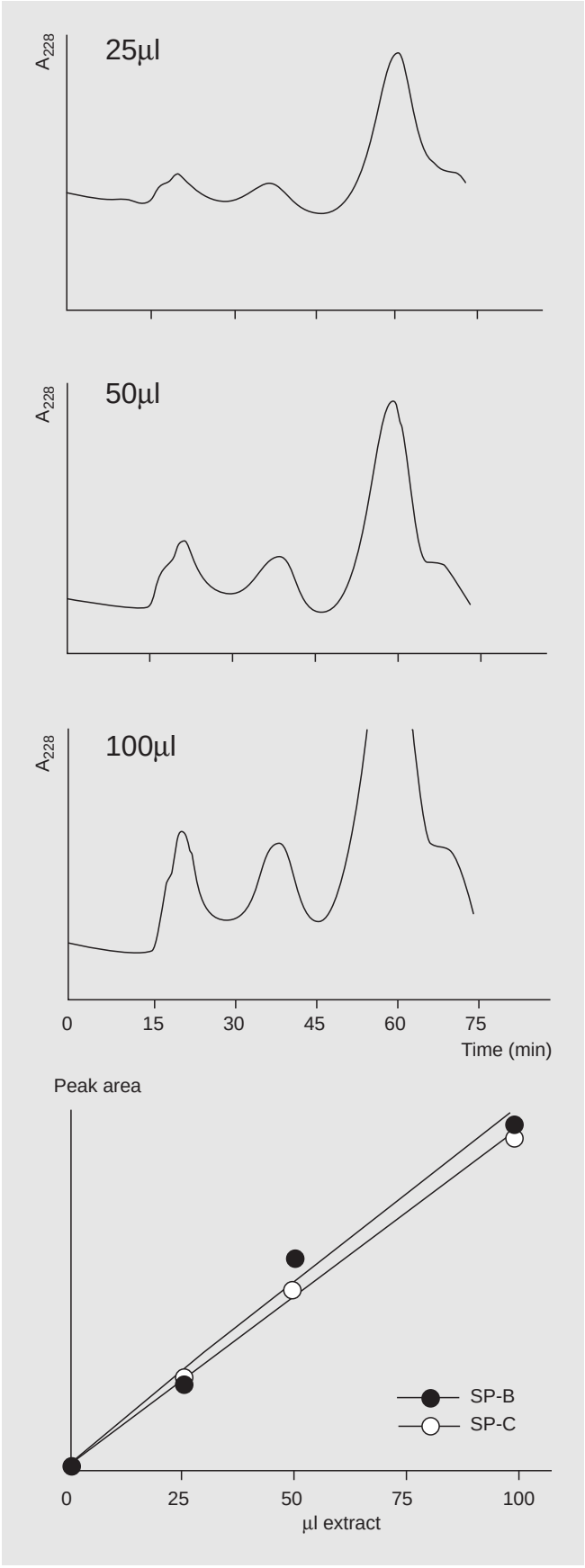

Fig. 4. Analysis of SP-B and SP-C in surfactant extract. Different amounts of butanol-extracted surfactant were applied to the Sephadex LH-60 column. (Reproduced with permission.)

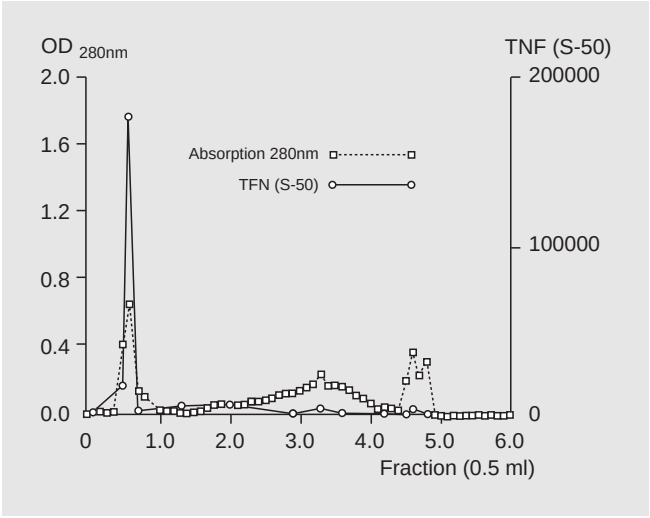

Fig. 5. Separation of trypsin-treated membranes on Sephadex LH-60. Fractions were subsequently monitored for macrophage-activating ability. (Reproduced with permission.)

Trypsin treated extracted membranes solubilized in 2-chloroethanol were fractionated by gel filtration on a column of Sephadex LH-60. Figure 5 shows the result. The active component(s) from the sharp peak had a 10-fold increase in specific activity when assayed for macrophage-activating ability at a range of concentrations. Further studies into the chemical and physical nature of the membrane-derived macrophage-activating factor may help facilitate future applications as a clinical immunomodulator.

Antimicrobial activity of pine cone extracts

Pine cone extracts of *Pinus parviflora* Sieb. et Zucc. show antitumor, antiviral and antimicrobial activity. As the bioactivity of extracted substances fractionated using previous procedures overlaps several fractions, a more clear-cut fractionation procedure was attempted with Sephadex LH-60.

One of the most potent alkaline extracts (PCna4-ppt) was further fractionated on a 3×46 cm column of Sephadex LH-60 equilibrated with 50% ethanol (8). The activity recovered from the column was almost exclusively found in the sub-fraction with the highest molecular weight and a relatively high sugar content. Since many lignin-related species have potent antimicrobial activity and many molecular species of lignin are available, further isolation and identification of the factors responsible is considered worthwhile.

References

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Ordering information

Product	Quantity	Code No.
Sephadex LH-60	150 g	17-0890-04
Sephadex LH-60	1 kg	17-0890-08

Related product

Product	Quantity	Code No.
Sephadex LH-20	100 g	17-0090-01
Sephadex LH-20	500 g	17-0090-02
Sephadex LH-20	5 kg	17-0090-03

Recommended columns

Product	Code No.
SR 10/50	19-2638-01
SR 10/50J*	19-1734-01
SR 25/45	19-0879-01
SR 25/100	19-0880-01

All SR Columns are fitted with two adaptors.
* Includes borosilicate glass jacket.

SR Column accessories are described in the Amersham Biosciences general products catalogue.

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