CHROM. 4863

A SYSTEM FOR PREPARATIVE SEPARATION OF A WIDE RANGE OF SMALL MOLECULES BY GEL PERMEATION CHROMATOGRAPHY IN ORGANIC LIQUIDS

J. L. MULDER AND F. A. BUYTENHUYS
AKZO Research Laboratories, Arnhem (The Netherlands)
(Received June 1st, 1970)

SUMMARY

This paper mainly deals with the description of the apparatus used; only a few examples of separations are given. More applications of the technique will be fully described in subsequent papers.

The procedures are applicable to high boiling compounds in the molecular weight range 100–1500.

For preparative purposes effective separations can be obtained with 30–100 mg amounts of mixture, using a single glass column, or at most a combination of 2–3 columns of 120 cm length and about 15 mm internal diameter, packed with gels of the Bio-Beads or Sephadex LH-20 type in organic liquids.

Low sensitivities of the $8–32 \cdot 10^{-5}$ R.I.U. full scale deflection of the detector, a differential refractometer, are normally used, the latter being thermostatically operated at 2° below room temperature.

Plate numbers of single columns are 3000–6000, HETP values being 0.4–0.2 mm at flow rates of 25–30 ml/h and *o*-dichlorobenzene as a test substance.

INTRODUCTION

Up to now only a relatively small amount of work has been reported in the literature on gel permeation chromatography (GPC) dealing with separations of low molecular weight substances in organic liquids. However, with the increasing interest during the last two years the literature on this matter has been growing steadily.

Apart from the basic literature on this subject[1–6], important studies about the elution behaviour of model compounds on polystyrene gels have been published by HENDRICKSON[6–8], EDWARDS AND NG[9], MOORE AND HENDRICKSON[10,11], CHANG[12,13], and by JOUSTRA *et al.*[14] and KRANZ[15] for alkylated dextran gels. Factors affecting the GPC of small molecules, mainly solvent–solute interactions caused by hydrogen bonding, are described by HENDRICKSON[16], CAZES AND GASKILL[17,18] and LARSEN[19]; adsorption effects of aromatic compounds on alkylated dextran gels are mentioned by WILK *et al.*[20], MAIR *et al.*[21], JANSON[22] and BROOK AND HOUSLEY[23].

Results obtained from separations on lipophilic dextran gels with different

methoxyl content are described by SJÖVAL AND NYSTRÖM[5]. These authors have also used complex solvent mixtures to obtain separations in the field of lipid analysis. ALTGELT[24] states that a small addition of a strong polar liquid to a less polar elution solvent decreases the adsorption effects. Using 2-propanol as mobile phase for GPC on Sephadex LH-20, OELERT[25] found that the elution order of aromatic compounds is given more by polarity than by the shape of the molecules. For applications of the technique to natural and commercial products, interesting information can also be obtained from the literature.

Separations of petroleum hydrocarbons on Sephadex LH-20 have been described by TALARICO *et al.*[26] and MAIR *et al.*[21]; separation of crude oil fractions on polystyrene gels is mentioned by COLEMAN *et al.*[27]. BOMBAUGH and coworkers[28–33] have described many separations in the field of hydrocarbons, alcohols, glycols, triglycerides, steroids, surfactants and ethylene oxide derivatives on polystyrene gels. SCHRÖDER AND MISCHE[33] give procedures for the separation of adipic plasticizers on lipophilic polydextran gels, while HAGEN AND SCHRÖDER[34] use polydextran as well as polystyrene gels for the fractionation of phenolic resins. The analysis of tall oil, using polydextran and polystyrene gels, is described by CHANG[35]. Other gel materials, *e.g.* isocyanate modified polydextran, cross-linked polymethyl- or polybutylmethacrylates and cross-linked polyvinylacetate have been used by HEITZ *et al.*[36] for the separation of oligomeric phenylenes and urethanes. The separation on a preparative scale of low molecular weight polystyrenes, polybutylmethacrylates, and polyethylene glycols on polystyrene gels is described by HEITZ AND ULLNER[37]; the optimal conditions and the limitations of GPC to the same oligomeric materials are given by HEITZ *et al.*[38].

Cyclo-oligomers from Nylon 6.6, 6.10 and 11 have been separated by ZAHN AND KUSCH[39] on a hydrophilic polydextran in acetic acid–water. These authors also separated oligomeric cyclo-glycolterephthalates on polystyrene and polydextran gels.

The papers cited above all deal with GPC in organic solvents; application of the technique in water or buffer solutions is mainly restricted to biological and biochemical analysis, using hydrophilic polydextran and polyacrylamide gels. Some examples of separation of low molecular weight commercial substances in aqueous solution are given by FEIST *et al.*[40], who separated polyethylene glycols on Sephadex G-50; the analysis of surfactants on Sephadex G-50 and G-75 is described by TOKIWA *et al.*[41–43].

Chromatography on Sephadex G-10, using two different eluants for the separation of an anionic detergent and an anionic non-detergent, combined with ion-exchange, is described by MUTTER[44].

In most cases cited above use is made of commercially available apparatus for liquid chromatography normally equipped with stainless steel columns, 4 ft. in length and 3/8 in. I.D., packed with polystyrene gels. For analytical purposes only a few mg of mixture can be separated with such an apparatus, using combinations of 2–5, sometimes as much as 10 and 40 columns, in series[28,29].

The use of glass columns is mainly coupled with the introduction of polydextran gels. Here, columns vary from 50–500 cm in length and from 16–50 mm in diameter; usually no exact description of the columns and other apparatus used is given. As far as we know, no commercial apparatus is used in combination with Sephadex gels. In addition the application of the technique using polydextran gels is mainly restricted to analytical separations. Preparative separations on a gram scale, using glass columns

of 200 cm length and 50 mm inside diameter, are described by HEITZ and coworkers[37,38].

Our purpose was to apply GPC to qualitative and quantitative analysis of mixtures from different origins. Generally, we only possess limited information about mixtures to be separated, based on an IR spectrum, a thin-layer chromatogram or/and the purpose for which such a mixture is or can be used.

For both qualitative and quantitative analysis, we need separation procedures, which have to be carried out on a larger scale than is possible with a commercial analytical GPC apparatus; furthermore, these apparatus are not flexible enough for our purpose.

As we are dependent on the solubility of the samples in organic liquids, and as in principle GPC is a simple technique[1,2], we thought an experimental apparatus, in which some types of different gels are used, each swollen in two or three solvents, to be the best suited to our analysis, *i.e.* separations on a 30–100 mg scale, which amount allows identification of the sample by means of IR and NMR, as well as quantitative gravimetric determination. Although this work has not yet been concluded, we thought it of interest to publish a description of the apparatus at this stage. In addition, some examples of separations are given.

CEL MATERIALS AND APPARATUS DETAILS

Gel materials

The choice of the gel is somewhat difficult, as the behaviour of polystyrene and polydextran gels is quite different. Association effects may occur in both cases. However, as the polydextran gel Sephadex LH-20 is not fully alkylated and possesses a certain number of hydroxyl groups, polarity of the substances to be separated plays an important role here. The effect may be diminished by the correct choice of the eluting solvent. An additional point is the adsorption of aromatics to polydextran gels. These phenomena may seem to be troublesome, but it is our experience that they can easily be overcome, and that it is sometimes even possible to exploit these properties and to obtain separation in cases where it was not expected.

A survey of commercially available gels, which may be used for the separation of small molecules, is given in Table I. For the sake of completeness some gels for use in aqueous solution are also mentioned here.

We have very good results with gels of the Bio-Beads and Sephadex LH-20 type. Comparative tests between the polystyrene gels of the Bio-Beads and Poragel type with approximately the same molecular weight exclusion limits showed that better results were obtained with the Bio-Beads gels; to obtain about the same separation we needed at least two Poragel columns, while with Bio-Beads only one column of the same dimensions was needed. It must, however, be mentioned, that the Poragels have a more rigid structure, which would probably allow the use of rather high pressures and facilitate the coupling of more columns in series.

Recently, Merckogel OR has been marketed. It is a gel based on cross-linked polyvinylacetate. According to the data from the manufacturer, the properties of this gel will probably lie between those of the polystyrene and the alkylated polydextran gels.

TABLE I

GEL MATERIALS FOR SEPARATION OF SMALL MOLECULES

Name	*Type*	*Swelling agent*[a]	*Mol. wt. exclusion limit*	*Manufacturer*
Sephadex LH-20	alk. polydextran	org.	5000	Pharmacia
Sephadex G-10	polydextran	H_2O	700	Pharmacia
Sephadex G-15	polydextran	H_2O	1500	Pharmacia
Sephadex G-25	polydextran	H_2O	5000	Pharmacia
Bio-Beads SX-1	polystyrene	org.	3500	Bio-Rad Laboratories
Bio-Beads SX-2	polystyrene	org.	2700	Bio-Rad Laboratories
Bio-Beads SX-3	polystyrene	org.	2000	Bio-Rad Laboratories
Bio-Beads SX-4	polystyrene	org.	1400	Bio-Rad Laboratories
Bio-Beads SX-8	polystyrene	org.	1000	Bio-Rad Laboratories
Bio-Gel P2	polyacrylamide	H_2O	1600	Bio-Rad Laboratories
Bio-Gel P4	polyacrylamide	H_2O	3600	Bio-Rad Laboratories
Bio-Gel P6	polyacrylamide	H_2O	4600	Bio-Rad Laboratories
Poragel A1	polystyrene	org.	1000	Waters Associates
Poragel A3	polystyrene	org.	3000	Wators Associates
Styragel 40 A	polystyrene	org.	1600	Waters Associates
Styragel 100 A	polystyrene	org.	4000	Waters Associates
Merckogel OR 750	polyvinylacetate	org.	750	Merck A.G.
Merckogel OR 1500	polyvinylacetate	org.	1500	Merck A.G.
Merckogel OR 5000	polyvinylacetate	org.	5000	Merck A.G.

[a] Swelling agents, according to the manufacturers, are: Sephadex LH20: DMF, water, alcohols, chloroform, *p*-dioxane, THF, acetone, ethyl acetate, toluene; polystyrene gels: benzene, DMF, chlorinated hydrocarbons, THF, *o*-dichlorobenzene; polyvinylacetate gels: THF, ethyl acetate, benzene, acetone, methanol.

The gels used in our experiments were the polystyrene gels Bio-Beads SX-1, SX-2 and SX-8 from Bio-Rad Laboratories, having exclusion limits of a mol. wt. of 3500, 2700 and 1000 respectively, and the alkylated polydextran gel Sephadex LH-20 from Pharmacia, having an exclusion limit of about 5000.

It should be mentioned that these limits, which are supplied by the manufacturers, have to be interpreted with caution, as it is our experience, that they are generally too high, and that the effective range for separation purposes starts considerably below these values. It should also be mentioned, that polystyrene as well as polydextran gels, having rather high exclusion limits of about 5000, may be successfully used for the separation of compounds in the mol. wt. range of 200–100 and lower.

The polystyrene gels were swollen in benzene and chloroform, the polydextran gel was swollen in methanol, acetone, or chloroform. In all cases, the gels were soaked for 24 h in an excess of solvent with occasional stirring. Before packing the columns, the suspension of gel is evaporated at room temperature for 1 h using a water jet pump.

Swelling agents

Liquids used for preparative GPC have to be absolutely free from nonvolatile matter; we therefore found it necessary to distil all solvents, even "pro analysi"

quality, until no residue could be detected by weighing after evaporation of 100 ml of solvent at 40° under N_2. The distilled solvents are stored in 30-l glass flasks, from which the liquids are pumped to the columns.

Solvents for preparative GPC should, on the one hand, be of low volatility but, on the other hand, it is desirable, that they are also good swelling agents; these two requirements considerably reduce the choice. In early experiments, tetrahydrofuran, the common solvent in GPC, was used. However, in spite of all the precautions to prepare and keep this solvent free from peroxides after removal of the stabilizer and distillation, so much peroxide was formed, where the solvent was exposed to air, *e.g.* in the fraction collector, that identification of the isolated substance was impossible. Therefore, this solvent was not used in further experiments.

Columns and plungers

Using stainless steel columns as in commercial apparatus, it is not possible to observe the gel bed. However, cracks and channels sometimes arise in the gel bed, even in columns which have been in use regularly for half a year and longer, so as we only intended to apply low pressures, it was decided to use glass columns, and so be able to observe the gel bed.

Experiences with commercially available columns were somewhat disappointing, except with the Sephadex SR column length 1 m and 1 in. I.D. which proved to be useful for separations on a 0.5–1 g scale under favourable circumstances. Resolution of such a column, however, is considerably lower than that of longer columns with smaller diameters.

We chose columns of 120 cm length and 14.5 mm I.D., as it appeared from our experiments, that good resolution could be obtained and that the capacity of these columns was amply sufficient for our purposes. As in the case of low-porosity polystyrene and polydextran gels, which are used for the GPC of small molecules, alterations in the packed gel bed are almost negligible. Columns, provided with only one small plunger, were made from Quickfit Visible Flow glass of the above-mentioned dimensions. Tolerances of the inside diameter of this type of glass tubing are narrow, so that the home-made, stainless steel plungers are interchangeable. Maximum pressure for these columns is 3.5 atm.

To avoid wall effects, the column walls are silylated with a 5 % (v/v) solution of dimethyldichlorosilane in chloroform for 1 h at room temperature; the columns are washed with chloroform, and then with synthetic detergent for complete removal of excess reagent and polysiloxanes formed. Column and plunger are shown schematically in Figs. 1a and 1b. Turning the nylon ring of the plunger pushes down the polyethylene ring over the oblique bottom part, and expands the ring firmly against the glass wall, so that it can easily withstand pressures up to 3 atm.

Coupling

For the connection of columns, plungers, and valves with flexible polyethylene tubing, stainless steel capillaries and hypodermic needles of exactly 1.3 mm O.D., about 0.8 mm I.D. and 4 cm length are used, over which a small piece of the said tubing, 2 mm O.D., 1 mm I.D. and 2 cm length is pushed. Capillary and tubing together are now pushed forcefully into the glass capillary at the bottom of the column (which has an O.D. of 9 mm and an I.D. of 1.9–2.0 mm); from this a piece of steel capillary

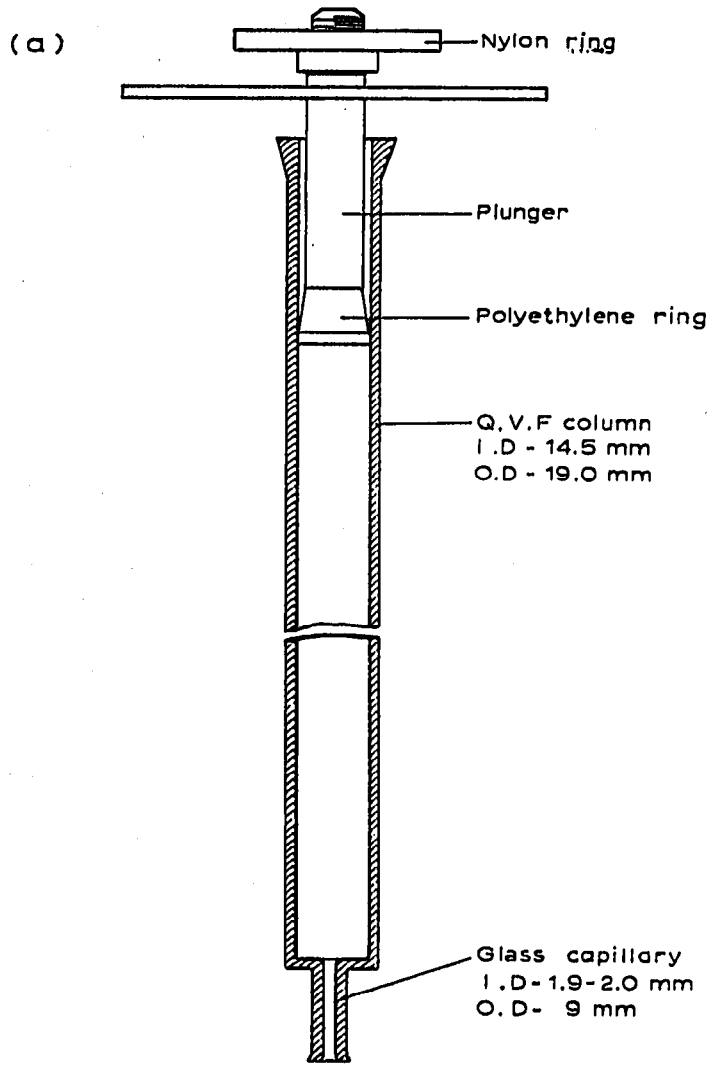


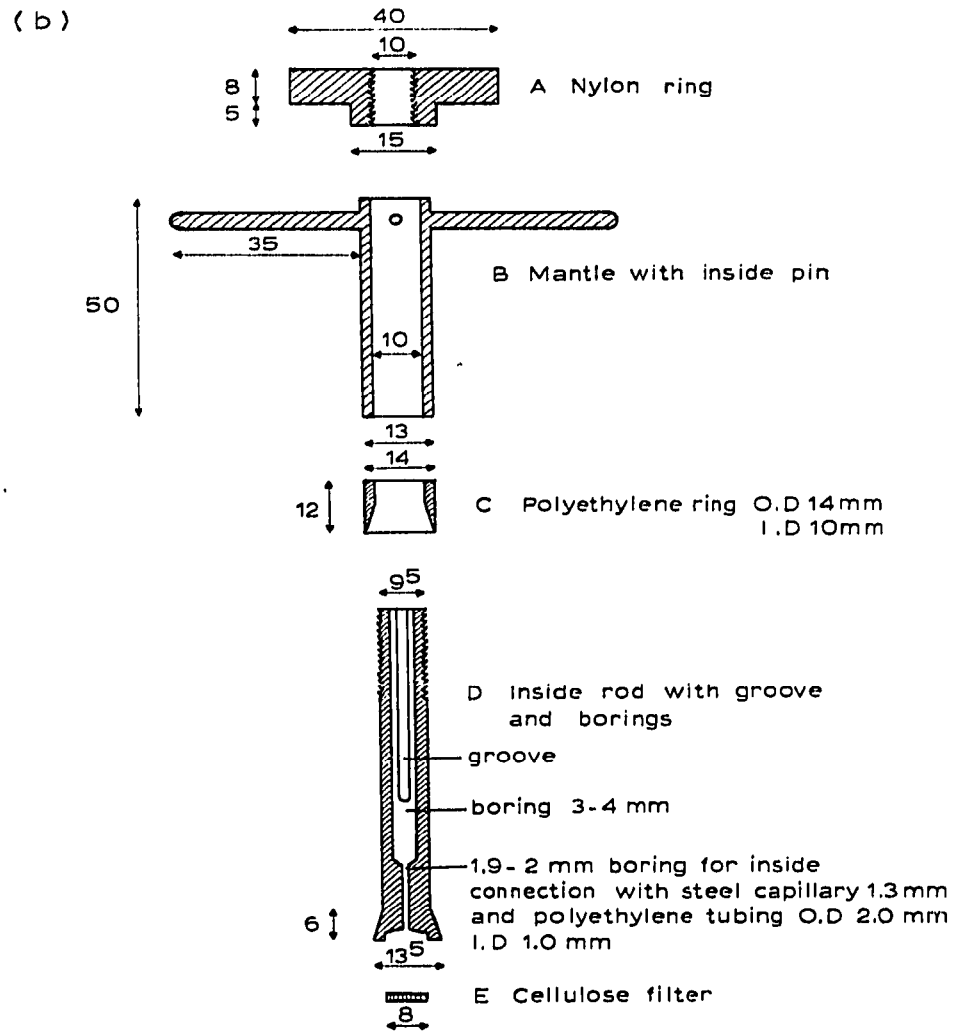
Fig. 1. Cross-section of (a) the chromatographic column and (b) the plunger for 14.5 mm I.D. chromatographic columns.

of about 2 cm, not covered with polyethylene tubing, extends. Over this free end the longer connecting tube is pushed over a length of 1.5–2 cm (Fig. 2a).

In the same way the plunger and its tubing are connected by means of a short piece of steel capillary in the 1.9–2.0 mm bore in the plunger (Fig. 2b). In our case, all columns are provided with Hamilton 3-way valves at the bottom; instead of steel capillaries, short hypodermic needles with Luer connections are used here, the valve being mounted on this (Fig. 2c). These connections, which we have used for years in column adsorption chromatography, have been found to be leak-proof. As with the plunger, pressures up to 3 atm. can easily be withstood here as well.

We prefer polyethylene tubing for flexible connections, as it is easier to handle than Teflon tubing; it is also equally stable against liquids such as benzene and chloroform at room temperature.

Before using the column, a very small wad of glass wool is pushed into the glass capillary above the stainless steel connecting piece, after which about 2 mm of sand



is placed in the sharply necked bottom of the column in order to obtain a real flat insert layer and thus to avoid irregularities in flow at the end of separation.

Column packing

Gel beds have to be carefully packed. Procedures have been described by DETERMANN[1], ALTGELT[24], HEITZ AND ULLNER[37,47], MOORE[45], FLODIN[46] and SIE AND VAN DEN HOED[48]. In our case no special procedures are used, however. In contrast to normal procedures, where a thin gel suspension is used, we used a rather thick slurry, which is poured into the column, in which 10–15 cm of solvent is already present. With the valve fully open at the bottom, the suspension above the settled particles is regularly kept moving by stirring. After the surface of the gel bed has reached the upper part of the column, the plunger is placed in position and liquid pumped down the column at a rate of about 60–80 ml/h until no decrease in volume of the gel bed can be observed. The plunger is now pushed down upon the gel bed, care being taken to avoid the entrance of air, until no dead volume between gel bed and plunger can be observed. Even with liquids such as chloroform and dichloromethane, in which the gels float, no difficulties arise.

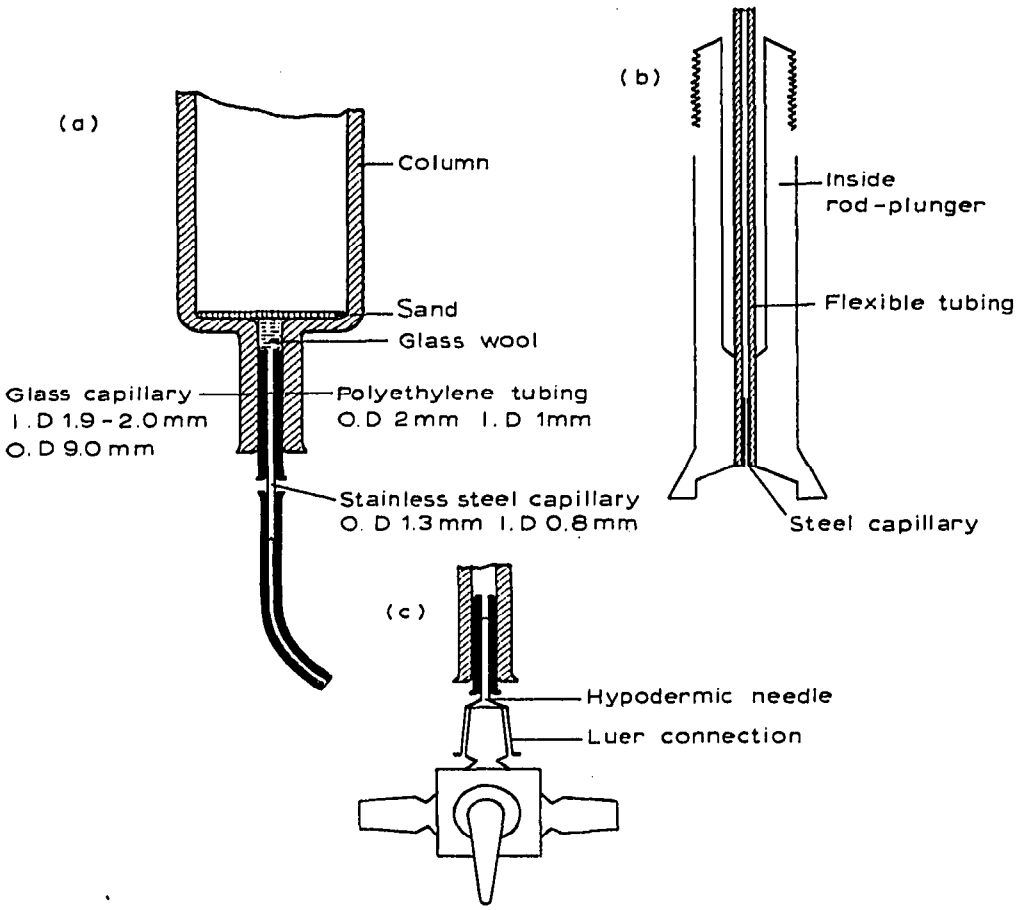


Fig. 2. Connection of (a) column–flexible tubing; (b) plunger–flexible tubing; and (c) column–Hamilton valve.

It appears that with this simple procedure good quality columns can be prepared; our polystyrene columns in benzene usually have plate numbers of 3000–6000, HETP values being 0.4–0.2 mm, obtained with *o*-dichlorobenzene as the test substance at flow rates of 25–30 ml/h. These values are comparable with those given in the literature[9,10,28,29].

The chromatographic system

Packed columns are inserted in the whole system, consisting of storage vessel, pump, pulsation damper, sample application system, thermostat, detectors, recorder, and fraction collector.

Liquid is pumped through the main liquid lines of heavy walled Teflon tubing (5 mm O.D. and 1 mm I.D.). Columns are placed between the main lines, making operation with one or two and three coupled columns easy. All connections between columns and main lines are as described above. A schematic diagram is given in Fig. 3.

Pumping; pulsation damping

The pumps are Hughes plunger type micro pumps with single short stroke mechanisms for each carrier solvent, operating at 20 strokes/min and having a maximum capacity of 185 ml/h.

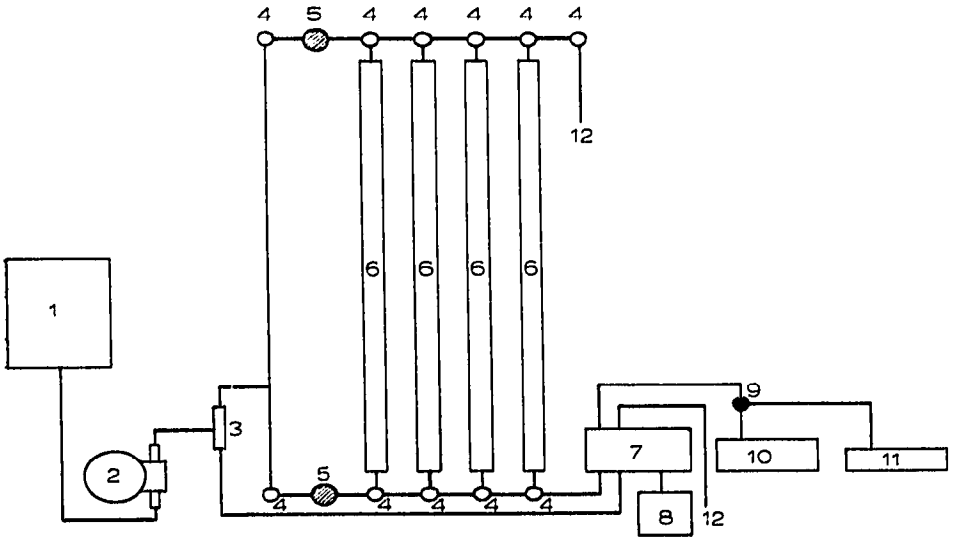


Fig. 3. Survey of apparatus. 1 = solvent reservoirs; 2 = micropump; 3 = pulsation damper; 4 = Hamilton 3-way valves; 5 = Chromatronix sample valves; 6 = columns; 7 = differential refractometer; 8 = recorder; 9 = stream splitter; 10 = fraction collector; 11 = TLC detector; 12 = waste.

It is necessary to damp the pulses of the pumps. This is possible by means of a shunt in the main line, provided with a manometer at the end. This construction is usually found in the commercial apparatus. However, it is also possible to damp pulses with the simple glass apparatus shown in Fig. 4a. It is essential, that the space between the air and liquid is separated by mercury, as in earlier experiments it appeared that, when the liquid was in direct contact with air, the latter was gradually dissolved, even in liquids such as benzene and chloroform, causing air bubbles at the exit of the column, where the pressure was reduced. With this apparatus it is also possible to measure pressures, as the longer and smaller tube acts as a manometer. A modification of this apparatus, now in use, is shown in Fig. 4b. In this case an air trap is also provided; a second improvement is a precision Teflon needle valve, which regulates the flow of the reference stream.

Sample application

Sample application systems in commercial apparatus are of two types. The first is derived from gas chromatographic injection systems, *i.e.* application of the sample with a hypodermic syringe *via* a septum; the second uses sample loops, which can simply be switched into the main liquid line. As the amounts of liquid we normally apply are 0.5–1.5 ml, we chose the latter system. In our apparatus we make use of several Chromatronix sample valves with stainless steel capillary sample loops. These valves have been in use for more than a year and we have never had any difficulties with them.

Formerly, we used a system of two Hamilton 4-way valves, provided with hypodermic needles and flexible Teflon tubing as a sample loop (Figs. 5a and 5b). It is true that a simple method of sample application is possible in this way, however, as Teflon tubing swells considerably in organic liquids, stainless steel capillary sample loops have to be used here. If columns are operated only by the gravity of the eluting

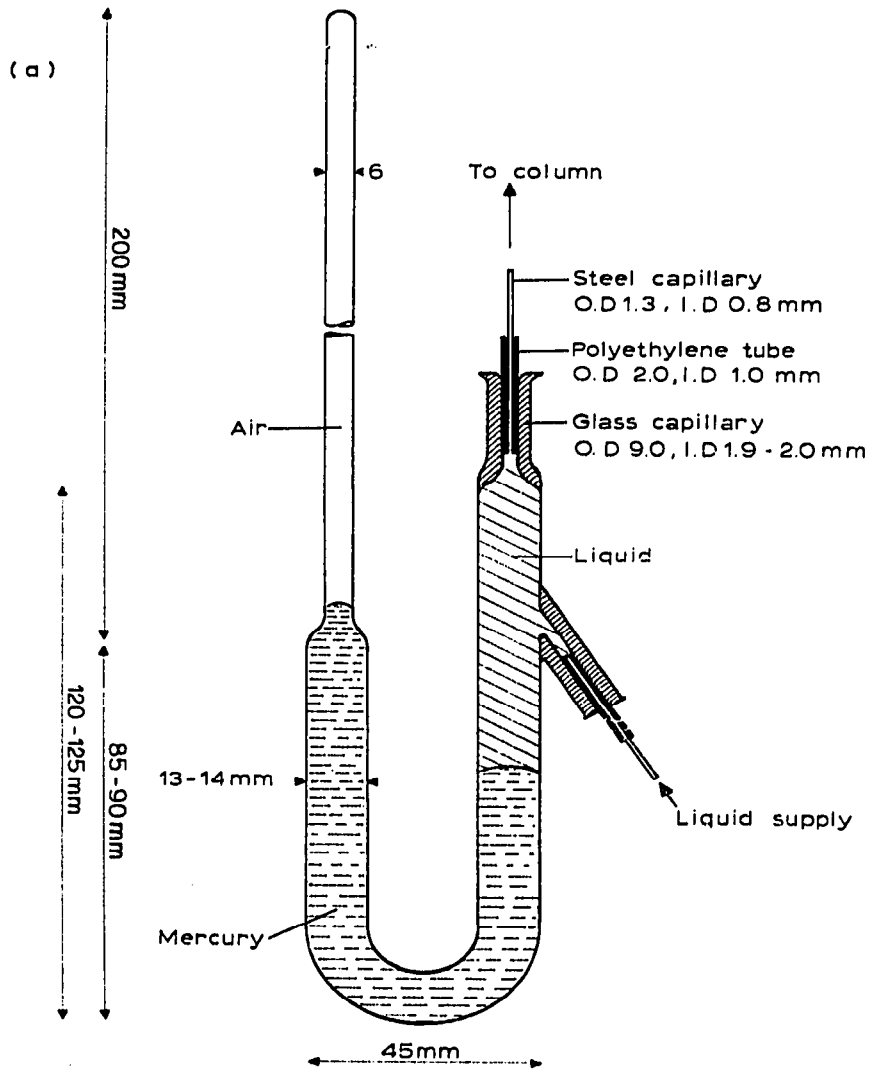


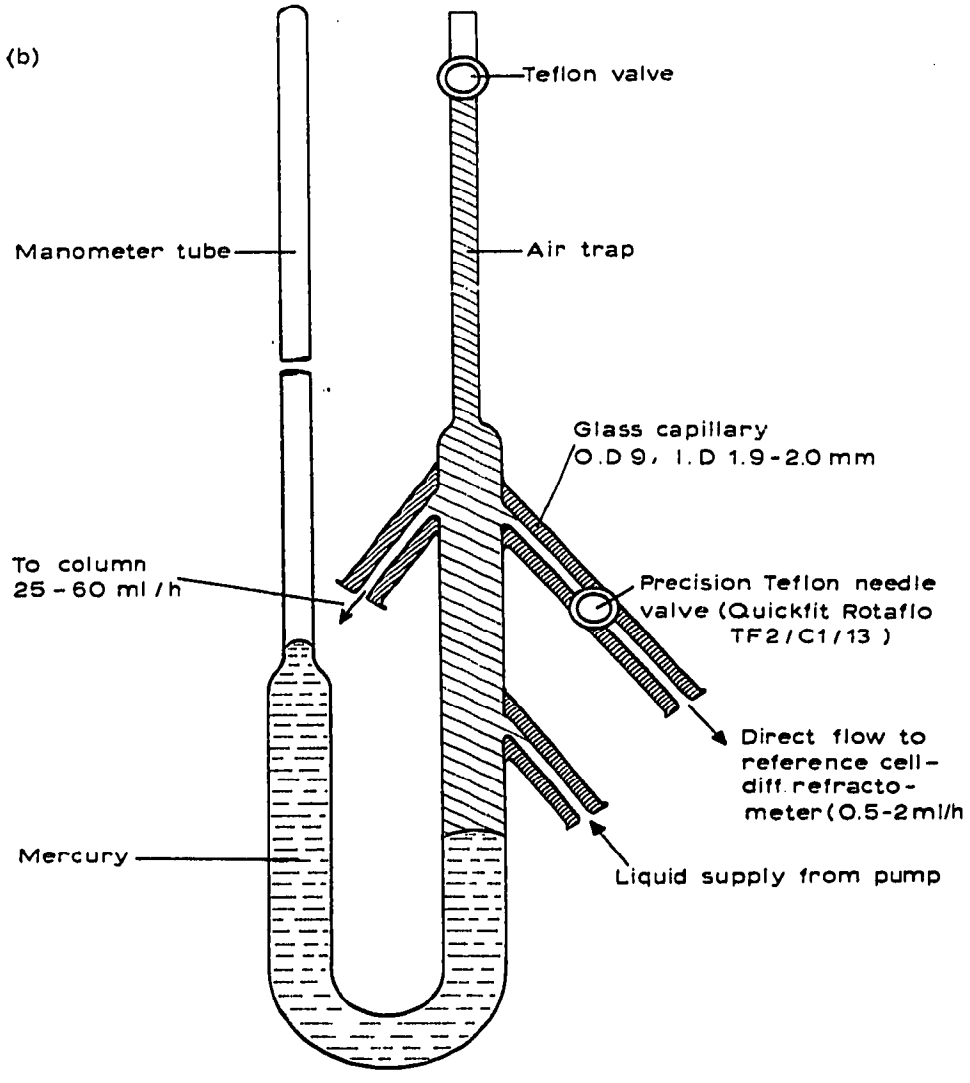
Fig. 4. Cross-section of the pulsation damper (a) and the modified pulsation damper (b).

solvent, a simple method of sample application is the use of a glass 3-way valve and a centrifuge tube, the latter containing the sample (Fig. 5c).

Detection

General methods for detection are flame ionization, heat adsorption, and differential refractometry. The last method was chosen, based upon the fact that this method seemed to be reliable, as it had been used for GPC of polymeric materials for years by several authors. However, flame ionization was also tried, but two types of apparatus, based on solvent transport by means of a chain, proved to be unreliable, and a third type, based on solvent transport upon a wire, had the disadvantage of inhomogeneous solvent application to the wire and also a somewhat irregular transport of the wire itself. We have not investigated heat adsorption detection.

For our purposes we are using three differential refractometers of the type R4 from Waters Associates, equipped with 70-μl cells, which have been in operation almost continuously for one year. Two of them are used in combination with poly-



styrene gels in benzene and chloroform, the third is used for polydextran gels in methanol, chloroform, and acetone.

For the amounts of mixture (30–100 mg) we usually apply for separation, the detectors can be used at the low sensitivities of the $8–32 \cdot 10^{-5}$ R.I.U. full scale deflection of the recorder, whereby the whole effluent passes through the sample cell of the refractometer without splitting. The refractometers operate at $2° \pm 0.01°$ below room temperature with the aid of precision thermostats. It is necessary to keep the temperature of the cells between such close limits, as otherwise no stable base line can be obtained; in addition it is also advisable to work a few degrees below room temperature, as in this way the appearance of gas bubbles in the cells is prevented. Nevertheless, gas bubbles will sometimes arise with the use of such low volatile eluants as chloroform and dichloromethane. In this case it is advisable to place the outlets of the flexible tubing, connected to the outlets of the cells, about 10 cm above the differential refractometer. In this way the appearance of gas bubbles can be fully suppressed. After distillation of the carrier solvents, no further degassing of the liquids

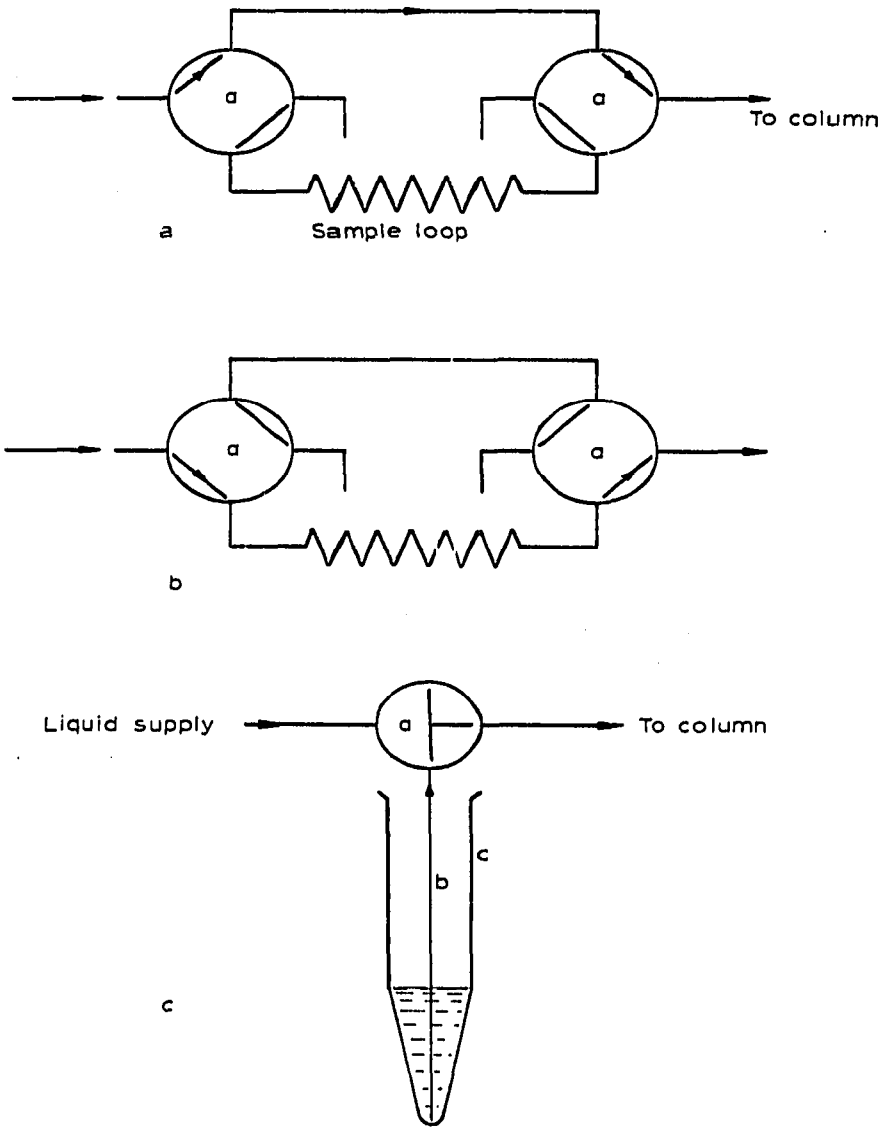


Fig. 5. Sample application using two Hamilton 4-way valves, hypodermic needles and flexible Teflon tubing (a + b); (a) flushing/loading, (b) sample application. (c) Sample application by gravity.

is carried out, the whole separation procedure being carried out at room temperature (21° ± 0.5°).

In contrast with GPC of polymeric materials, the eluant only passes through the columns which are used for separation, no reference column is used. Reference liquid is taken from the apparatus of Fig. 4b and directed to the reference cell at the low flow rates of 0.5–2 ml/h. The flow rate of the eluate itself amounts to 30–60 ml/h. As long as the ratio between the flow rates remains constant, no deflections of the recorder pen can be observed, and a stable baseline is obtained.

Normally, the whole effluent is guided to a time-operated fraction collector, but we split 2–3% from the effluent leaving the differential refractometer off to a new type of chromatographic detector, described by VAN DIJK[49]. The principle of this cheap method of detection is the continuous application of column effluent to a

series of TLC plates. This apparatus runs synchronously with the fraction collector. After development and visualization of the TLC plates, the spots are correlated with the corresponding tubes. Full details are given in the above-mentioned paper.

Flow rates

Flow rates formerly used were 15–25 ml/h. Under these conditions the separation procedures were carried out in 8–15 h, using the columns as described, having a total volume of about 200 ml. LITTLE and coworkers[50], however, have proved, that the elution volume of a chromatographic peak is less dependent upon the flow rate than expected, and it is demonstrated, that at very high flow rates peak widths are not really increased. Though the apparatus used was a modified analytical gel permeation chromatograph, operating at 2200 p.s.i. and 80°, we thought this finding interesting enough to be applied to our procedures. Though our working conditions are totally different, it appeared that our flow rates could be easily increased by at least 2–3 times without considerable increase in elution volume and loss in resolution. Normal procedures are now carried out at flow rates of about 60 ml/h, reducing the separation time to about 3 h. Further increases in flow rate are now being studied; the preliminary results are promising, as we have already been able to separate completely 75 mg of a test mixture containing 7 components in about 45 min, pumping at a rate of 120 ml/h. In this case a column of 120 × 0.9 cm, packed with Bio-Beads SX-2 in benzene, was used.

APPLICATIONS

Experiments with GPC of small molecules usually start with the investigation of test substances. Therefore, we thought it to be useful to give some examples in this field.

Fig. 6 shows the results of a separation of triglycerides and hydrocarbons, using a combination of 2 columns with Bio-Beads SX-1 and SX-2 in benzene, *i.e.* gels suitable for separations in the mol. wt. range of about 3000 and lower. Except in the

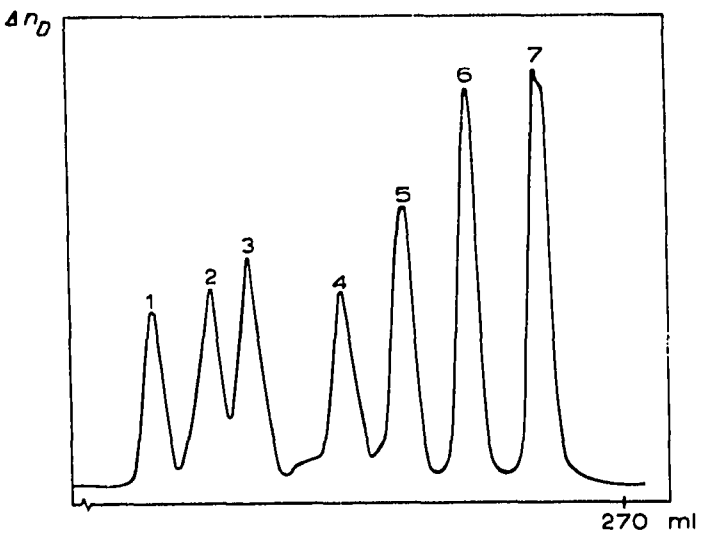


Fig. 6. Separation of test substances. Bio-Beads SX-1 + SX-2 in benzene; 2 columns. Sample application, 70 mg; attenuator, 32 ×; flow rate, 20.5 ml/h. 1 = tristearin; 2 = trimyristin; 3 = trilaurin; 4 = tricaprylin; 5 = tricaproin; 6 = hexadecane; 7 = undecane.

case of trimyristin (mol. wt. 723.2) and trilaurin (mol. wt. 639.0), a practically complete separation of all the components was obtained. The resolution of the substances in the higher molecular weight range seems to be somewhat disappointing, as has already been stated. On the other hand, however, separation of the lower molecular weight components is satisfactory beyond expectation.

It is interesting to compare here the results of BOMBAUGH and coworkers[28,29]. In an analytical separation of a number of the same substances, combinations of 10 and 40 columns packed with Styragel are used, at flow rates of 66 and 24 ml/h, respectively. These authors, however, used THF as the carrier solvent. In spite of the fact that we used only two columns and applied 70 mg of mixture, our results can compete fully with those of the above authors.

A separation on a single column of Bio-Beads SX-8 in benzene (exclusion limit mol. wt. 1000) is given in Fig. 7. Here, too, the limit given is rather high, as tristearin is eluted with the void volume. Good separation is obtained with the lower molecular weight components, even *n*-amyl and isoamylbenzene tend to separate. Using more columns in series, one can obtain a complete separation in such a case.

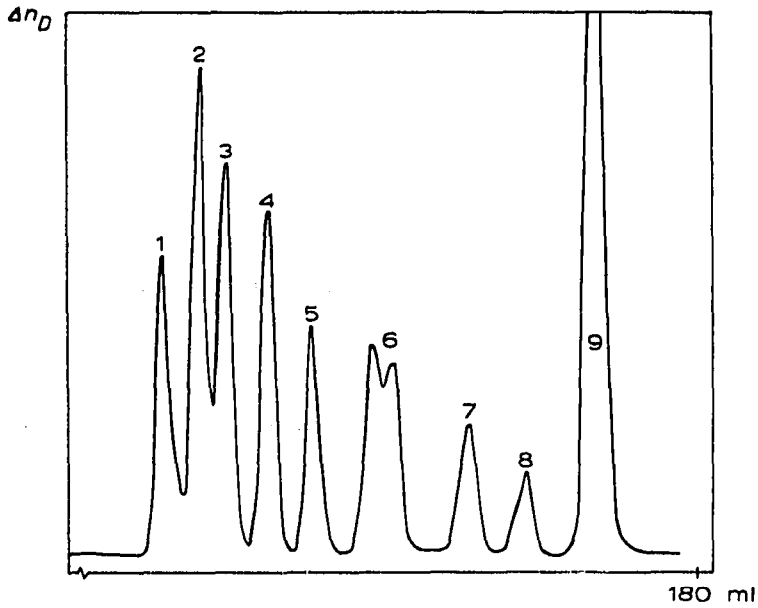


Fig. 7. Separation of test substances. Bio-Beads SX-8 in benzene; 1 column. Sample application, 41 mg; attenuator, 16 ×; flow rate, 24.5 ml/h. 1 = tristearin; 2 = tricaprylin; 3 = nonadecylbenzene; 4 = tridecylbenzene; 5 = nonylbenzene; 6 = *n*-amylbenzene + *iso*-; 7 = *n*-butylbenzene; 8 = toluene; 9 = methanol.

Sephadex LH-20 in methanol is used in the last example of separation with test substances (Fig. 8). The first tall peak originates from a propylene oxide–ethylene oxide copolymer, Pluronic F 68, having a molecular weight of about 4600. We have used this substance for the determination of the void volume of all Bio-Bead and Sephadex LH-20 columns, as it readily dissolves in all the solvents used.

The adsorptive properties of Sephadex for aromatics are clearly demonstrated in this case, as *p*-dichlorobenzene is eluted after water.

The following examples are all related to problems we met in practice. Investigating commercial mixtures of long chain acid alkylphosphates, neutralized with

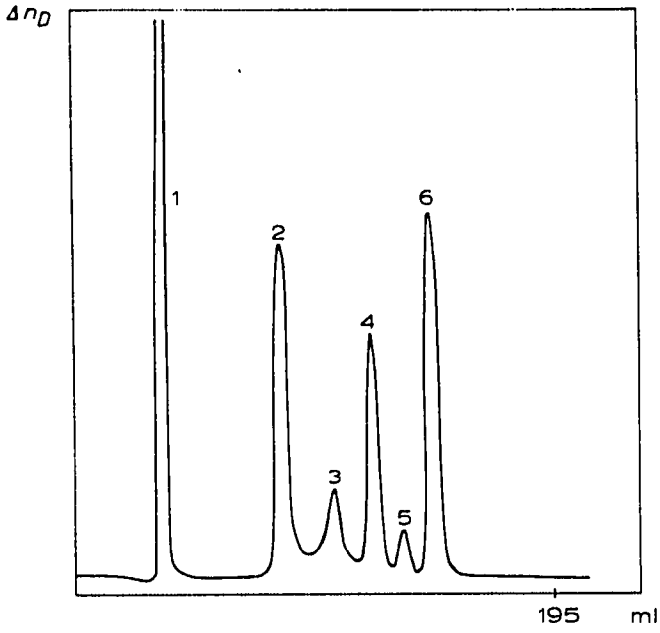


Fig. 8. Separation of test substances. Sephadex LH-20 in methanol; 1 column. Sample application, 15 mg; attenuator, 16 ×; flow rate, 24.5 ml/h. 1 = polypropylene oxide–polyethylene oxide; 2 = tristearin; 3 = stearic acid; 4 = ethylene glycol; 5 = water; 6 = *p*-dichlorobenzene.

bases such as morpholine or ethanolamines, we used ion exchange in an alcoholic solution for removal of the cationic part. The residue of the eluate, containing anionic and probably nonionic substances, was subjected to GPC in methanol on Sephadex LH-20 in methanol, but no complete separation was obtained, probably because of solute–solute interactions. However, after methylation of the residue, a complete separation was obtained on 3 columns of Sephadex LH-20 in methanol, and after isolation of the separated substances it appeared from NMR analysis that the main

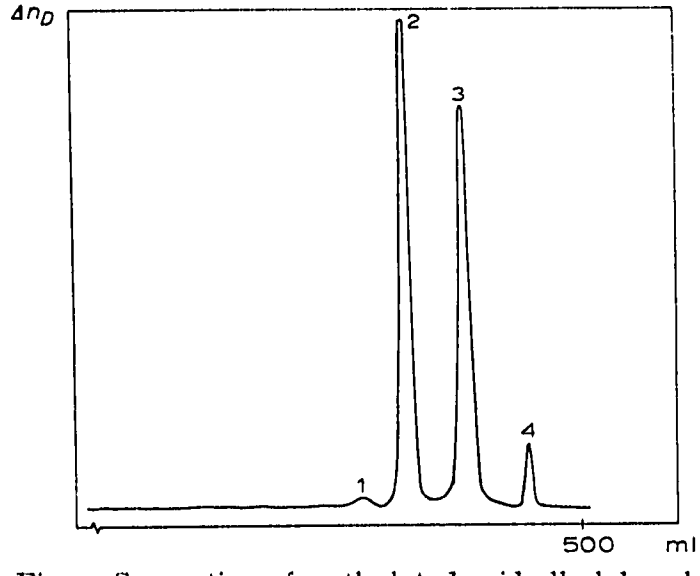


Fig. 9. Separation of methylated acid alkylphosphates. Sephadex LH-20 in methanol; 3 columns. Sample application, 60 mg; attenuator, 32 ×; flow rate, 40 ml/h. 1 = tridodecylphosphate; 2 = methyl-didodecylphosphate; 3 = dimethyl-dodecylphosphate; 4 = *n*-dodecylalcohol.

components in the mixture were mono- and didodecylphosphates. Also a small amount of the free alcohol was identified. Only one small peak, possibly from a triester, escaped identification (Fig. 9).

Dealing with oligomeric materials, we had the problem of separating cyclic oligomers, present in extracts from polyamide 6. In this case we were able to separate the oligomers up to the octamer on a simple combination of 2 columns Sephadex LH-20 in methanol (Fig. 10). By partial removal of the excess of lactam, the monomer, it is even possible to detect the nonamer. For quantitative analysis of the oligomer composition we use 3 columns in series, whereupon complete separation of all the oligomers is obtained. As GPC is only a part of a rather complex analysis procedure, further details of this procedure will be described in a subsequent paper.

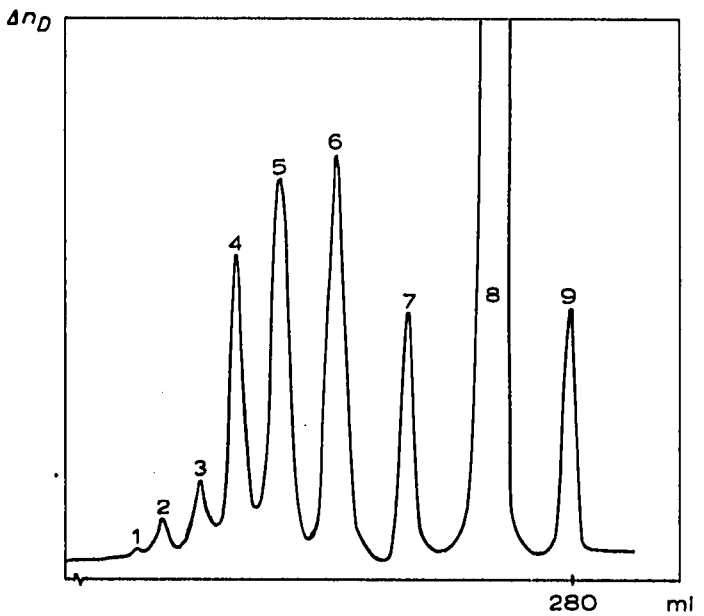


Fig. 10. Separation of cyclic caprolactam oligomers. Sephadex LH-20 in methanol; 2 columns. Sample application, 30 mg; attenuator, 32 ×; flow rate, 30 ml/h. 1–7 = octamer up to dimer; 8 = caprolactam; 9 = water.

In an investigation of the composition of deposits on machine parts, originating from an industrial yarn, a chloroform solution of the deposits was passed through a single column of Bio-Beads SX-2 in chloroform, and a usable separation was already obtained (Fig. 11). As in the case of the polyamide oligomers, an important improvement in resolution may here, again be expected by using more columns in series. However, with one column alone, several components in the mixture could be isolated.

Finally, two other examples are given in Figs. 12 and 13. In neither case was it our intention to identify the components separated. In Fig. 12 the separation is shown for components present in a polyester, prepared from a substituted diol and adipic acid. The tall peak at the end of the chromatogram, which comes from a small amount of chloroform added to the methanolic solution to dissolve a cloudy precipitate, is remarkable. Fig. 13 shows the chromatogram of a complex surfactant mixture, containing long chain acid salts of ethanolamines, free acid, and polyethyleneoxide derivatives.

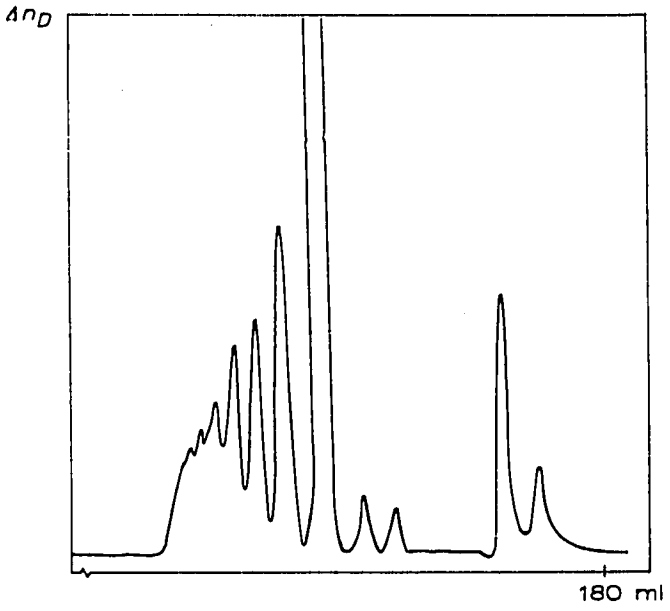
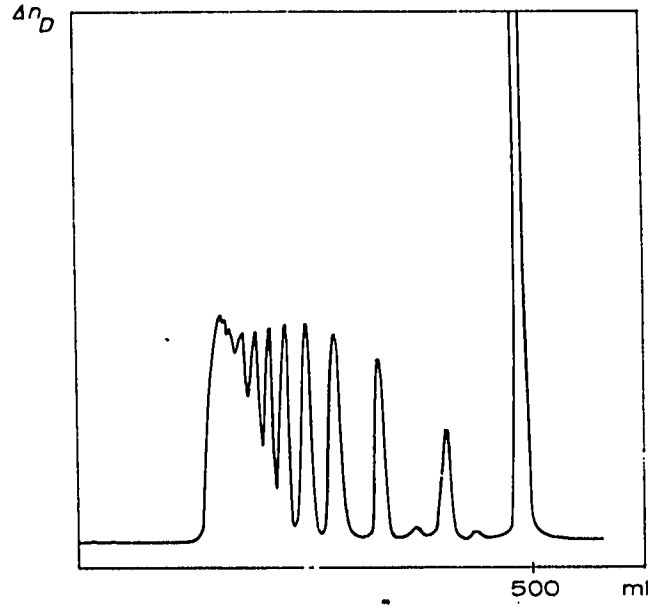


Fig. 11. Separation of components, present in deposits. Bio-Beads SX-2 in chloroform; 1 column. Sample attenuation, 30 mg; attenuator, 16 ×; flow rate, 47 ml/h.



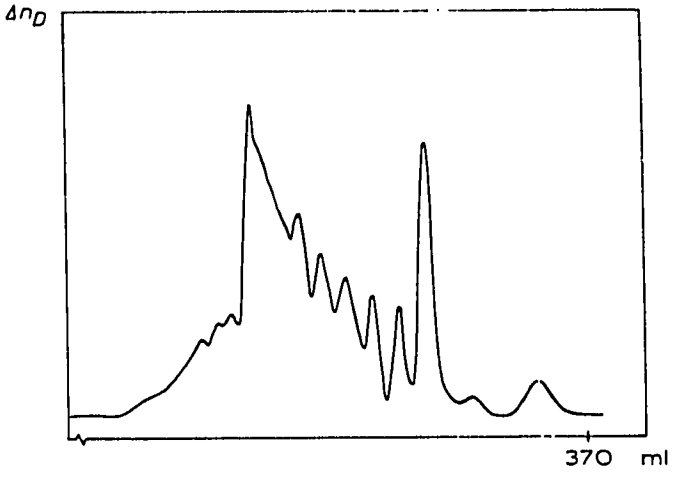


Fig. 12. Separation of low molecular weight polyester components. Sephadex LH-20 in methanol; 3 columns. Sample application, 30 mg; attenuator, 16 ×; flow rate, 53 ml/h.

Fig. 13. Separation of surfactant mixture. Bio-Beads SX-1 + SX-2 in benzene; 2 columns. Sample appication, 100 mg; attenuator, 32 ×; flow rate, 28 ml/h.

We believe we have demonstrated with these few examples, that GPC may be applied to many kinds of separations. Other applications in our laboratory are the analysis of glycols, plasticizers, sulphur compounds, resinous mixtures, competetive samples, and purifications.

It should be mentioned, that our apparatus seems to be rather complex, which is due to the many different kinds of samples we have to analyse. When, however, the field of analysis is limited, it is possible to obtain very satisfactory separations with one or two columns, which are operated by the gravity of the elution solvent only. In such cases sample application is carried out with the simple apparatus of Fig. 5c and detection by means of TLC can be carried out.

CONCLUSIONS

Gel permeation chromatography of small molecules in organic liquids is a versatile and efficient separation technique, which in principle may be carried out with simple apparatus.

As there is little or no irreversible adsorption of sample to the gel materials, repeated use of columns is possible.

Resolution is high and in some respects comparable with analytical GLC, even when using semi-preparative procedures. The technique is still more time-consuming than GLC and is in this respect more comparable with preparative GLC, but gel permeation chromatography is more versatile, as volatility of substances is of no account, and decomposition of sensitive substances is not to be feared.

In comparison with classic adsorption chromatography, sample loading capacity is high, polarity of substances plays a less important role, and separation time is short.

REFERENCES

1 H. DETERMANN, *Gelchromatographie*, Springer Verlag, Berlin, 1967.
2 H. DETERMANN, *Chimia (Aarau)*, 23 (1969) 94.
3 L. FISCHER, *Laboratory Techniques in Biochemistry and Molecular Biology*, North Holland Publ. Comp., Amsterdam, 1969.
4 J. F. JOHNSON, R. S. PORTER AND M. J. R. CANTOW, *Rev. Macromol. Chem.*, 1 (1966) 393.
5 J. SJÖVALL AND E. NYSTRÖM, *Advan. Chromatog.*, 6 (1968) 119.
6 J. G. HENDRICKSON, *4th Int. Seminar on GPC*, (1967) 112.
7 J. G. HENDRICKSON, *4th Int. Seminar on GPC*, (1967) 130.
8 J. G. HENDRICKSON, *4th Int. Seminar on GPC*, (1967) 139.
9 G. D. EDWARDS AND Q. Y. NG, *J. Polymer Sci.*, Part C, No. 21 (1968) 105.
10 J. C. MOORE AND J. G. HENDRICKSON, *J. Polymer Sci.*, Part C, No. 8 (1965) 233.
11 J. C. MOORE AND J. G. HENDRICKSON, *J. Polymer Sci.*, Part A-1, No. 4 (1966) 167.
12 T. L. CHANG, *Anal. Chim. Acta*, 39 (1967) 519.
13 T. L. CHANG, *Anal. Chim. Acta*, 42 (1968) 51.
14 M. JOUSTRA, B. SÖDERQVIST AND L. FISCHER, *J. Chromatog.*, 28 (1967) 21.
15 D. KRANZ, *Kolloid Z.*, 227 (1967) 41.
16 J. G. HENDRICKSON, *4th Int. Seminar on GPC*, (1967) 125.
17 J. CAZES AND D. R. GASKILL, *Separation Sci.*, 2 (1967) 421.
18 J. CAZES AND D. R. GASKILL, *Separation Sci.*, 4 (1969) 15.
19 F. N. LARSEN, *Appl. Polymer Symp.*, 8 (1969) 111.
20 M. WILK, J. ROCHLITZ AND H. BENDE, *J. Chromatog.*, 24 (1966) 414.
21 B. J. MAIR, P. T. R. HWANG AND R. G. RUBERTO, *Anal. Chem.*, 39 (1967) 838.
22 J. C. JANSON, *J. Chromatog.*, 28 (1967) 12.
23 A. J. W. BROOK AND S. HOUSLEY, *J. Chromatog.*, 41 (1969) 200.
24 K. H. ALTGELT, *Makromol. Chem.*, 88 (1965) 75.
25 H. H. OELERT, *Z. Anal. Chem.*, 244 (1969) 91.
26 P. C. TALARICO, E. W. ALBAUGH AND R. E. SNYDER, *Anal. Chem.*, 40 (1968) 2192.
27 H. J. COLEMAN, D. E. HIRSCH AND J. E. DOOLEY, *Anal. Chem.*, 41 (1969) 800.
28 K. J. BOMBAUGH, W. A. DARK AND R. F. LEVANGIE, *Z. Anal. Chem.*, 236 (1968) 443.
29 K. J. BOMBAUGH, W. A. DARK AND R. F. LEVANGIE, *Separation Sci.*, 3 (1968) 375.
30 K. J. BOMBAUGH, L. E. MALEY AND B. A. DENENBERG, *Dechema Monograph.*, 62 (1967) 295.

31 K. J. BOMBAUGH, W. A. DARK AND R. N. KING, *Res. Dev.*, (1968) 28.
32 K. J. BOMBAUGH, W. A. DARK AND R. F. LEVANGIE, *J. Chromatog. Sci.*, 7 (1969) 42.
33 E. SCHRÖDER AND W. MISCHE, *Plaste Kautschuk*, 15 (1968) 258.
34 E. HAGEN AND E. SCHRÖDER, *Plaste Kautschuk*, 16 (1969) 335.
35 T. L. CHANG, *Anal. Chem.*, 40 (1968) 989.
36 W. HEITZ, H. ULLNER AND H. HÖCKER, *Makromol. Chem.*, 98 (1966) 42.
37 W. HEITZ AND H. ULLNER, *Makromol. Chem.*, 120 (1968) 58.
38 W. HEITZ, B. BÖMER AND H. ULLNER, *Makromol. Chem.*, 121 (1969) 102.
39 H. ZAHN AND P. KUSCH, *Textilind.*, 69 (1967) 880.
40 W. C. FEIST, C. F. SOUTHERLAND AND H. TARKOW, *J. Appl. Polymer Sci.*, 11 (1967) 149.
41 F. TOKIWA, K. OHKI AND I. KOKUBO, *Bull. Chem. Soc. Japan*, 41 (1968) 2845.
42 F. TOKIWA, K. OHKI AND I. KOKUBO, *Bull. Chem. Soc. Japan*, 41 (1968) 2285.
43 F. TOKIWA, K. OHKI AND I. KOKUBO, *Bull. Chem. Soc. Japan*, 42 (1969) 575.
44 M. MUTTER, *Chromatographia*, 2 (1969) 208.
45 J. C. MOORE, *J. Polymer Sci., A-2*, (1964) 835.
46 P. FLODIN, *J. Chromatog.*, 5 (1961) 103.
47 W. HEITZ, *7th Int. Seminar on GPC*, (1969) 214.
48 S. T. SIE AND N. VAN DEN HOED, *J. Chromatog. Sci.*, 7 (1969) 257.
49 J. H. VAN DIJK, *Z. Anal. Chem.*, 247 (1969) 262.
50 J. N. LITTLE, J. L. WATERS, K. J. BOMBAUGH AND W. J. PAUPLIS, *5th Int. Symp. on Separation Methods, Lausanne, 1969*, preprints.