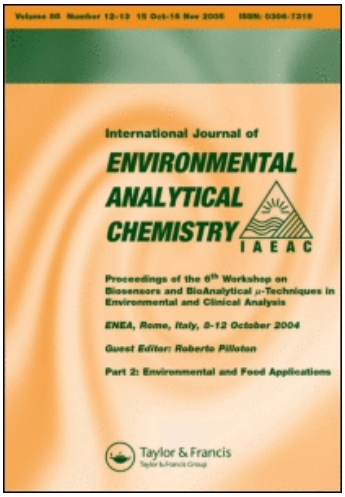


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# The Comparison of Elution Profiles of Polychlorinated Biphenyls and Fats in Various Gel Permeation Chromatographic Systems

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# THE COMPARISON OF ELUTION PROFILES OF POLYCHLORINATED BIPHENYLS AND FATS IN VARIOUS GEL PERMEATION CHROMATOGRAPHIC SYSTEMS

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An optimisation of gel permeation chromatography (GPC) is presented for separation of polychlorinated biphenyls (PCBs) from fats. The results of testing of Bio Beads S-X3 gel eluted with different mobile phases (mixtures of chloroform and cyclohexane) are evaluated with respect to the use of GPC as a single, rapid and efficient clean-up technique suitable for routine analyses of PCBs. Relations between theoretical assumptions and achieved results are discussed.

Although the elution profiles for individual PCB congeners differed, various tested fats eluted in similar volumes. Several of investigated systems can be used for isolation of PCBs from fats. Nevertheless, for practical reasons a system with chloroform as a single mobile phase, in which PCBs were eluted in the lowest volume as a narrow fraction, has been chosen.

**KEY WORDS:** Gel permeation chromatography, Bio Beads S-X3, PCBs, fats, clean-up.

## INTRODUCTION

Many systems consisting of various types of columns, gels and solvents have been utilised since the introduction of gel permeation chromatography (GPC) as a clean-up technique into the field of organic trace analysis. The significant feature of these systems is the frequent use of polystyrene-divinylbenzene copolymers—mainly Bio Beads S-X3 gel has found wide use in routine practice. The first complex study of GPC by Stalling *et al.*[1] demonstrated a potential application of this technique for a separation of organic contaminants such as non-ionic pesticides from substances with a higher molecular weight represented by lipids extracted from fish. Specht and Tillkes[2,3] investigated the elution volumes of a large number of organic compounds in their GPC systems. Steinwandter[4,5] tested various GPC systems for separation of more than 100 pesticides and industrial chemicals from fats and oils. Patterson[6] optimised the system by reducing the size of the GPC column. Rhijn and Tuinstra[7]

dealt with a miniaturisation of size exclusion chromatography. Grob's and Kälin's[8] experiments were aimed at the on-line coupling of a miniaturised GPC system with a gas chromatograph.

Examination of several types of GPC systems represented by various gels and mobile phases were carried out in our previous study[9]. The best results were achieved in experiments employing Bio Beads S-X3 gel. Further study, therefore, was focused on optimisation of the mobile phase composition in GPC system with Bio Beads S-X3 column. Chloroform, which is in use in our laboratory at present[10], meets best the requirements for routine clean-up.

The objective of the present study was to demonstrate the strategy for the optimisation of GPC systems conducted on the basis of an application of both experimental data and theoretical assumptions.

## EXPERIMENTAL

### *Chemicals*

Chloroform, cyclohexane, isooctane and hexane p.a. (Lachema Brno, CR) were redistilled before use. Anhydrous sodium sulphate (Lachema Brno, CR) was heated 4 h at 500°C. PCB congeners (dichlorobiphenyls: cong. 15, trichlorobiphenyls: cong. 28, tetrachlorobiphenyls: cong. 52, pentachlorobiphenyls: cong. 101 and 114, hexachlorobiphenyls: cong. 129, 137, 138 and 153, heptachlorobiphenyls: cong. 171, 180, 183, 185, 189 and 191, octachlorobiphenyls: 200, 201 and 203, nonachlorobiphenyls: cong. 206 and decachlorobiphenyl: cong. 209) were obtained from National Research Council, Canada.



### *Materials*

The investigated fats were: sunflower oil, lard, duck fat, beef tallow and milk fat.

### *Gel permeation systems*

All tested systems consisted of a stainless steel column 50 × 0.8 cm i.d. (Tessek, CR) filled with 200–400 mesh Bio Beads S-X3 gel (Bio Rad, USA), which was swollen in the corresponding mobile phase. A linear solvent delivery system (pump HPP 5001—Laboratorní přístroje Praha, CR) and valve Rheodyne 7125 with injection loop 1 ml (Rheodyne, USA) was used. In all systems the flow rate was 0.6 ml/min at the head column pressure 0.4 MPa. Fractions were collected using graduated cylinders. Table 1 characterises the tested systems.

### *Investigation of fats and PCBs elution profiles*

Raw fats were dissolved in hexane and obtained solutions were dehydrated by filtration

**Table 1** Characterisation of tested GPC systems

| *System number* | *Composition of mobile phase* | *Solubility parameter (δ)*)* |
|---|---|---|
| 1 | Cyclohexane | 8.20 |
| 2 | Cyclohexane:chloroform (3:1, v/v) | 8.47 |
| 3 | Cyclohexane:chloroform (1:1, v/v) | 8.75 |
| 4 | Cyclohexane:chloroform (1:3, v/v) | 9.02 |
| 5 | Chloroform | 9.30 |

*) Values calculated according to Shepherd[11]

through a layer of anhydrous sodium sulphate. After evaporation of hexane fat samples were dissolved in the respective mobile phases so that the concentrations were approximately 250 mg/ml. Solutions of PCB congeners were prepared from aliquot part of stock solution (after removal of isooctane by a gentle stream of nitrogen) by dissolving in the respective mobile phases, their concentrations ranged from 0,019 to 0,040 μg/ml with exception of congener 15 (0,697 μg/ml). The testing of the optimised system was carried out by fats spiked on corresponding levels. Injection volume into GPC system was 1 ml.

The content of the fats in each fraction was determined by gravimetric analysis after removal of mobile phase. Determination of PCBs after mobile phase evaporation and dissolving in isooctane was carried out by gas chromatography using electron capture detector (GC-ECD).



### *GC-ECD determination of PCBs*

Gas chromatograph Hewlett Packard 5890 Series II equipped with nickel electron capture detector ($^{63}$Ni-ECD), autosampler Hewlett Packard 7673 and capillary column ULTRA 2 (5% phenyl methyl silicon, 50 m × 0.2 mm × 0.33 μm) were used under following conditions: carrier gas—nitrogen at the flow rate 0.8 ml/min at 60°C, make up gas—nitrogen at the flow rate 30 ml/min, splitless period—2.5 min, injector temperature—225°C, detector temperature—300 °C and oven temperature profile—initial temperature 60°C (2.5 min), increase 30°C/min to 220°C, increase 1°C/min to 280°C, hold 23 min at 280°C. Injection volume was 1μl.

## RESULTS AND DISCUSSION

In five GPC systems, that were tested in our experiment, the composition of solvent (mobile phase) represented the variable parameter. Figure 1 illustrates an increase of swelling capacity of Bio Beads S-X3 with the rise of chloroform content in the solvent. Simultaneously (because of the fixed volume of GPC column) the contents of gel (expressed as the weight of dry polymer) in systems decreased. As can be seen, the distinct change in swelling capacity was recorded, when a small amount of chloroform was added to cyclohexane. This phenomenon is in accordance with Shepherd's theory[11] based on assumption that maximum swelling of the gel occurs when the properties of both solvent (solvents mixture) and sorbent

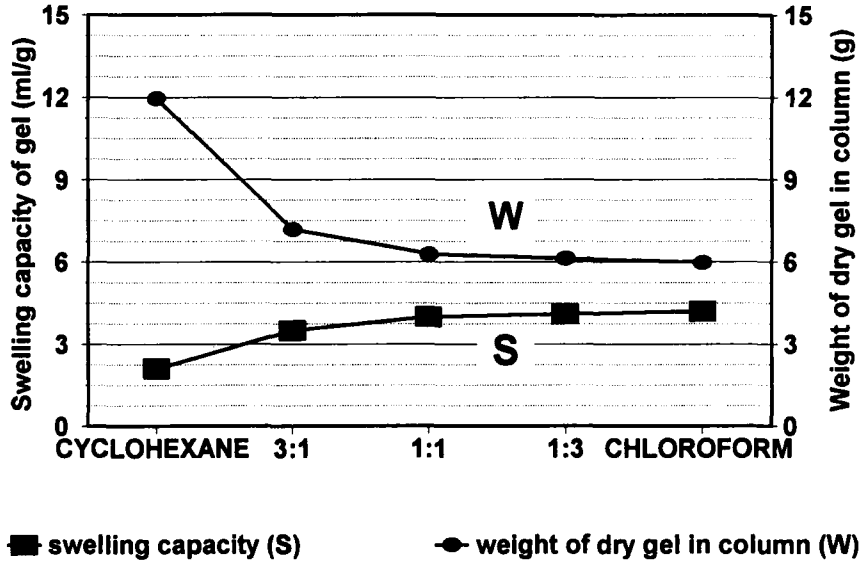


**Figure 1** Relation of swelling capacity and weight of gel in column to mobile phase composition



characterised by means of solubility parameter ($\delta$) are equal. Shepherd[11] introduced an estimated value of solubility parameter for polystyrene-divinylbenzene ($\delta$ = 9.1–9.4) and for chloroform ($\delta$ = 9.3), which correspond to observed high swelling capacity of Bio Beads S-X3 in chloroform. On the other hand, in cyclohexane ($\delta$ = 8.2) is swelling capacity of Bio Beads S-X3 in comparison with chloroform lower.

Applicability of individual experimental systems for separation of PCBs and different kinds of fats is documented in Figure 2. In the system No. 1 (non-polar eluent cyclohexane) both groups of substances were eluted in wide bands and, consequently, their overlap occurred. Addition of chloroform to the mobile phase reduced simultaneously band broadening and elution volumes, which resulted in improved separation. These observations are comparable with results achieved by Steinwandter[4] for GPC systems using several mixtures of cyclohexane and dichloromethane as solvents.

As regards the elution of various kinds of fats in individual GPC systems no significant differences were observed, although tested fats differed both in the content of unsaturated fatty acids and in their molecular weights (molecular weight of triglycerides approximately ranged from 600 to 1000). In fact, with exception of pure cyclohexane, all the systems with mobile phases consisting of various mixtures of cyclohexane-chloroform as well as pure chloroform were thus proved to be suitable for the practical use.

Figure 3 shows the elution profile of individual PCBs in pure cyclohexane. In this system (No. 1) the most remarkable differences in chromatographic behaviour of PCBs were recorded: e.g. congener 209 (decachlorobiphenyl) was eluted completely in 24 ml while congener 15 (dichlorobiphenyl) was eluted in 39 ml, nevertheless, the width of elution bands

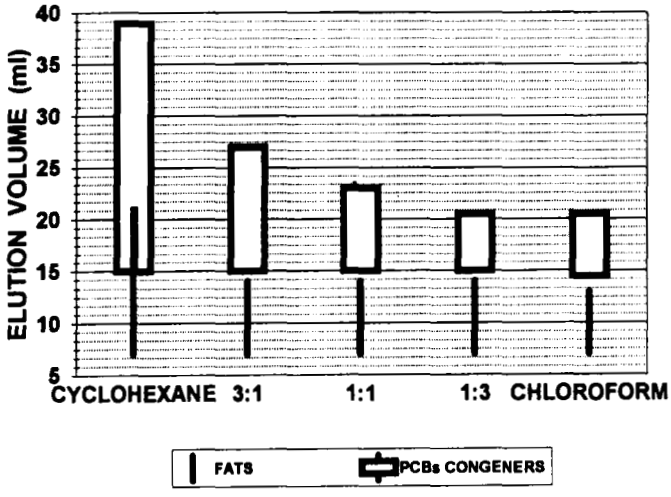


**Figure 2** Elution profile of fats and PCBs



was similar (10 ml for PCB 15; 9 ml for congener 209). Differences were observed even in the case of congeners with identical molecular weight. PCBs 183, 185 and 189 were completely eluted in 27 ml, 30 ml and 33 ml, respectively. It is evident that with increasing of chloroform content in the mobile phase differences in elution profiles of analytes were gradually diminished and their elution bands become narrower. Figure 4 demonstrates the

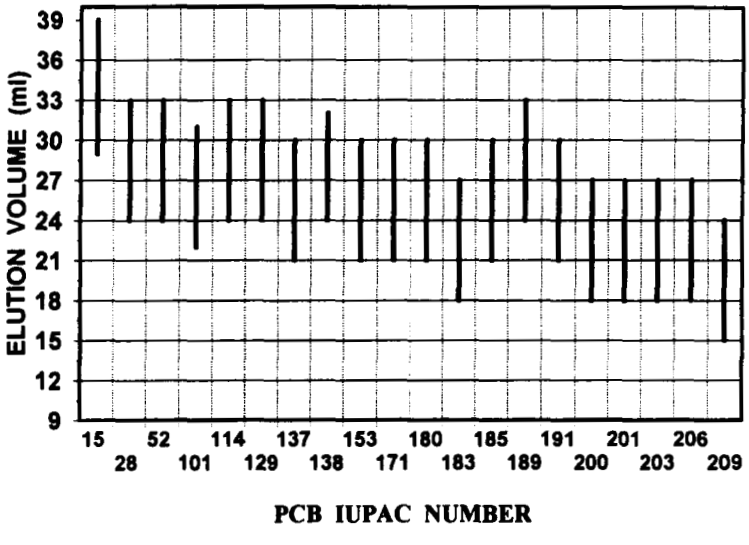


**Figure 3** Elution profile of tested congeners in cyclohexane

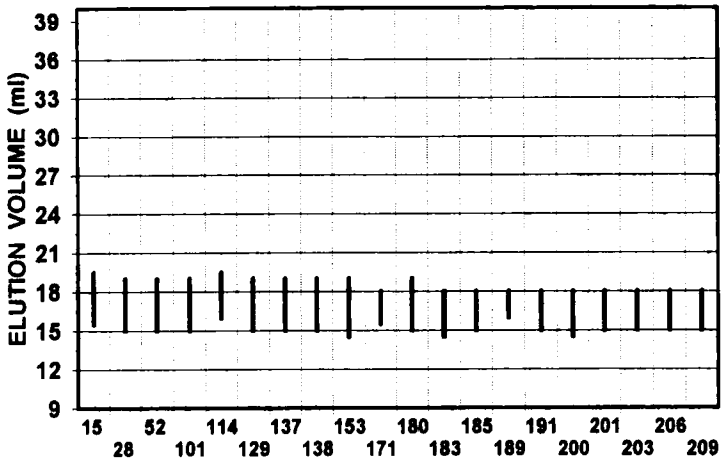


**Figure 4** Elution profile of tested congeners in chloroform



elution profiles of investigated PCB congeners in system No. 5 employing chloroform as a mobile phase. Under these conditions the differences in elution volumes and in width of elution bands were negligible.

We can draw from the experiment following generalisations:

1) increasing of gel swelling capacity and thereby pore size with increasing of the chloroform concentration in mobile phase
2) decreasing of the elution volumes of investigated compounds with increasing of the chloroform concentration in mobile phase
3) reduction of the elution band width with increasing of the chloroform concentration in mobile phase
4) diminishing of differences in behaviour of individual PCBs with increasing of the chloroform concentration in mobile phase.

The possibility to utilise these observations for a prediction of chromatographic behaviour of investigated compounds was considered. Tabulated data (solubility parameters) as well as experimentally measured characteristics of systems (gel swelling capacity) were used for this purpose.

Shepherd[11] pointed at the possibility of a linkage of solubility parameter theory and chromatographic properties through the concept of activity coefficients. From these considerations he derived an expression for the capacity ratio ($K_i$) of solute i:

$$\ln K_i = (V_{m,i}/RT)\,(\delta_m + \delta_s - 2\delta_i)\,(\delta_m - \delta_s) + \ln\,(n_s/n_m) \qquad \text{(eq.1)}$$

$V_{m,i}$—molar volume of solute i
R—general gas constant
T—absolute temperature
$\delta_i$—solubility parameter of solute i
$\delta_s$—solubility parameter of stationary phase
$\delta_m$—solubility parameter of mobile phase
$n_s$—number of moles of stationary phase in column
$n_m$—number of moles of mobile phase in column

When $\delta_s$ and $\delta_m$ are equal, the first term becomes zero and we get simplified equation 1:

$$K_i = (n_s/n_m) \qquad \text{(eq.2).}$$

Size-exclusion mechanism of separation is then the dominating phenomenon in such systems.[11] The resolution of PCBs (in our experiment molecular weights ranged between 223.1 and 498.5) and fats (molecular weights can be approximately assumed to the range between 600 and 1000) improved with an increase of chloroform content in a mobile phase. The principle of separation in GPC systems No. 4 and No. 5 can be almost characterised as "pure" size—exclusion mechanism ($\delta_s$ and $\delta_m$ are approximately identical). For a routine analyses of PCBs GPC system No. 5 was chosen—chloroform represents a single, one-component mobile phase, which is more convenient for everyday use.



## CONCLUSION

An evaluation of presented results proved the possibility to assume the chromatographic behaviour of investigated compounds prior to practical testing of GPC systems. In practice it is feasible to estimate the elution volumes of analytes and coextracts on the basis of measured and tabulated parameters (gel swelling capacity, solubility parameter). Optimal GPC conditions for obtaining efficient clean-up can be designed for a given purpose in advance thus the extent of experimental work is significantly reduced.

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