GEL FILTRATION IN ORGANIC SOLVENTS

M. JOUSTRA, B. SÖDERQVIST AND L. FISCHER*
Pharmacia Fine Chemicals, Uppsala (Sweden)

Gel filtration in aqueous systems as developed by FLODIN AND PORATH in the years 1958–1960 has many valuable features, the most important of which are:

(1) Fractionation according to molecular size.
(2) Easy handling of the material.
(3) Good stability of the chromatographic bed.
(4) Possibility of chromatography of labile substances without denaturation.
(5) Permissibility of high sample concentrations because of the linearity of partition isotherms.

There is therefore considerable interest in extending the technique to include organic solvents.

It was very soon found that Sephadex® of the G-series, the classical material for gel filtration in aqueous solutions, could be used in some polar organic solvents. Suitable solvents include glycol, formamide and dimethylsulphoxide, although these have not been frequently used. The use of mixtures of water and the lower alcohols, notably ethanol has, however, often been reported in the literature.

In 1960, VAUGHAN reported the use of cross-linked polystyrene as a chromatographic medium with aromatic hydrocarbons as solvents[1]. This material could be used for the determination of molecular weight, for distribution analysis etc. CORTIS-JONES also used the same material[2], while BREWER used vulcanized rubber latex[3]. Since then, polystyrene gels have been developed by MOORE for use mainly with non-polar organic solvents[10]. DETERMANN *et al.* have prepared a copolymer of methyl methacrylate and ethyleneglycol dimethacrylate that was used for fractionation of low molecular weight polystyrenes[11].

A quite diffrerent approach to the problem of gel filtration in organic solvents was taken by NYSTRÖM AND SJÖVALL[4,5]. These authors modified the existing types of Sephadex to make them suitable for work in polar organic solvents. Methylation was performed with dimethylsulphate by a rather laborious method involving several steps. The most highly methylated Sephadex can be used even with non-polar organic solvents such as hydrocarbons. With the modified Sephadex, SJÖVALL and coworkers have been able to separate a considerable number of lipids, steroids[4], protected oligopeptides[6] and vitamins of the K group[7]. In some experiments they have used a chromatographic tedchnique very similar to that used in gas chromatography, with a 1.5 mm diameter teflon tube, approximately 2 m long, as the column.

At the same time that NYSTRÖM AND SJÖVALL developed their methylated Sephadex, Pharmacia Fine Chemicals independently developed another derivative of Sephadex for use with organic solvents. This derivative has properties fairly similar to those of the methylated Sephadex of NYSTRÖM AND SJÖVALL. It is commercially

available under the name Sephadex LH-20. It is produced by the hydroxypropylation of Sephadex G-25 and has a solvent regain value of approximately 2 ml/g in many solvents. The swelling properties and other technical data for Sephadex LH-20 are presented in Table I. The fractionation range is slightly different in different solvents. In most solvents the exclusion limit falls somewhere between 2,000 and 10,000.

TABLE I

SOLVENT REGAIN FOR SEPHADEX LH-20 IN DIFFERENT SOLVENTS

Solvent	*Approximate solvent regain (ml solvent/g dry gel)*	*Approximate bed volume (ml/g dry gel)*
Dimethylformamide	2.2	4.0–4.5
Water	2.1	4.0–4.5
Methanol	1.9	4.0–4.5
Ethanol	1.8	3.5–4.5
Chloroform, stabilized by 1 % ethanol	1.8	3.5–4.5
Chloroform	1.6	3.0–3.5
n-Butanol	1.6	3.0–3.5
Dioxane	1.4	3.0–3.5
Tetrahydrofuran	1.4	3.0–3.5
Acetone	0.8	
Ethyl acetate	0.4	
Toluene	0.2	

It is well-known that in aqueous solutions aromatic substances are retarded on Sephadex of the G-series[12]. This effect is particularly noticed in the highly cross-linked gels G-10, G-15 and G-25. This retardation of aromatic compounds is found for Sephadex LH-20 with alcoholic solutions, although the effect is far less pronounced than with aqueous solutions. In chloroform solutions the effect is not noticeable[8].

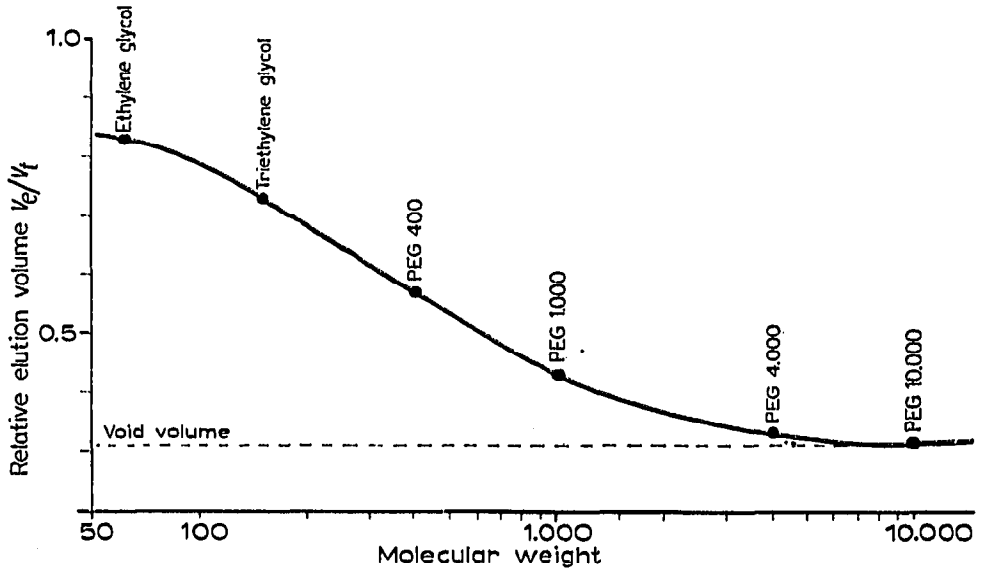

Fig. 1. Correlation between relative elution volume and molecular weight for polyethylene glycols on Sephadex LH-20 in ethanol.

It has been noticed that in chloroform solutions substances containing carboxyl groups and hydroxyl groups are retarded relative to the corresponding substances not containing these groups. This specific retardation may, in some cases, be used to obtain fractionation of substances which are otherwise closely similar. Some examples of fractionations obtained with Sephadex LH-20 will now be discussed:

The relationship between elution volume and molecular weight for polyethylene glycols on Sephadex LH-20 follows the expected pattern (see Fig. 1). At low molecular weight K_D values approach unity and decrease within the fractionation range to $K_D = 0$ at approximately mol. wt. = 5,000 in ethanol solution. In chloroform solution, very high K_D values are obtained at low molecular weight. This may be explained by the presence of terminal hydroxyl groups in the polyethylene glycol chains. The relationship between elution volume and molecular weight for polyethylene glycols in chloroform on LH-20 is illustrated in Fig. 2.

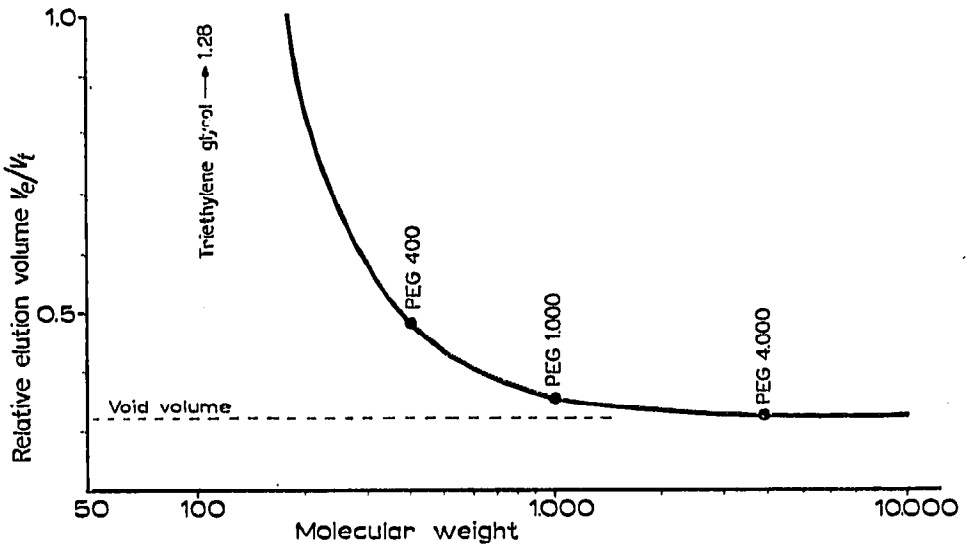

Fig. 2. Correlation between relative elution volume and molecular weight for polyethylene glycols on Sephadex LH-20 in chloroform.

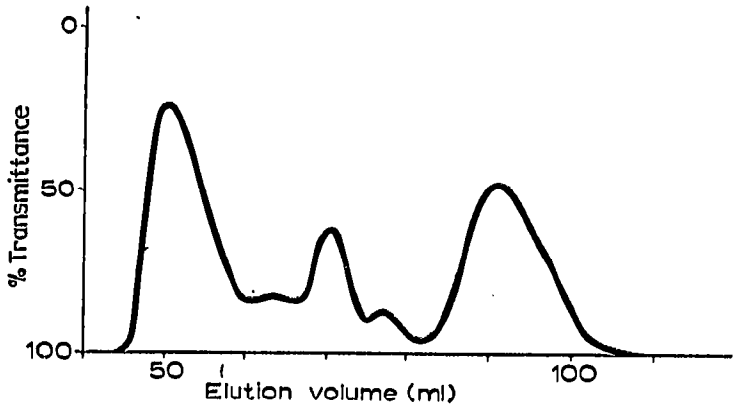

Fig. 3. Elution diagram of a commercial polystyrene (Dow Resin PS-3) on Sephadex LH-20 in chloroform.

Separation of substances of high and low molecular weight can be used for analytical purposes. Fig. 3 shows the separation of a commercially available polystyrene, Dow-Resin PS-3, on Sephadex LH-20 in chloroform. The first peak eluted corresponds to the polymer and the last peak corresponds to the monomer. The second peak probably corresponds to the plasticizer or some similar material.

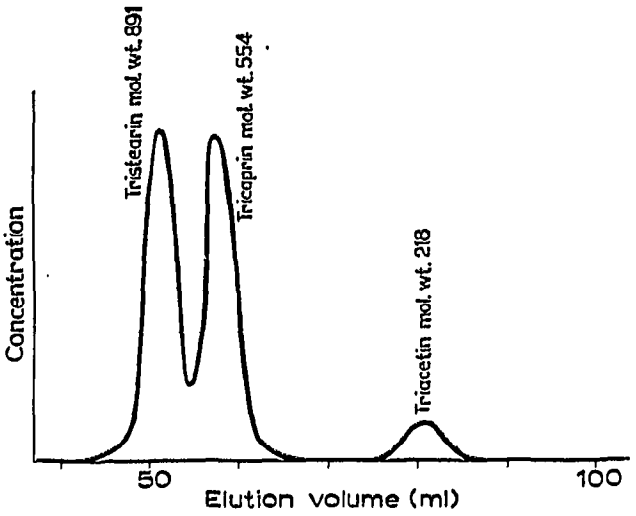

Fig. 4. Elution diagram for glycerol esters on Sephadex LH-20 in chloroform.

The separation of molecules according to molecular weight is illustrated in Fig. 4, giving the separation of tristearin, tricaprin and triacetin on Sephadex LH-20 in chloroform solution.

The separation of dipalmitins with primary and secondary hydroxyl groups illustrated in Fig. 5, is based on the structure rather than on the molecular size of the substances.

Finally the use of mixed solvents should be mentioned. VIKHO has reported the separation of cholesterol and dehydroepiandrosterone sulphate[9] on Sephadex LH-20 in a mixture composed of chloroform, methanol and water. In this case, the content of water in the solvent mixture has a profound influence on the separation obtained.

In mixed solvents, the composition of the solvent inside and outside the gel

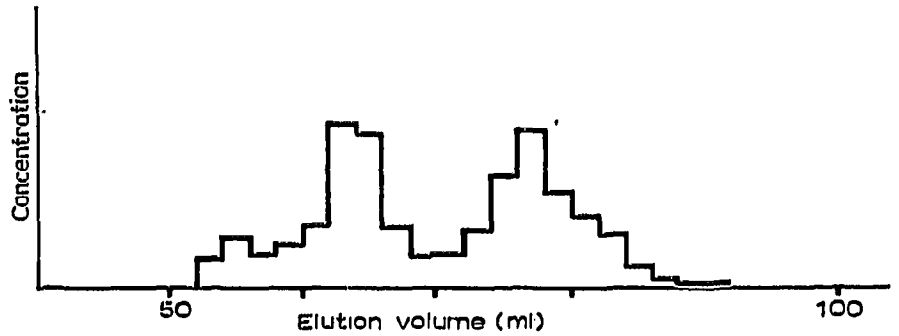

Fig. 5. Elution diagram for dipalmitins with primary and secondary hydroxyls on Sephadex LH-20 in chloroform.

grain is different. Thus, in addition to the sterical factors, partition of a solute between the mobile and the gel phase is also influenced by the difference in the composition of the liquids.

SUMMARY

The development of gel filtration in organic solvents is briefly reviewed. The properties of Sephadex LH-20 in some organic solvents are discussed in more detail, *viz.* solvent regain values, the elution behaviour of polyethylene glycols in ethanol and chloroform, the retardation of hydroxyl containing substances in chloroform.

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