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## SYNTHESES AND PROPERTIES OF GEL CHROMATOGRAPHY MATERIALS

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## SUMMARY

The effect of the mode of synthesis on the characteristics of cross-linked polymers is examined for cross-linked copolymers of ethylvinylbenzene-divinylbenzene and vinyl acetate-divinyl adipate. For homogeneously cross-linked gels the amount of cross-linking agent determines the "pore size" of these gels in the swollen state. Decreasing the amount of the divinyl component results in gels with an increase in the excluded molecular weight. The mechanical stability sets the experimental limit of their usage, so this type of gel is mostly suited for the separation of oligomers; polymers, however, may be separated by use of heterogeneously cross-linked gels. The latter type of gel is prepared by using high amounts of cross-linking agent and polymerising in the presence of an inert compound. It is demonstrated that in case of a heterogeneously cross-linking polymerisation the heterogeneity of the copolymers increases while at the same time the solvating ability of the inert component decreases; this leads simultaneously to an increase in the excluded molecular weight.

A decrease in the quality of the solvent using a non-polar non-solvent may result in gels having a dense shell. Gels with such balloon-like structures separate over a narrow range of molecular weights. As to be expected by theory, substances which are totally excluded ( $V_e = V_0$ ) and substances which can easily penetrate these gels give sharp peaks, whereas substances in the separation range give broad peaks.

## INTRODUCTION

Gel chromatography is a method, whereby the separation of a mixture takes place due to differences in molecular weight. Like any other chromatographic procedure gel chromatography may be defined as a method where a mixture of substances suspended in a mobile phase passes over a stationary phase and an exchange of matter is effected. In gel chromatography the stationary phase is a porous material. The pore size and particle size distribution must match any function of molecular size.

The aim of this paper is to elucidate some points which are important for the optimal construction of these polymer networks. On eluting a mixture of substances on a column filled with swollen gel particles the first substances to appear in the eluate are those substances having molecular sizes which prevent them entering into the gel.

They are eluted in a volume of eluate, corresponding to the volume between the gel particles which we will call  $V_0$ . With decreasing molecular weight the accessible volume increases, that is, the substances are eluted in the sequence of their decreasing molecular weight. Neglecting specific interactions with the gel for the moment, the last substance will appear at the end of the column after an additional volume  $V_t$ , corresponding to the volume of the solvent in the gel particles, has passed through the column. If we plot the logarithm of the molecular weight against elution volume we get, over a fairly large range, a linear relationship within the given limits  $V_0$  and  $V_0 + V_t$ . The gels may be characterised by their excluded molecular weight which is obtained by definition when extrapolating this straight line to  $V_0$ .

The separation efficiency obtainable in gel chromatography is greatly restricted by the limited volume in which the separation occurs. For example the peak at  $V_0$  corresponds to the air peak in gas chromatography. The total volume of the column  $V_t$  may be divided into three parts.  $V_0$  which, if we assume a statistical arrangement of spheres, is equal to  $0.35 V_t$ ,  $V_t$  and  $V_p$  the volume of the polymer network.  $V_p$  is found experimentally to be in the range of  $0.1$  to  $0.3 V_t$ , that is, in a column of  $100$  ml total volume the separation occurs in  $35$  to  $55$  ml. And without any experimental work we can say that the smaller  $V_p$  is, the better the separation would be. That is, gels which can be highly swelled should be used; then not very much cross-linked copolymer is needed for packing the column. However, we have another experimental requirement: the gels must be mechanically stable. As the mechanical stability decreases with increasing pore volume within the gel we have to find the optimal conditions by experiment.

#### PRINCIPLES OF PREPARATION

It is a common practice to use the gels as spherical particles as they are obtained in this form by suspension polymerisation. In principle there are three ways of obtaining gels which fit gel chromatography requirements. The first possibility is to cross-link a linear polymer. The first gel to be used for a separation according to molecular size was of this type<sup>1</sup>. It was a cross-linked dextran now available under the trade mark Sephadex. These gels are prepared by cross-linking dextran with epichlorohydrin. The pore size of these gels is controlled by the concentration and the molecular weight of the linear polymer and the amount of cross-linking agent. This type of preparation has the disadvantage that gels with high excluded molecular weights swell to extreme limits.

The second mode of synthesis consists in starting with the pure monomers. Copolymerising a monovinyl compound with a certain amount of cross-linking agent results in a cross-linked copolymer where only the amount of cross-linking agent determines the mesh size of the network. This is only true if the polymerisation is performed in absence of solvent. In the dry state these copolymers have no porosity. On swelling these cross-linked copolymers, the single polymer chains become solvated and form a loose statistical network, the porosity is only present in the swollen state and is called the swelling porosity. The mean working range of these gels corresponds, within an order of magnitude, to the mean mesh size of the network as calculated from the amount of cross-linking agent. Thus we may conclude that these gels are — in a statistical sense, of course — homogeneously cross-linked. This statement is

questionable, but some results which might bring us to better criteria will be discussed later.

With decreasing amount of cross-linking agent the excluded molecular weight rises. Nevertheless these gels can only be used for the separation of oligomers; as already pointed out with a decreasing amount of cross-linking agent the swelling is greater and they do not have good mechanical stability. Similar problems existed in the preparation of ion exchangers accessible to large ions. This problem was solved by the process of heterogeneous cross-linking polymerisation. The first patent dealing with ion exchangers of this type came from CORTE in 1957<sup>2</sup>. MOORE first published results of the successful use of this type of copolymer in 1964<sup>3</sup> in gel chromatography. The basic principle of this method is the polymerisation of a monomer mixture which could be cross-linked in presence of an inert and soluble compound. This inert compound should have the following qualities: it must be soluble in the monomer mixture and should not be chemically bound to the network during polymerisation. After the polymerisation it should be easily removable from the polymer.

As suspension polymerisation is the main mode of synthesis the inert compound may not be miscible with water. Many organic liquids fulfill these conditions, acting as a solvent or non-solvent for the polymer being produced. Even the use of polymers has been described<sup>4</sup>.

When polymerising a mixture of a monovinyl and a divinyl compound the growing polymer has only a limited swelling capacity. So a phase separation must occur assuming that the system is in a thermodynamic equilibrium. It is obvious that this phase separation is the more pronounced the higher the amount of cross-linking agent and the lower the solvating power of the solvent.

In the extreme case we have a process like a precipitation polymerisation in each polymerising droplet and the microgel particles which are formed within these droplets at an early stage of the polymerisation gradually grow together.

After removing the inert compound, *e.g.* by steam distillation, we get a cross-linked polymer which is in most cases porous even in the dry state. This porosity will be called permanent porosity, but besides this permanent porosity these copolymers have a swelling porosity. Because of the heterogeneous structure of these copolymers we will call this method heterogeneous cross-linking polymerisation. The main factors governing the heterogeneity are the amount of cross-linking agent as well as the kind and amount of the inert compound. The heterogeneity of these cross-linked copolymers may be easily seen under a normal microscope<sup>5</sup> and with an electron microscope<sup>6</sup>.

Gels prepared in the presence of increasing amounts of inert substance in which they are insoluble show an increasing turbidity when observed by microscopy. The electron microscope allows one to see the pores directly and to measure the pore size distribution. Much more detail is obtained by scanning electron microscopy<sup>7,8</sup>. Here the steric arrangement of the pores is visualised much more clearly.

#### *Homogeneously cross-linked gels*

Fig. 1 shows the elution behaviour of homogeneously cross-linked poly(vinyl acetate) gels with different amounts of divinyl adipate as cross-linking compound. The same substances were tested with all gels in tetrahydrofuran. Log  $M$  is plotted against  $V_e$ . Over their linear part, the curves always have approximately the same slope, that is, from a given difference in molecular weight the same difference in elution

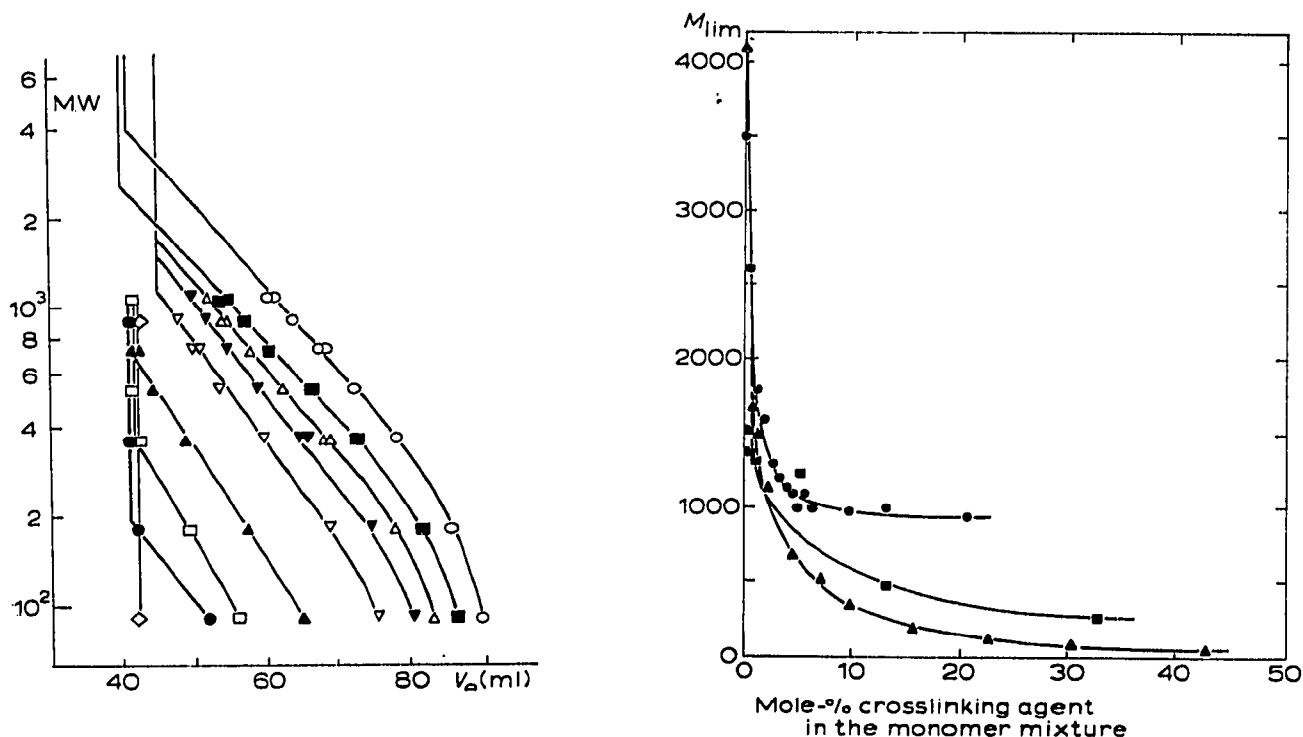


Fig. 1. Elution behaviour of homogeneously cross-linked copoly(vinyl acetates) Wt.-% divinyl adipate  $\circ$ , 0.5;  $\blacksquare$ , 1;  $\triangle$ , 2;  $\blacktriangledown$ , 3;  $\nabla$ , 5;  $\blacktriangle$ , 10;  $\square$ , 20;  $\bullet$ , 30;  $\diamond$ , 63.

Fig. 2. Excluded molecular weight of homogeneously cross-linked gels in dependence of the amount of cross-linking agent.  $\bullet$ , vinyl acetate-butenediol divinylether;  $\triangle$ , vinyl acetate-divinyl adipate;  $\blacksquare$ , methyl methacrylate-ethyleneglycol dimethacrylate.

volume results. With decreasing amount of cross-linking agent the excluded molecular weight gets larger but the mechanical stability decreases due to larger amount of swelling in these gels. The limit of practical use is in the range of 1% cross-linking agent and the corresponding excluded molecular weight is approximately 3000. With 63% divinyl adipate the cross-linking density is so high that even toluene cannot enter the pores of this network, toluene is eluted with  $V_0$ . The results of plotting the excluded molecular weight *versus* the amount of cross-linking agent are shown in Fig. 2.

Values for vinyl acetate gels cross-linked with butanedioldivinylether and with divinyl adipate, respectively, as well as methyl methacrylate gels are given. Each kind of gel gives a different curve. Obviously the copolymerisation behaviour of the cross-linking compound has a marked influence. Vinyl ethers have a poor tendency to copolymerise with vinyl acetate, that is, the cross-linking density is not very high even with high amounts of divinyl ether. The porosity of the homogeneously cross-linked gels is effected by the swelling. The specific gel bed volume is a measure of the swelling.

Plotting the excluded molecular weight *versus* the gel bed volume results in an approximately linear relationship. Values for 2 types of polyvinyl acetate gels, polymethyl methacrylate gels and polystyrene gels are on the same curve. The fact that the two types of polyvinyl acetate gels have the same dependence is remarkable but due to their chemical similarity this conclusion is fairly safe.

Using these homogeneously cross-linked gels a number of oligomers could be separated<sup>9</sup>. Only typical examples are given here. Fig. 4 shows a separation of a low molecular weight polystyrene, prepared with butyl lithium. The figures at the peaks

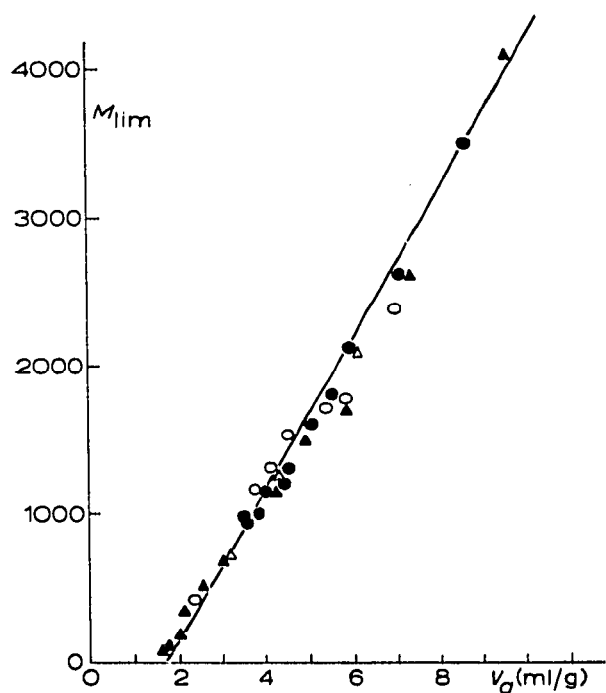


Fig. 3. Excluded molecular weight *vs.* gel bed volume for homogenously cross-linked gels. ●, vinyl acetate-butenediol divinyl ether; ▲, vinyl acetate-divinyl adipate; Δ, methyl methacrylate-ethylene glycol dimethacrylate; ○, styrene-divinylbenzene.

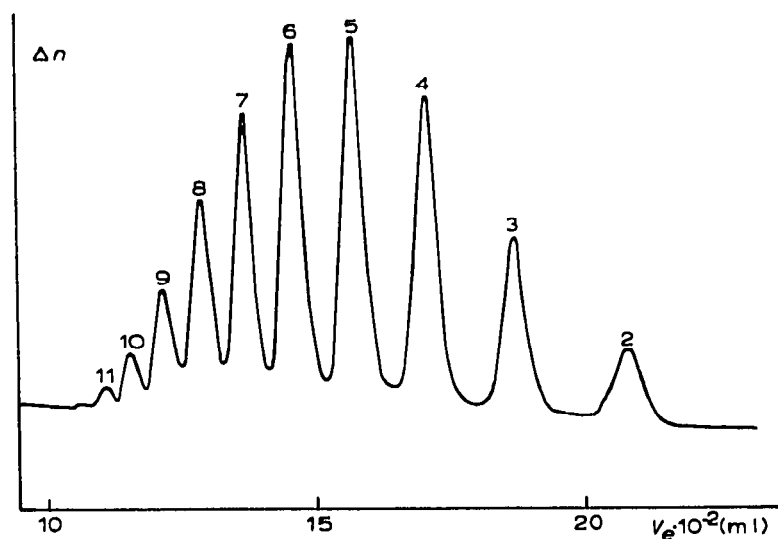
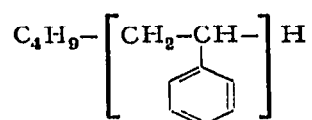


Fig. 4. Gel chromatogram of



( $M_n = 580$ ). Experimental conditions: polystyrene gel cross-linked with 2% divinylbenzene, column:  $200 \times 5$  cm, eluent: tetrahydrofuran.

correspond to the degree of polymerisation. Integration of the peak area gives an  $\bar{M}_n$ -value of 580 in good agreement with value of 583 obtained by vapour phase osmometry. Fig. 5 shows a separation of a polyethylene oxide. The lower curve shows the separation of a polyethylene oxide with  $\bar{M}_n \approx 600$ . Here again the figures at the peaks correspond to the degree of polymerisation. To obtain curve b, polymerisation degrees of 9 and 15 were added together resulting in an enlargement of these peaks. In Fig. 6 the limits of separation can be seen. Although we are well below the excluded molecular weight the high molecular weight end of this distribution is not resolved.

Similar separations can be obtained with tensides (Fig. 7) and epoxides (Fig. 8).

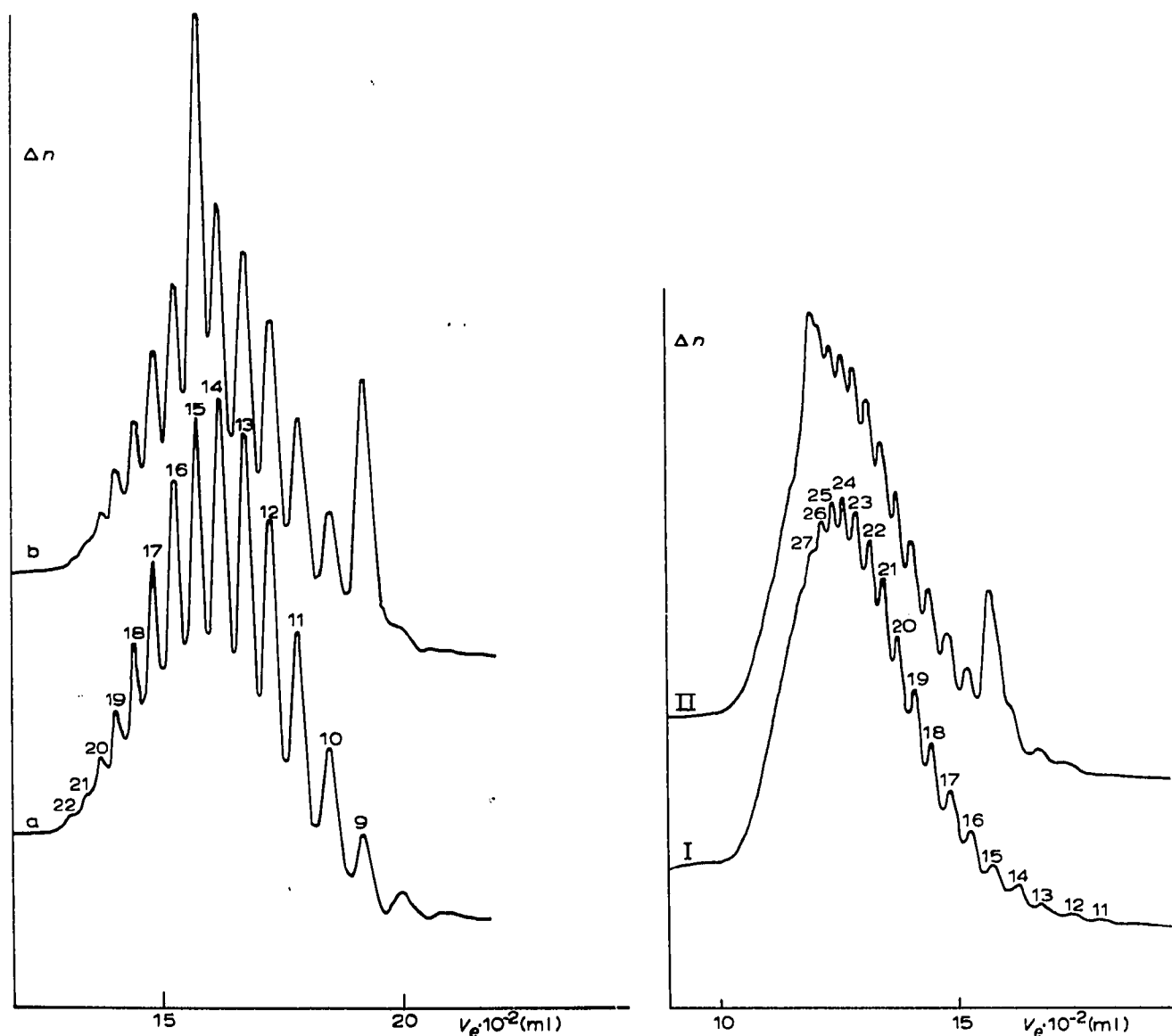


Fig. 5. Gel chromatogram of  $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$ . Curve a:  $\bar{M}_n \approx 600$ ; curve b:  $\bar{M}_n \approx 600 + n = 9$  and 15.

Fig. 6. Gel chromatogram of  $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$ . Curve I:  $\bar{M}_n \approx 1000$ ; curve II:  $\bar{M}_n \approx 1000 + n = 15$  and 27.

The separation of the epoxides (Fig. 8) shows that 2 series of oligomers are present, their peaks are differing in size and in alternating sequence.

The examples given show that a separation of the low members of a polymeric

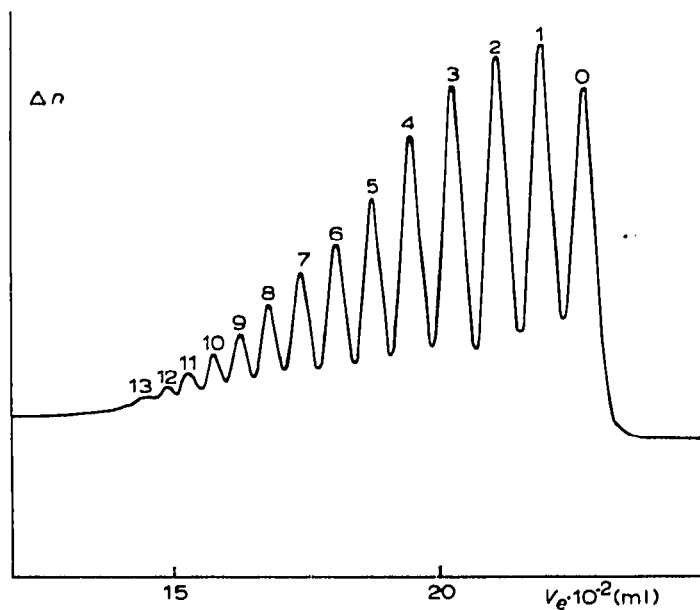


Fig. 7. Gel chromatogram of a tenside  $R-O(CH_2CH_2O)_nH$ .

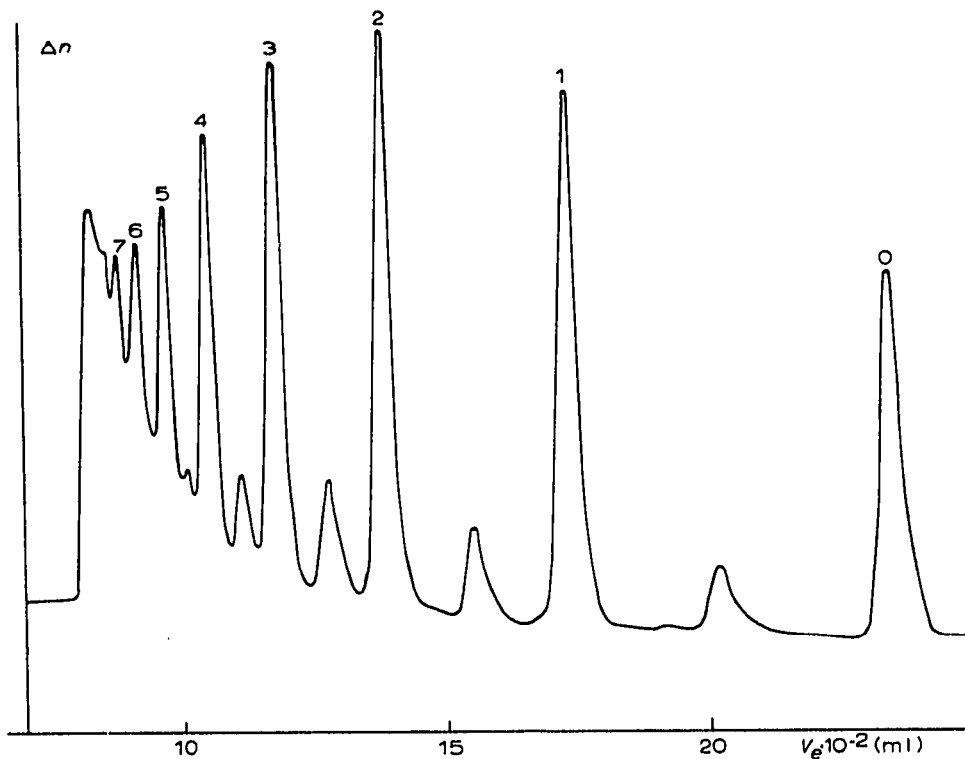
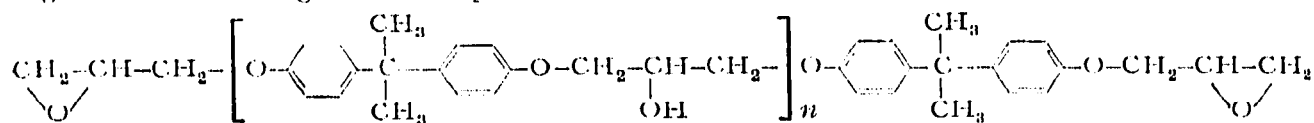


Fig. 8. Gel chromatogram of an epoxide resin



homologous series is possible. However, due to the limited volume there is no way of extending this range very much. An unresolved peak is obtained which consists of a dense sequence of overlapped peaks. Furthermore with homogeneously cross-linked gels the excluded molecular weights attained are too low. The method of heterogeneously cross-linking polymerisation must therefore be used to prepare suitable gels.

#### *Heterogeneously cross-linked gels*

The most important factors governing the heterogeneity of the network are the kind and the amount of inert compound as well as the cross-linking density. The copolymerisation of vinyl acetate with divinyl adipate was investigated with butyl acetate as a good solvent and *n*-octane as a non-solvent. Fig. 9 shows the influence of the amount of inert compound on the apparent density. The apparent density is the density of the copolymer plus the air in the pores. Independent of the amount of butyl acetate the apparent density of the copolymers from vinyl acetate and 40% divinyl adipate is constant although the excluded molecular weight rises sharply between 40 and 50% butyl acetate. This change in heterogeneity causes no change of the apparent density, that is, they have no permanent porosity. The effect of *n*-octane is completely different. Copolymers prepared in presence of more than 20% *n*-octane show permanent porosity as can be seen from the change in apparent density. The gel chromatographic properties of these gels are discussed later.

The influence of the concentration of the cross-linking compound using a good solvent as the inert compound is shown in Figs. 10 and 11. Between 40 and 50% divinyl adipate the apparent density changes sharply, but this change amounts to about 10%, although during polymerisation about 2/3 of the organic phase consisted of butyl acetate, that is, the permanent porosity is only a small fraction of this amount. The uptake of an organic liquid by a cross-linked copolymer is called solvent regain.

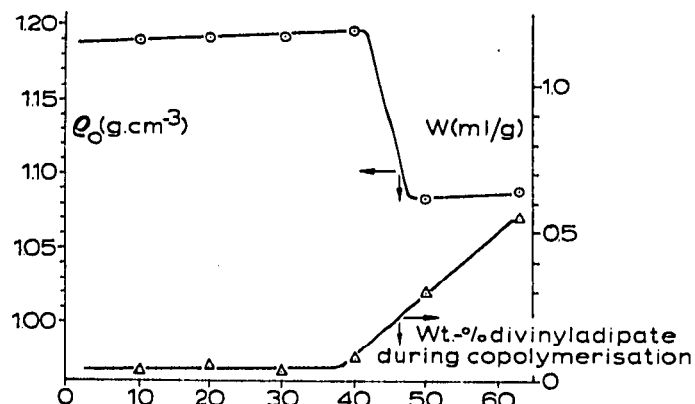
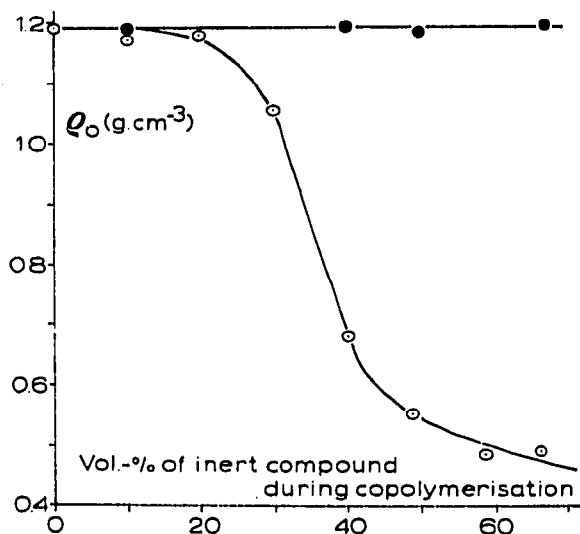


Fig. 9. Apparent density  $\rho_0$  of heterogeneously cross-linked polyvinyl acetate gels ○, *n*-octane, 20 wt.-% divinyl adipate; ●, *n*-butyl acetate, 40 wt.-% divinyl adipate.

Fig. 10. ○, Apparent density  $\rho_0$  and △, *n*-heptane regain *W* for copolymers of vinyl acetate-divinyl adipate, polymerised in the presence of 67 vol.-% *n*-butyl acetate.



When a polymer with permanent porosity is brought into contact with a non-solvent this can only enter the permanent pores, that is, the solvent regain for the non-solvent also characterises the heterogeneity of a gel structure. The lower curve shows the solvent regain obtained with *n*-heptane. Only if the amount of cross-linking compound is greater than 40% do we get appreciable *n*-heptane regain. But this *n*-heptane regain is higher than expected from the apparent density. This means that even the non-solvent *n*-heptane is able to swell this cross-linked copolymer of vinyl acetate.

The volume swelling was determined by the volume ratio of the gel in the swollen and unswollen state. With increasing amount of divinyl adipate the volume swelling decreases but at about 30% we get a sudden break in this dependence. Assuming a homogeneous distribution of the network density the excluded molecular weight should decrease with increasing amount of cross-linking compound. But this curve shows that the excluded molecular weight increases with increasing amount of cross-linking agent and this clearly shows that heterogeneity increases. Even using a good solvent such as butyl acetate the excluded molecular weight obtainable is only in the range of about 100000. If higher excluded molecular weights are required non-solvents must be used as inert compounds.

Using higher amounts of *n*-octane the gels have permanent porosity, as shown by lower values for the apparent density. The copolymers show permanent porosity if more than 20% *n*-octane is added. But the conclusion that these gels have high excluded molecular weights is rejected by Fig. 12. In both cases curves with maxima are obtained. These maxima agree with the onset of permanent porosity. This maximum is found if a non-polar non-solvent is used as an inert compound. Similar behaviour has been found by MOORE with polystyrene gels<sup>3</sup>. If the hydrophobic properties of the inert compound are higher than those of the copolymer, the microgel particles which form at an early stage of polymerisation tend to accumulate in the phase boundary. The assumption that these gels have a dense shell is supported by their behaviour in gel chromatography. Fig. 13 shows the behaviour of such a gel

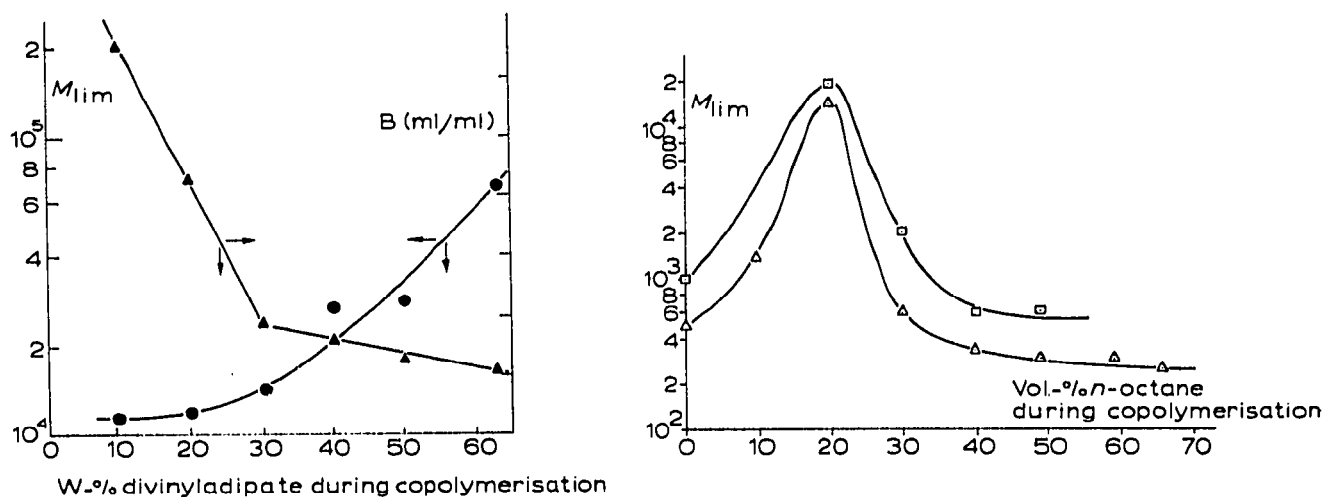


Fig. 11. ●, Excluded molecular weight  $M_{lim}$  and ▲, volume swelling B of copolymers of vinyl acetate-divinyl adipate, polymerised in presence of 67 vol.-% *n*-butyl acetate.

Fig. 12. Excluded molecular weight  $M_{lim}$  of copoly(vinyl acetates) prepared in presence of □, 10 and Δ, 20 wt.-% divinyl adipate.

together with the corresponding peak forms. From the elution volume of a low molecular weight substance one might extrapolate to an excluded molecular weight of  $2 \cdot 10^4$ . But substances with molecular weights higher than 800 are all eluted with  $V_0$ . The broad and unsymmetrical shape of the curves show that these gels have a kinetically controlled behaviour. Such behaviour is to be expected if the gels have the proposed structure. All gels on the right hand side of the maximum in Fig. 12 show a similar behaviour. Furthermore these gels are brittle and are easily ground to a powder. If polar non-solvents such as *n*-heptanol are used this effect is not observed. In this case excluded molecular weights of  $10^7$  and higher are obtainable. These gels have a good mechanical stability and separation characteristics for polymers.

Fig. 14 shows a separation in the medium molecular weight range using a column length of 25 cm. Analysis time is less than 1 h. The gel has an excluded molecular weight of 11000. The peaks are symmetrical. This separation was done as a preliminary experiment in order to optimise analysis time.

In accordance with the theory of van Deemter and Giddings a normalised presentation of separation efficiency is obtained if reduced quantities are used<sup>10</sup>. Due to the small diffusion coefficient of the polymers the values for the reduced velocity  $v$  ( $v = vd_p/D$ ;  $h = H/d_p$ ;  $h =$  reduced plate height,  $v =$  linear velocity;  $H/d_p$ ;  $H =$  HETP,  $d_p =$  mean particle diameter;  $D =$  diffusion coefficient of the solute in the mobile phase) are high. This allows this dependence to be approximately represented by a straight line through the origin ( $h \sim d_p$ ). So we have  $H \sim d_p^2$ .

If we decrease the particle size by a factor of two we can reduce the column

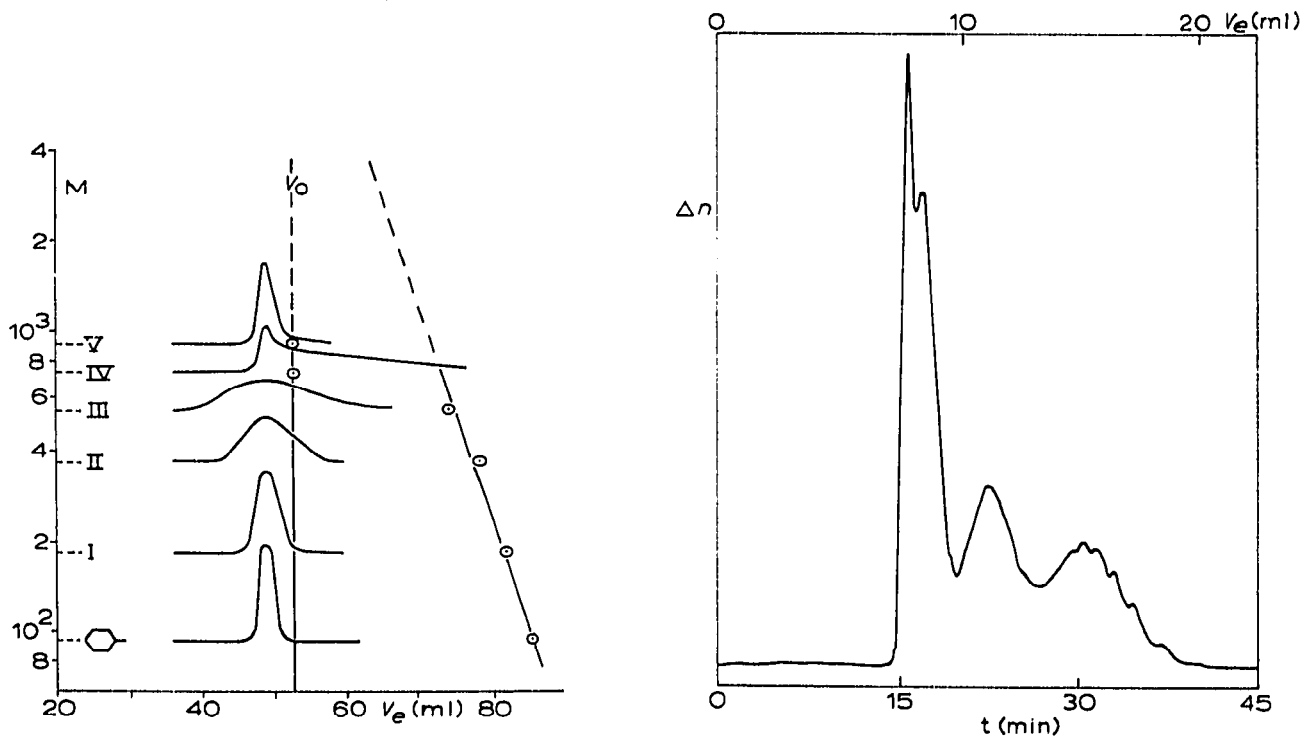


Fig. 13. Elution behaviour of copoly(vinyl acetates) prepared with 40 vol.-% *n*-octane in the organic phase and 10 wt.-% divinyl adipate in the monomer mixture.

Fig. 14. Separation of polystyrenes  $\bar{M}_n = 173000$ ; 10300; 2000; 600. Column length 25 cm.

length by a factor of 4. Using the same linear elution velocity the analysis time is also reduced to 1/4. As the pressure drop  $\Delta p \sim l/d_p^2$ ,  $l =$  column length, we get no increase in pressure. These two effects cancel out.

Homogeneously cross-linked gels show a linear dependence between excluded molecular weight and the specific gel bed volume. For heterogeneously cross-linked gels the swelling and therefore the specific gel bed volume is not so characteristic. The relative increase in volume is a more characteristic quantity. This increase in volume can be determined by the ratio of the gel bed volume in the swollen and unswollen state. We will call this quantity volume swelling.

This quantity can never be smaller than 1 and furthermore characteristic behaviour should be expected for the volume swelling if the network density is very homogeneous.

Fig. 15 shows the dependence of  $\log M_{lim}$  resulting from volume swelling for poly(vinyl acetate) gels. Curve I represents the homogeneously cross-linked gels; the experimental points are omitted here. The deviation from curve I is a measure of the heterogeneity of the gel structure. A variation in the concentration of the inert compound results in the curves II, III and IV. With increasing amount of inert compound we are approaching area *d*. A variation in the concentration of the cross-linking compound results in the curves V and VI. With increasing amount of cross-linking compound we go onto these curves from the right to the left. The dotted line VII marks the boundary between copolymers with permanent porosity and swelling porosity. Copolymers with permanent porosity are marked by a big circle. The lowest possible value for volume swelling is 1, that is, no values can be found in field *a*, furthermore no values were found in field *b*. If any one succeeds in preparing gels with uniform mesh size of the network they would belong to area *b*. This shows a difficulty

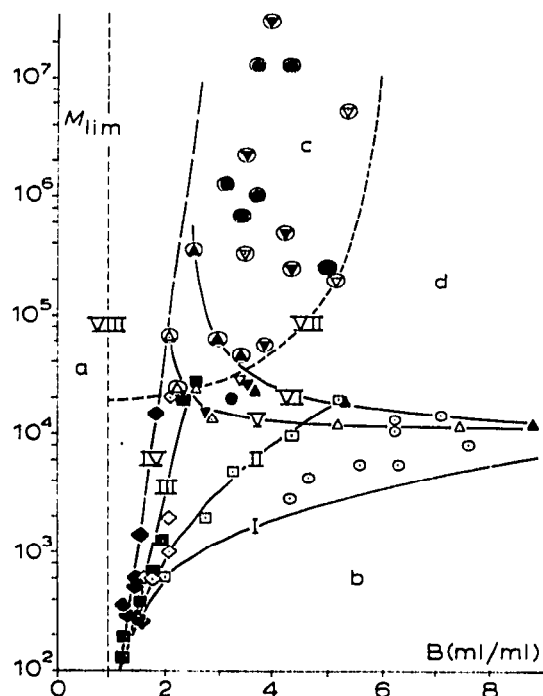


Fig. 15. Excluded molecular weight  $M_{lim}$  vs. gel bed volume  $B$  for copoly(vinyl acetates). For explanation see text.

necessarily connected with this problem: The gels should have a large swelling capacity if appreciable excluded molecular weights are wanted. In area  $d$ , limited by curves VI and VII we also found no values. Too large a dilution of the monomer mixture either results in the formation of microgels or we get syneresis; that is the inert compound is expelled with contraction of the gel.

From different points of view it is of interest to investigate gels where the swelling porosity is negligible, that is, gels which occupy the same volume in the swollen and unswollen state. Styrene homogeneously cross-linked with 55 % divinylbenzene has practically no swelling capacity. For this reason the corresponding heterogeneously cross-linked copolymers were investigated. Fig. 16 shows some data obtained with heterogeneously cross-linked polystyrenes using 55 % of divinylbenzene. The monomer mixture was composed of 400 ml mixture of monomers and the amount of inert compound is given as the abscissa. On the left of the double line the values of the homogeneously cross-linked copolymer are given. In all the other cases the ratio of monomer/inert compound was 1:2. The inert compound consisted of amyl alcohol and toluene. Increasing the amount of amyl alcohol means a decrease in the solvent power.

The homogeneously cross-linked gel has an apparent density of 1.16. All copolymers prepared in presence of inert compounds have a low apparent density, but only in this range does the decrease in density agree with the amount of inert compound. As a ratio of monomer to inert compound of 1:2 was used the apparent density should be reduced to 1/3 of the value of the homogeneously cross-linked copolymer if the inert compound is decisive for the pore volume.

The values of the inner surface were determined by the BET-method. The homogeneously cross-linked gel has a surface area lower than 1 m<sup>2</sup>/g. That is the surface of the compact spheres. With decreasing solvent power the inner surface increases to values higher than 500 m<sup>2</sup>/g and then it decreases. This behaviour is just as would be expected. With increasing pore size we need less pores for the same volume, that is, the inner surface becomes smaller. The decrease observed when using toluene alone

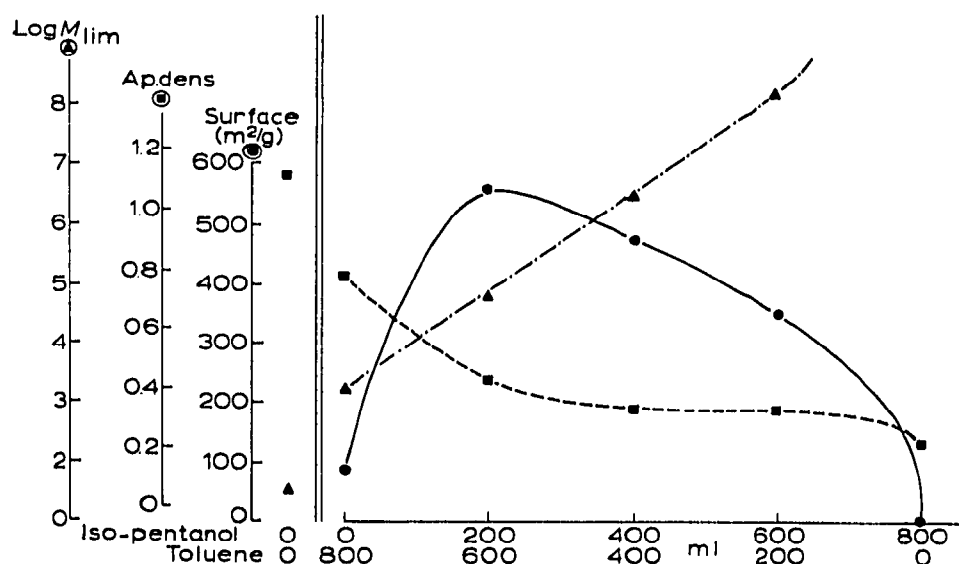


Fig. 16. Properties of heterogeneously cross-linked polystyrenes (55 % divinylbenzene). Each run composed of 400 ml monomer mixture and the amount of inert compound *cf.* abscissa.

is caused by a lower pore volume as the lower apparent density shows. The excluded molecular weight increases with decreasing solvent power to values of  $10^6$ ,  $10^8$  and finally to a value which cannot be extrapolated.

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