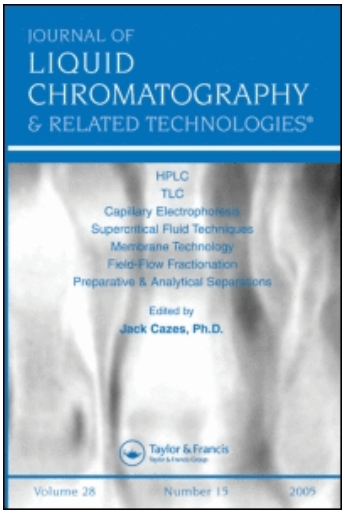


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# The Use of Polystyrene Gels in Preparative Recycle-HPLC for the Separation of Structurally Related Molecules

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# THE USE OF POLYSTYRENE GELS IN PREPARATIVE RECYCLE-HPLC FOR THE SEPARATION OF STRUCTURALLY RELATED MOLECULES*

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## ABSTRACT

This paper deals with the use of polystyrene gels in high performance liquid chromatography for preparative separations of small molecules with very similar structures. The preparative chromatograph, equipped with a recycle device and made in our laboratory, is described. The column set, consisted of three columns packed with low porosity polystyrene gel. The mobile phase was diisopropylether. Three particle sizes (10μ, 20μ, 50μ) were studied for improving the preparative performance. We checked the very high efficiency of our recycling system versus sample load. High sample loadings (up to 100 grams) can be injected when the separation does not require a large number of plates but typical sample loads are 10-20 grams for high performance separations. Some examples of separation of diastereoisomers, configurational isomers or related structure molecules are given. The advantages of our preparative system are discussed.

*Presented at the 3rd International Symposium on Column Liquid Chromatography - Salzburg, September 1977.

## INTRODUCTION

Initially, during its early developmental period, liquid chromatography was a preparative technique. A mixture of compounds was introduced into a chromatographic system, and components were recovered separately after elution. However, the very important technological advances of the ten pasts years have been focused on the analytical aspects of chromatography. High performance liquid chromatography can now be used to perform analyses of milligram quantities only in a few minutes. During this period, preparative aspects were almost completely neglected, except for the specific problem of polymer fractionation by gel permeation chromatography. A preparative instrument was supplied by Waters Associates ([1]) and hot-GPC preparative fractionation of polyolefins was performed by Peyrouset and Panaris ([2]) leading to the commercialization, by S.N.E.A.P. ([3]), or narrow fractions of polyethylene.



Suppliers of chromatographic instrumentation only recently showed serious interest in preparative liquid chromatography. Two preparative instruments simultaneously appeared on the market about four years ago :

- The Chromatospac-prep 100 of Jobin-Yvon ([4]) which uses silica beads under axial compression.
- The prep LC/system 500 of Waters Associates ([1]), using radial compression technology, with prepacked cartridges filled with silica and, more recently, with C 18-silica bonded phase packing.

Both instruments are primarily recommended for chromatography with silica-based materials. At the present time, there is no possibility of preparative chromatography on polymeric materials such as cross-linked polystyrenes packings. Therefore we have studied, in the present work, the preparative separation of molecules with very similar structures (isomers, configurational isomers and diastereoisomers) previously performed at the semi-preparative scale with polystyrene packings ([5-10]).

## EXPERIMENTAL

Polystyrene packings, such as Poragel and Styragel, supplied by Waters Associates, are porous material for gel permeation chromatography. They are very efficient packings for polymer fractionation by steric exclusion, over a very broad range of molecular weights. Particularly, low porosity materials are efficient for species having molecular weights smaller than 2 000. They are swollen by organic solvents, allowing the steric exclusion process but there is often also a partition mechanism between mobile phase and swollen gel ([11]).

This last effect, considered as a disturbing phenomenon in GPC, is often used to one's in liquid chromatography. However, the selectivity of these materials is low, since molecules are eluted between the dead volume $V_0$ and approximately 2 $V_0$. Therefore, capacity factor k' are between 0 and 1, exceptionally 2 when the partition process is strong. On the other hand, this low selectivity can be dramatically enhanced by the recycling method ([8])([12]) which consists in eluting solutes several times on the same column in succession, artificially increasing the column length.



### Selection of the stationary phase.

A preliminary study was first carried out to select the most suitable stationary phase for preparative work. We have thus compared three styrene-divinylbenzene packings whose performances, efficiency versus sample loading, are plotted in Figure 1.

- Poragel 60 Å, particle size 37-75μ (curve C). This classical material was previously used in our semi-preparative work with diastereoisomers and configurational isomers ([5-10]). Its low efficiency (≃ 400 plates per foot) leads to very long runs.

- Microstyragel 100 Å, particle size 10μ (curve A), which is a high speed GPC packing, is very efficient. Unfortunately, its very high cost makes its use impossible at a preparative scale.

- Styragel 100 Å, particle size 15-25μ (curve B). The performance of this intermediate material is located between the two first ones.

Figure 1 shows that variations of packing efficiencies versus their particle sizes are in a good agreement with theory ; the higher the efficiency, the greater is the effect of overloading.

For these reasons, its seems that the intermediate material is the most appropriate one for preparative work.

Selection of the mobile phase.

A commonly used mobile phase for styrene-divinylbenzene packings is tetrahydrofuran (THF) ; it is a good solvent for many polymers. For preparative chromatography the two most important mobile phase properties are :

- Volatility, because following chromatographic separation, solutes have to be recovered from very dilute solutions ; solvent evaporation is the most commonly used recovery method.

- Purity and stability. If non-volatile solvent impurities are present, they will become concentrated during evaporation and thus contaminate the prepared sample fractions.

Tetrahydrofuran is volatile but not stable. It forms peroxides which lead to non volatile compounds and restrict its use in preparative chromatography. We replaced it with another ether, diisopropylether, a more stable compound which does not have these drawbacks. This ether, a poorer solvent for polystyrene than THF, swells the Styragel less than does THF (about 15%). Therefore solvent exchange is impossible and columns have to be packed with a diisopropylether-polystyrene slurry.

On the other hand, stationary phase-solute interactions increase in this mobile phase, particularly for aromatic compounds, and partition and adsorption mechanisms occur in addition to the

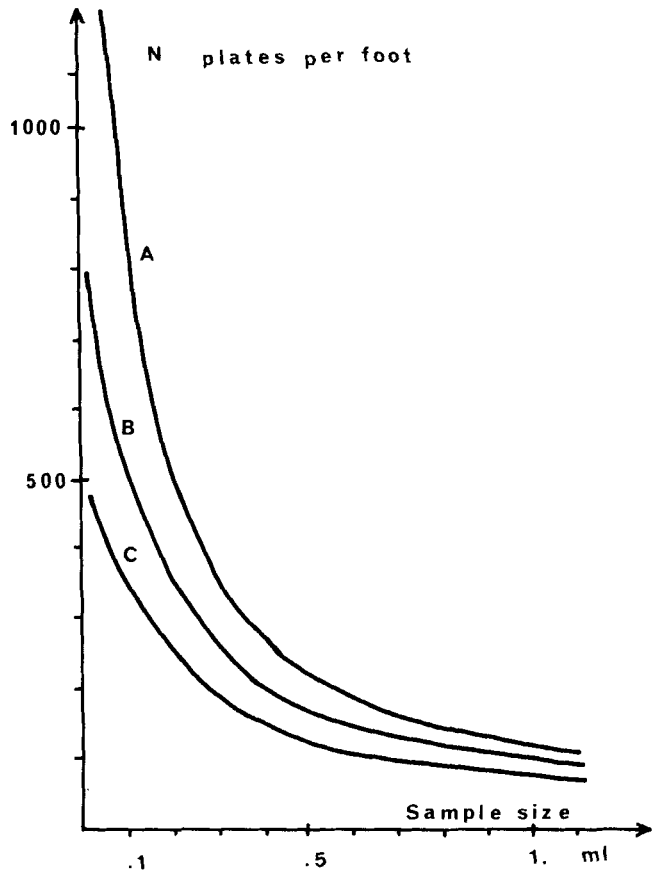



Figure 1 : Plots of cross-linked polystyrene packing efficiencies (A = microstyragel 100 Å, 10μ - B = styragel 100 Å, 15-25μ - C = Poragel 60 Å, 37-75μ) versus sample size. 3 columns (3' length, 3/8" O.D.) - Mobile phase : diisopropylether 2 ml/mn. Solute : pure cyclohexane.

steric exclusion process ; thus the Styragel-diisopropylether system suggests great possibilities in a broad range of separation problems.

Apparatus.

As commercially available preparative instruments are not generally suitable for organic packings, we built an apparatus whose capacity is about 15 times higher than the instrument that was used for semi-preparative preliminary separation ([5-10]). The schematic diagram of this apparatus is given in Figure 2. It consists of :

- a high capacity solvent reservoir (1) (≃ 10 liters), with a filter (2).

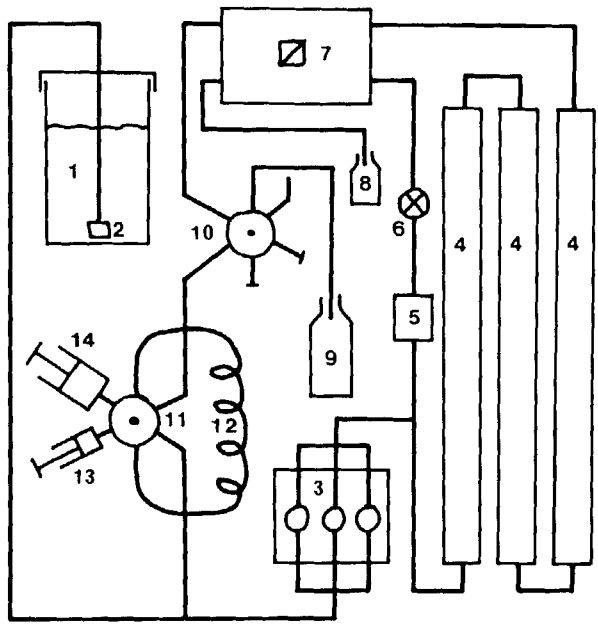



Figure 2 : Schematic diagram of the preparative instrument. 1. solvent reservoir - 2. Filter - 3. Pumping system - 4. Columns - 5. Pressure transducer - 6. Valve - 7. Differential refractometer - 8. Waste - 9. Fraction collector - 10. Recycle 6-port valve - 11. Injection 6-port valve - 12. 100 ml loop - 13. Sample syringe - 14. Solvent syringe.

- a diaphragm pump "Orlita" (3) with three heads at 120°. This pumping system has a low dead volume suitable for recycling and can operate at desired flow rates (up to 80 ml/mn) under high pressure (≃ 3 000 P.S.I.).

- a pressure transducer (5), connected in series with a valve (6) for purging the detector reference line.

- a set of 3 columns (4). Each column is 150 cm length and 2.6 cm I.D.. Clamp end fittings are provided with 3 microns porosity porous metal discs.

- a Waters R 404 refractometric detector (7) fitted for the high concentration that are usually encountered in preparative chromatography.

- a 6 port-valve (10) for recycling. In "recycle" position, eluates flow towards the pumping system and in "collect" position, towards a fraction collector (9).

- an injection system composed of a 6 port-valve (11), a 100 ml loop (12), a sample syringe (13) and a solvent syringe (14). The loop can be partly or completely filled. Recycling permits insertion of this device into the recycle line ahead of the pumping system, where the pressure is low. This position is advantageous to avoid usual high pressure when injector is located between the pump and columns and the only drawback is that the eluates must pass through the pumping system one time more.



The columns were packed by the slurry packing method. Gel is suspended in diisopropylether and allowed to swell. The column, topped with a 80 cm long precolumn, is filled with the slurry. Gel packing is achieved at a flow rate of 50 ml/mn for about 4 hours with recycling to avoid excessive consumption of solvent.

## RESULTS AND DISCUSSION

### Performance of preparative columns.

Column set was composed of 3 columns packed with Styragel 100 Å, particle size 15-25µ. Efficiency was checked with injec-

tions of pure heptane at various flow rates. Figure 3 shows the variations of the theoretical plate number versus sample size of heptane at 4 flow rates. Efficiency dramatically decreases with overloading or when flow rate increases. These classical phenomena lead to the best efficiency at low flow rates and small sample loadings. These criteria, useful in analytical chromatography, are

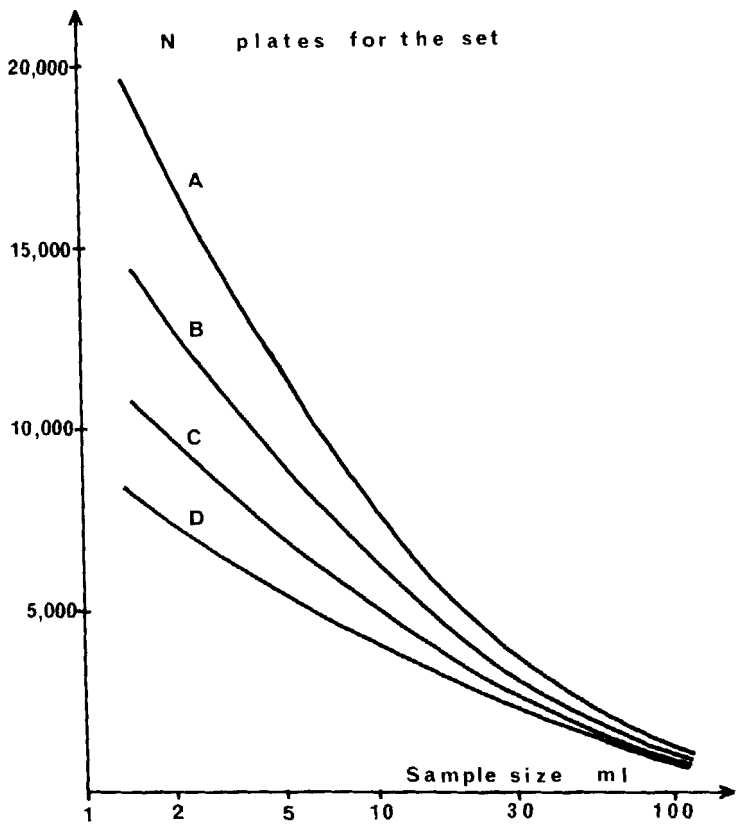



Figure 3 : Plots of preparative column efficiencies versus sample size. 3 columns (150 cm length, 2.6 cm I.D.) packed with Styragel 100 Å 15-25µ. Mobile phase : diisopropyl-ether (A : 5 ml/mn - B : 10 ml/mn - C : 25 ml/mn - D : 40 ml/mn). Solute : pure heptane.

not convenient in preparative chromatography where throughput per unit time is of prime importance. We have, thus, studied both of these factors : time and loading.

In order to emphasize the influence of run time, we have plotted, in Figure 4, the plate number per minute versus flow rate for various sample loagings. This parameter increases with flow rate and, therefore, preparative runs have to be performed at high flow rates. Moreover, these curves show that best efficiency is obtained with the smallest loading which does not lead to optimization of the amount of material purified per unit time. Consequently, we have plotted in Figure 5, the plate number x grams per minute for various loadings versus flow rate. In fact, this parameter takes into account both run time and amount of purified material and seems to be more convenient for preparative chromatography. It is obvious that maximum efficiency is



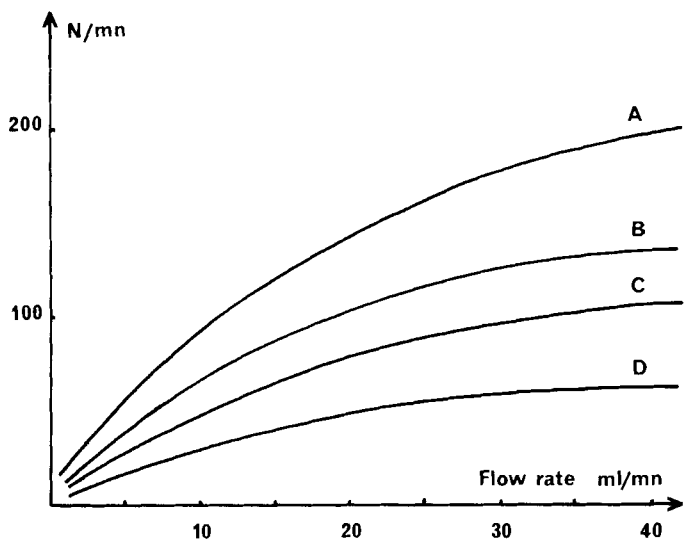



Figure 4 : Plots of the plate number per minute versus flow rate. Same conditions than in figure 3 with (A : 2 ml - B : 5 ml - C : 10 ml - D : 30 ml).

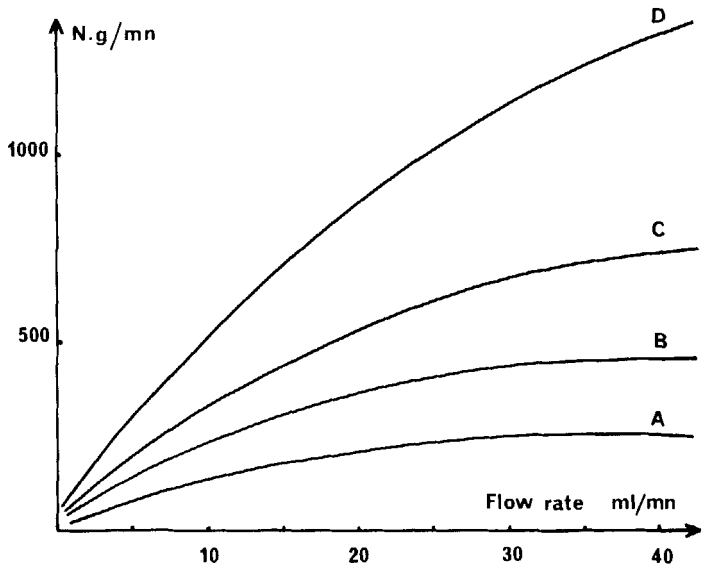



Figure 5 : Plots of the plate number x gram per minute versus flow rate. Same conditions than in figure 4.



obtained both for highest loadings and flow rates. However, we have to consider that the number of theoretical plates strongly decreases under these conditions and that it must be greater than the minimum plate number needful for adequate resolution. In recycle chromatography, we have established simple relationships leading to resolution for a couple of compounds ([7])([8]).

$$N \geq \frac{32\ R^2}{\delta} \text{ with } n \leq \frac{16\ R^2}{N\delta^2}$$

where N is the plate number, R the resolution, n the cycle number, $\delta = \Delta V/V$, the relative difference between elution volumes. The $\delta$ factor represents separation difficulty and can be expressed with classical separation factor $\alpha$ and capacity factor k' by :

$$\delta = \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{k' + 1} \text{ and } R = \frac{1}{4}\ \delta\ \sqrt{N}$$

We have fixed the resolution value R = 1.5, which corresponds approximately to the complete separation of two peaks and calculated optimum conditions which lead to resolution versus $\delta$. The number of grams per minute separable by the column set is plotted on Figure 6 versus $\alpha$ and $\delta$ factors. The yield of our instrument is high (few grams per minute) for easy separations ($\alpha > 2$), but quickly decreases as difficulty increases. It is only 1 g/mn for $\alpha = 1.5$, 0.4 g/m for $\alpha = 1.25$ and becomes very weak for very difficult separations (0.04 g/mn for $\alpha = 1.06$).

Experimental Performance.

We experimentaly checked performance described in Figure 6 using simple mixtures. These results, presented in Table 1 are in a good agreement with the curve of Figure 6.

Let us look at another interesting example of optimization of experimental conditions. This involves purification of practical divinylbenzene. This compound, widely used in polymer chemistry, is a mixture of isomeric divinylbenzenes (DVB) and ethylvinylbenzenes (EVB) in about 60-40 ratio. Figure 7 shows the chromatogram obtained with a sample loading of 30 grams and Figure 8 with a sample loading of 5 grams. Experimental values are given in the Table 2.

With a sample size of 5 grams, resolution is rapidly obtained after only 2 cycles (Fig. 8) and corresponds to .06 g/mn. But, if experimental conditions are optimized according to the curve of Figure 6, we can inject 30 grams (Fig. 7). Resolution is obtained after 4 cycles but yield is now .19 g/mn, that is, 3 time higher. On the other hand, 5 grams loading is convenient for the separation of meta and para isomers of DVB ($\alpha = 1.06$). After collection in the $2^{nd}$ cycle of the peak corresponding to ethylvinylbenzene isomers, complete resolution is obtained after 18 cycles, providing 7 milligrams per minute. As a general rule, optimum recycle conditions lead to the complete resolution of a couple of peaks just before their remixing.

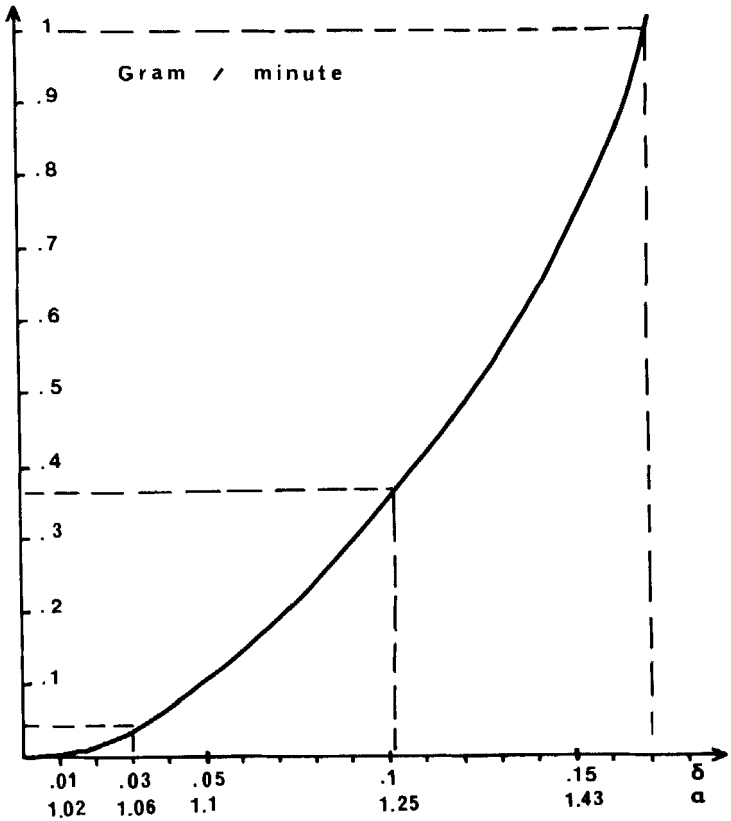



Figure 6 : Yield of the preparative instrument (in gram per minute) as a function of the separation difficulty ($\alpha$ or $\delta$ factors).

TABLE 1

| Solutes (50ml - 50ml) | $\delta$ | $\alpha$ | n | g/mn |
|---|---|---|---|---|
| Heptane - Benzene | .27 | 2.2 | 1 | > 3 |
| Heptane - Cyclohexane | .13 | 1.65 | 2 | 1 |
| Heptane - Hexadecane | .11 | 1.27 | 5 | .4 |
| Heptane - Dodecane | .05 | 1.10 | 10 | .1 |

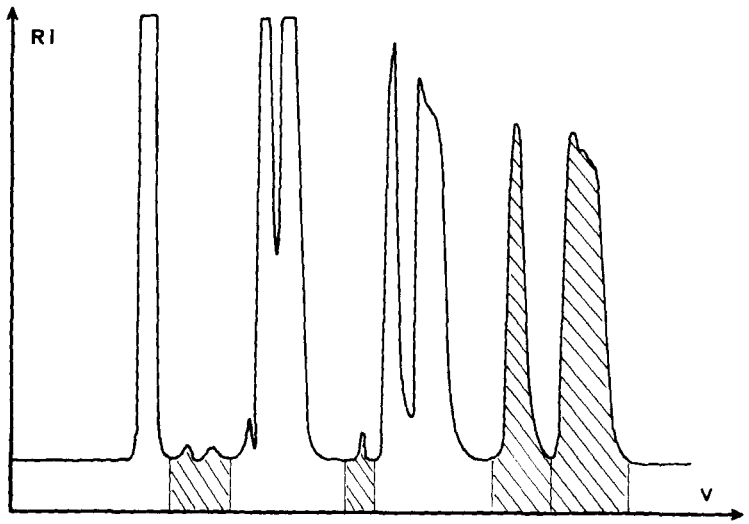



Figure 7 : Recycle chromatogram of DVB - EVB mixture, in same conditions than in figure 3 with flow rate : 40 ml/mn. Sample size : 30 grams.

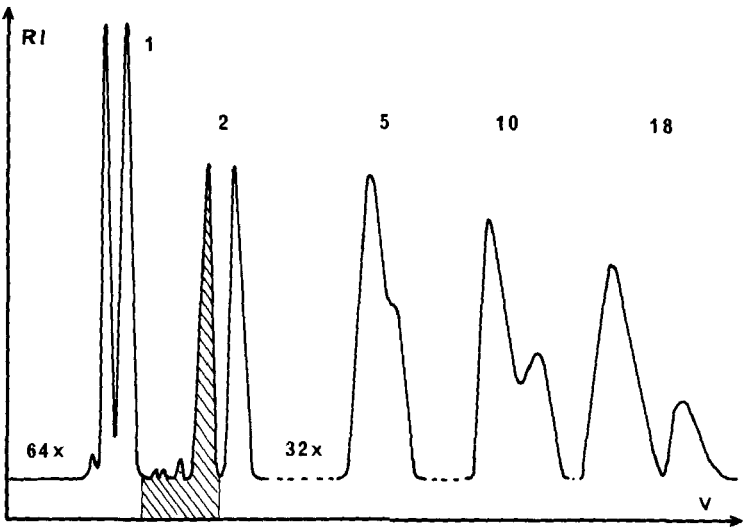



Figure 8 : Recycle chromatogram of DVB - EVB mixture in same conditions than in figure 3 with flow rate : 40 ml/mn. Sample size : 5 grams.

TABLE 2

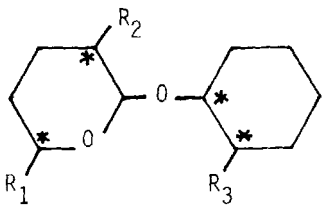
| Solutes | Sample size | δ | α | n | g/mn |
|---|---|---|---|---|---|
| EVB - DVB | 30 g | .1 | 1.25 | 4 | .19 |
| EVB - DVB | 5 g | .1 | 1.25 | 2 | .06 |
| meta DVB - para DVB | 5 g | .03 | 1.06 | 18 | .007 |

Separation of related structure molecules.

We performed some separations, with our instrument, of structurally related molecules such as diastereoisomers, configurational isomers and positional isomers.

*Diastereoisomers*

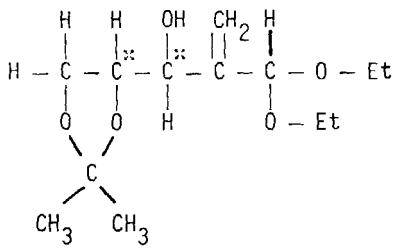
The model molecules of polysaccharides with the general formula shown below :



with $R_1$, $R_2$, $R_3$ = H, OH or $CH_2OH$

have two or more asymetric carbon atoms and are consequently mixtures of diastereoisomers. We achieved the preparative separation of a number of these mixtures ([10]).

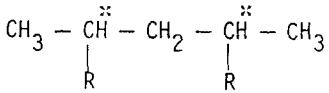
An other example is given by the diastereoisomer mixture of the molecules :



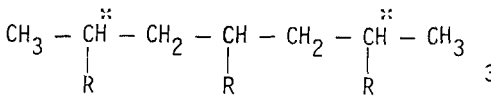
The two diastereoisomers ($\delta$ = .06, $\alpha$ = 1.14) are easily separated after 18 cycles at a flow rate of 40 ml/mn. Sample size of 15 grams leads to a yield of .05 gram per minute.

*Configurational isomers.*

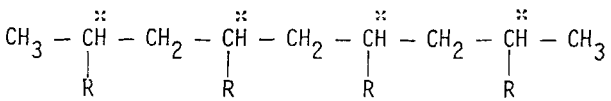
We performed the separation of mixtures of configurational isomers, representing model compounds of vinylic polymers :



2 asymetric carbon atoms
2 isomers (1 iso, 1 syndio)

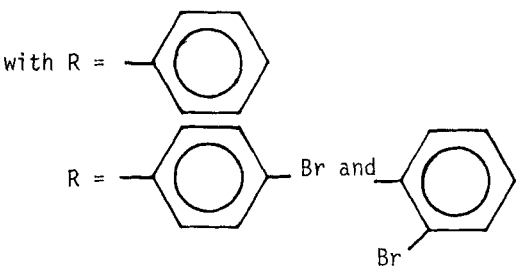


2 asymetric carbon atoms
3 isomers (1 iso, 1 hetero, 1 syndio)

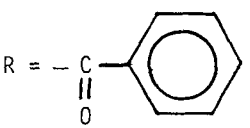


4 asymetric carbon atoms - 6 isomers (1 iso, 4 hetero, 1 syndio).

(6)(8)




(9)




*Positional isomers and very similar structure molecules*

An example was described in an earlier paragraph. This is the purification of practical divinylbenzene, a mixture of D.V.B. and E.V.B. shown in Figures 7 and 8. Separation of DVB from EVB is achieved with 30 grams sample loading and a yield of 0.19 gram per minute. The difficult separation of meta and para isomers of DVB is performed with 5 grams sample loading in 18 cycles

which corresponds to a purified material amount of 7 milligrams per minute.

Interpretation.

Mechanisms occuring in the described separations are complicated. If size exclusion, typical of GPC process, surely arises, it is obvious that other processes as partition and adsorption phenomena in relation with solute-stationary phase interactions, also take place. Furthermore, these interactions are stronger than in regular use of polystyrene gels where the mobile phase is a good solvent for polystyrene. Diisopropylether is a poor solvent for polystyrene and, as a mobile phase, leads to stronger solute-stationary phase interactions. For these reasons, aromatic compounds are easily separated. Interactions occuring with the polystyrenic matrix are probably of an electronic nature. For example, DVB, which has two vinylic functions, is eluted after EVB which has only one function. Likewise, the separation of meta and para isomers of DVB can be explained on this basis. Meta-DVB, with two conjugated vinylic functions, is eluted slightly after para isomer whose vinylic functions are not conjugated. Thus, aromatic character seems to be an important parameter in the described separations.



## CONCLUSION

We have built a preparative liquid chromatograph, different from commercially available instruments since it uses a special kind of chromatography (reverse phase chromatography on macromolecular stationary phase). Its advantages are :

- structurally very similar molecules such as diastereoisomers, configurational isomers and positional isomers can be separated.
- the yield of this apparatus can reach a few grams per minute and we can easily consider that increasing internal diameter of columns up to 10 cm would lead to few ten grams per minute with few hundred grams sample loading.

- solvent consumption is low because of recycling. Solvent consumption only occurs when collecting peaks or impurities. A classical separation would consume about 3 liters of diisopropyl-ether, more if many impurities are in the mixture.

- instrument uses only one stationary phase-mobile phase pair ; therefore, only one column set, efficient in a broad range of separations.

- column life is very long. By contrast to chromatography on silica packings, irreversible adsorption phenomena which gradualy modify stationary phase properties do not occur. After more than 3 years running, we have not noticed any aging of our column set.



## ACKNOWLEDGEMENTS

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