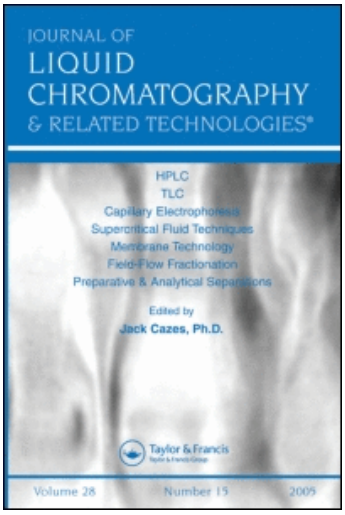

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Sulfated Oligosaccharides From K-Carrageenan and Oligogalacturonic Acids Separation By H.P.L.C.

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SULFATED OLIGOSACCHARIDES FROM K-CARRAGEENAN AND OLIGOGALACTURONIC ACIDS SEPARATION BY H.P.L.C.

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SUMMARY

Sulfated oligosaccharides produced from enzymic hydrolysis of K-carrageenan have been separated up to DP 13 on a column of μ-Bondapak C-18 or a Radial Pak cartridge "Dextropak" with sodium nitrate as the mobile phase. Moreover, the separation of oligogalacturonic acids has been performed by the ion pair method on the same supports with 0.05 % dodecyltributylammonium chloride in a sodium nitrate solution as eluent.

INTRODUCTION

High performance liquid chromatography has been widely used in determination of sulfated disaccharides produced from chondroitin sulfates (1-3) or heparan sulfate and heparin (4). Meanwhile, there is no paper on the separation of the sulfated oligosaccha-

rides from kappa-carrageenan . Recently, we have shown that fractionation up to DP 10 was obtained on a column of Bio-Gel P6 (5) but classical gel permeation chromatography is time consuming ; so it was interesting to solve this problem with a rapid and performant method. In a same way, we have tried to separate by H.P.L.C. the oligogalacturonic acids. These oligosaccharides have been studied with several chromatographic systems, and the most results have been summarized in a large review dealing with the separation of mono and oligosaccharides by liquid chromatography (6) ; high performance liquid chromatography has never been used for this fractionnation.

EXPERIMENTAL

Materials

Kappa-Carrageenan is a copolymer $(ab)_n$ purified as previously described (7-8). The enzymic hydrolysis was performed at pH 8.2 in 0.1M NaCl - 0.005M $NaHCO_3$ at 40°C with a kappa-carrageenase kindly given by Dr. Yaphe from Mc Gill University (Canada). The oligogalacturonic acids were obtained as a much appreciated gift from J.F. Thibault (Nantes - France) (9).

Apparatus

All separations were made using Waters Associates equipment : model M-6000 A solvent delivery system, model U6K universal injec-

tor, and a model R 401 refractometric detector. The recorder used was a Servotrace instrument (Sefram - France). The chromatographic supports were a prepacked column (4 x 300 mm) of a μ-Bondapak C-18 and a Radial Pak cartridge "Dextropak" for the radial compression system RCM-100 manufactured by Waters.

RESULTS AND DISCUSSION

Separation of sulfated oligosaccharides

In gel permeation chromatography process when a charged solute is chromatographed on different supports an electrostatic exclusion effect has to be taken into account and it is necessary to screen it by addition of an electrolyte to the eluent (10-12). The same phenomenom is observed on a reverse phase support ; so with the aim to suppress ionic effect and to obtain the same properties of the C-18 phase as found in the separation of neutral mono and oligosaccharides (13) a sodium nitrate solution has been used as the mobile phase in the concentration range 5.10^{-2}M - 1M. The chromatograms obtained are reproduced in Figure 1. The resolution is improved by increasing the ionic strength and in a 1 M $NaNO_3$ eluent sulfated oligosaccharides of kappa-carrageenan can be obtained up to DP 13. In opposite, there is no separation on the Dextropak cartridge when the salt concentration of the eluent is under 1M $NaNO_3$; the results are shown on Figure 2. The mechanism of separation of carbohydrate on a reverse phase column is due to hydrophobic interactions (13). Considering

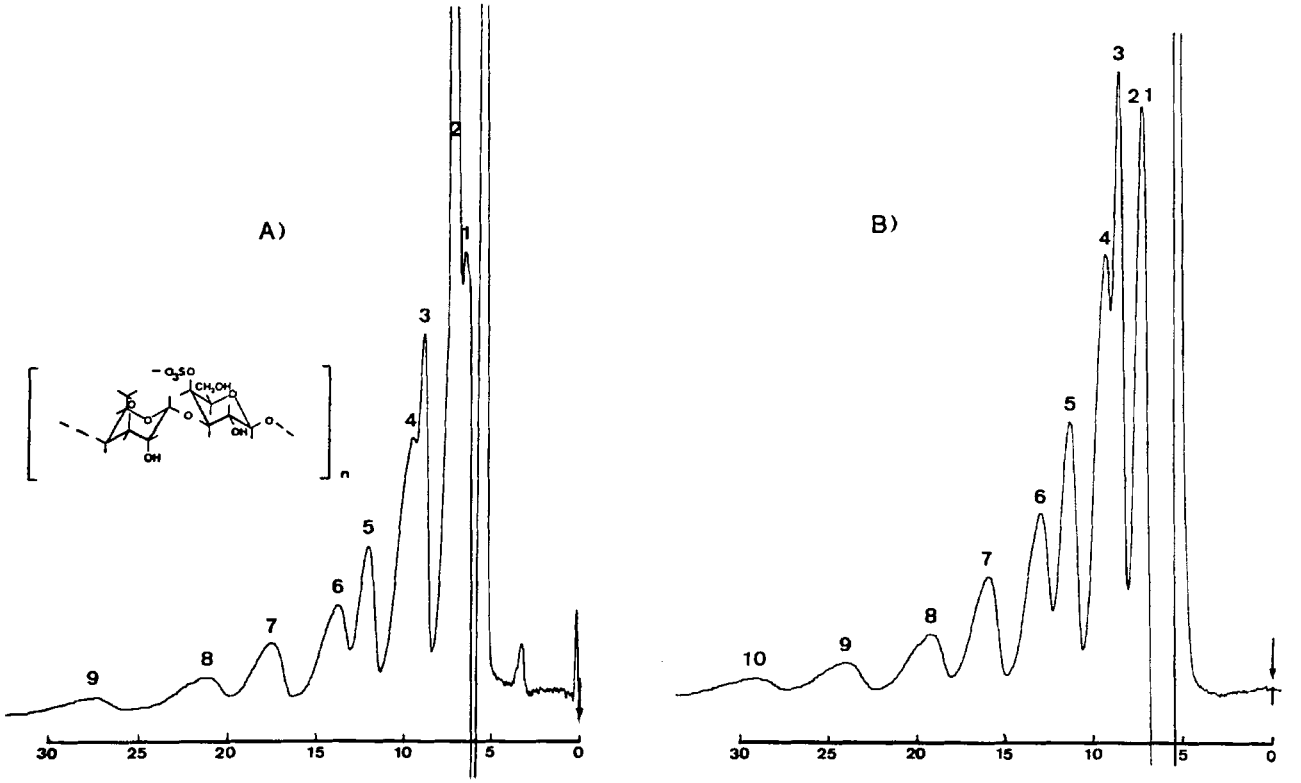

Figure 1 : Separation of K-carragennan oligosaccharides on C-18 μ-Bondapak column.
A - eluent : 0.1 M $NaNO_3$; flow rate : 0.5 ml/mn ; T = 20°C
B - eluent : 1 M $NaNO_3$; flow rate : 0.5 ml/mn ; T = 20°C.
The degree of polymerization is indicated by numbers over the peaks.

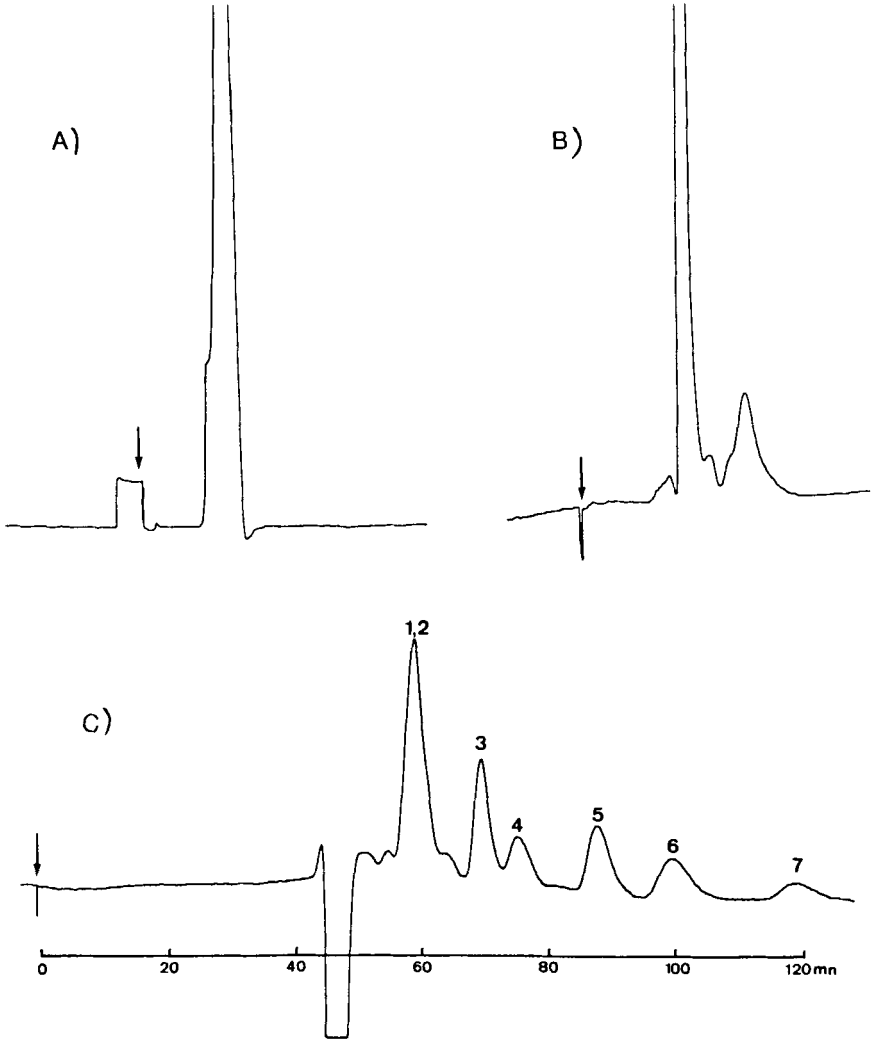

Figure 2 : Separation of K-Carrageenan oligosaccharides on "Dextropak" cartridge.
A - eluent : H_2O ; flow rate 1 ml/mn ; T = 20°C.
B - eluent : 0.1 M $NaNO_3$; flow rate 1 ml/mn ; T = 20°C
C - eluent : 1 M $NaNO_3$; flow rate 0.1 ml/mn ; T = 20°C
The degree of polymerization is indicated by numbers over the peaks.

the structure of the molecule (Figure 1) the hydrophobic anhydrogalactopyranosyl unit promotes the interactions ; the interaction strength with the support is controlled by the salt concentration. The sulfated polysaccharide is not kept back on the phase but it is excluded and eluted before the DP 1. In this case it seems that the charge density is too high and the hydrophobic interactions do not control the elution.

The behaviour observed on both columns can be explained by differences in hydrophobic interactions and structure of the two phases. In Dextropak, the paking is formed by more regular μ-spherical silica particles ; the surface silanol groups are modified by reacting with dimethyloctodecylchlorosilane but μ-Bondapak C-18 is more hydrophobic (14) due to an additional treatment with trimethylsilyl group on residual silanol groups.

As it has been found, hydrophobic bonds are enhanced when the salt concentration is increasing (15) ; so in 1 M $NaNO_3$ eluent, the hydrophobicity of both paking should become nearly the same.

Separation of oligogalacturonic acids :

The galacturonic acid unit has a weak hydrophobic character, due to the presence of the carboxylic sites, so no separation occurs in $NaNO_3$ media. The reverse phase system based on the ion-pair chromatography (16) can be applied. In this method, the ionic form of the solute is suppressed by addition of an appropriate

counterion to the mobile phase. Generally the retention time is depending on the pH of the eluent and the nature and the concentration of the counterion used. All experiments have been performed on the "Dextropak" cartridge. We have tested, without success, tetramethylammonium and tetrabutylammonium ions ; whatever the pH is, there is no retention. A counterion more hydrophobic has been tried : with 0.05 % dodecyltributylammonium ion in water, the complex formed is so strong and so hydrophobic that he can't be eluted from the column. So we have eluted with 0.05 % dodecyltributylammonium chloride in sodium nitrate solution. The results obtained in 0.1 M and 0.2 M $NaNO_3$ are illustrated in Figure 3. When the concentration of the sodium nitrate is 0.1 M, the first five DP are resolved. In 0.2 M, the resolution of the upper DP is increased but in 1 M $NaNO_3$ there is no more separation. It was concluded that, by adding electrolyte, the complex is destabilized by a competition between the electrolyte and the hydrophobic counterions.

CONCLUSION

High performance liquid chromatography on C-18 supports has been proved to be an excellent method to analyse the oligosaccharides produced from kappa-carrageenan by enzymic hydrolysis or to fractionate a mixture of oligogalacturonic acids. In the first case, the mechanism is based on hydrophobic interactions between C-18 chains and the anhydrogalactosyl unit after suppression of the ionic exclusion with elution by a sodium ni-

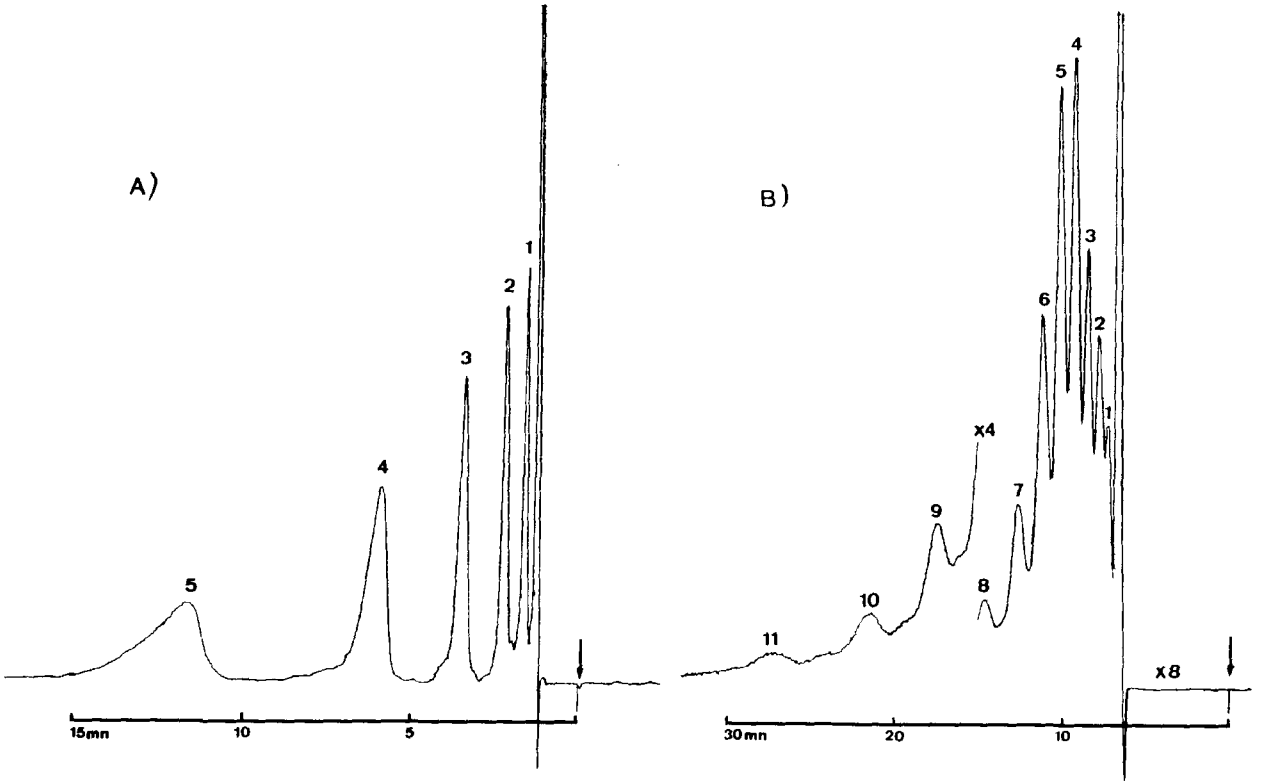

Figure 3 : Separation of oligogalacturonic acids on "Dextropak" cartridge

A - eluent : 0.1 M $NaNO_3$ + 0.05 % dodecyltributylammonium chloride ; flow-rate : 2 ml/mn.

B - eluent : 0.2 M $NaNO_3$ + 0.05 % dodecyltributylammonium chloride ; flow-rate : 0,4 ml/mn.

The degree of polymerization is indicated by numbers over the peaks.

trate solution. This system is not available with oligogalacturonic acids, but the separation can be performed by the ion-pair method ; the hydrophobic counterion used is dodecyltributylammonium. A concentration of 0.05 % in a sodium nitrate as the mobile phase allows to get good results, the resolution of different DP being checked by the ionic strength.

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