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2-1-2. Shodex SUGAR Series

A strong feature of the Shodex SUGAR series columns resides in the mechanism of separation in the ligand exchange mode. Ligand exchange refers to a mode of separation based on the interaction (ligand exchange potential) between hydroxyl groups and metal ions to form a complex. Saccharides have a 5-membered (furanose) or 6-membered (pyranose) ring structure, containing a large number of hydroxyl groups. These hydroxyl groups bind together either equatorially or axially with respect to the carbon plane. This conformation of the hydroxyl groups differs depending on the kind of saccharide.
Figure 2-3 shows the relationship between hydroxyl group conformation and counter ion interaction.

In Figure 2-3(a), three hydroxyl groups form a complex with the metal ion. In Figure 2-3(b), only two hydroxyl groups form a complex with the metal ion due to the hydroxyl group conformation. Hence, ligand exchange potential is higher for (a) than for (b).

The complex formation potential also differs depending on the kind of metal ion.

* When using a column of the SUGAR series, analysis should be performed at increased column temperatures to prevent anomer separation. Under strongly alkaline conditions, saccharides are likely to isomerize with the fear of decomposition of polysaccharides.

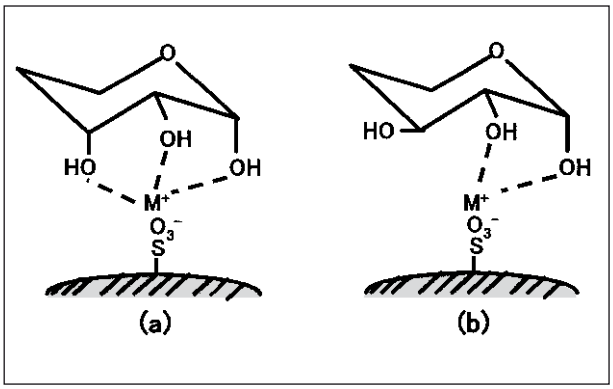

Fig. 2-3 Difference of counter ion interaction

2-1-3. Shodex Asahipak NH2P-50 Series

Columns of the Shodex Asahipak NH2P-50 series are greatly improved amino columns which not only maintain the high separation performance of the conventional silica-based amino columns, but also solve the problem of declines in retention over time. This is due to stable chemical bonding of polyamine with hydrophilic polymer gel. Other advantages are:

* Analysis under moderate conditions (around pH 7 and room temperature) is possible.
* Sharp, near-symmetric peaks can be obtained for a wide variety of saccharides.
* Accurate quantitative determination can be made.
* A wide range of eluents, such as various buffer solutions, alkaline solutions, or acidic solutions can be used.
* Alkaline washing of columns is possible.

With amino columns, saccharides elute in order of increasing polarity due to the function of normal phase chromatography. Usually, a mixed solvent of acetonitrile and water is used as the eluent. When the mixing ratio of acetonitrile is increased, the polarity of the eluent becomes lower. This results in a stronger interaction between saccharides and the column and a larger elution volume.

As NH2P-50 columns have weak alkaline amino groups, the condition inside the column is alkaline. This enables saccharides to be analyzed without causing separation of anomers even at room temperature.

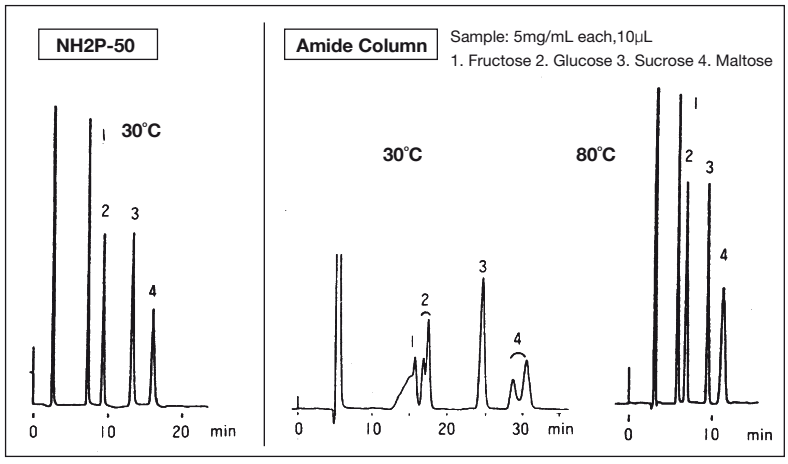

Fig. 2-4 Effects of Column Temperature on Elution Patterns (Comparison with Amide Column)

There are columns, called amide columns, which are used for analysis of saccharides under the same elution conditions as those for amino columns. Although amide columns have acrylamide groups introduced, analysis has to be made at high temperatures because the acrylamide group is not alkaline. (Fig. 2-4)

Column	: Shodex Asahipak NH2P-50 4E (4.6x250mm) Amide Column from Company-A (4.6x250mm)
Eluent	: CH_3CN/H_2O=75/25
Flow Rate	: 1.0mL/min
Detector	: Shodex RI
Column Temp.	: 30°C, 80°C