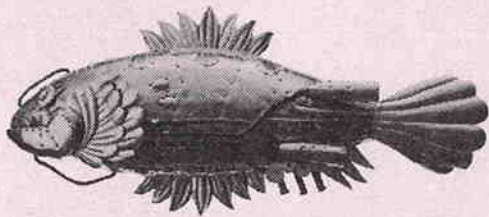


Shodex® NH2P-50 series columns

Analysis of saccharides in food industry



Shodex®

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1. Basic characteristics and features of NH2P-50

1-1. What is the NH2P-50 series?

Methods of analysis of monosaccharides and oligosaccharides using high performance liquid chromatography (HPLC) include normal phase, ligand exchange, size exclusion, and ion exchange chromatography. As amino columns, which are used for normal phase chromatography, provide high resolution of saccharides under simple analytical conditions, they are widely used in areas such as the food industry. Conventional silica-based amino columns, however, have a problem with chemical instability, which leads to (i) declines in retention power with time, and (ii) shorter column life.

Shodex Asahipak NH2P-50 series of columns are greatly improved amino columns which not only maintain the high separation power 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.

<Features of NH2P-50 series>

- * They are new amino columns in which polyamine has been bonded to a hydrophilic polymer gel (polyvinyl alcohol gel).
- * They have the inherent chemical stability of a polymer gel, solving the problem of deterioration over time that plagues conventional silica-based amino columns.
- * Stable chromatograms can be obtained for a long period of time by using these columns.
- * 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.

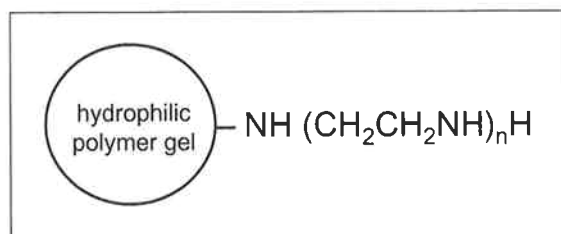


Fig. 1-1 Schematic drawing of NH2P-50 gel

Table 1-1 Specifications for Shodex Asahipak NH2P series

Product code	Product name		Column size	Average	Theoretical	Usable pH range
			ID x L (mm)	particle size (μm)	plate number (TP/column)	
F7630002	Analytical Scale Columns	Asahipak NH2P-50 4D	4.6 x 150	5	$\geq 5,500$	2~13
F7630001		Asahipak NH2P-50 4E	4.6 x 250	5	$\geq 7,500$	2~13
F6710016		Asahipak NH2P-50G 4A	4.6 x 10	5	guard	2~13
F7630006		Asahipak NH2P-50 2D	2.0 x 150	5	$\geq 3,500$	2~13
F6713000		Asahipak NH2P-50G 2A	2.0 x 10	5	guard	2~13
F6830001	Preparative Scale Columns	Asahipak NH2P-50 10E	10.0 x 250	5	$\geq 10,000$	2~13
F6830031		Asahipak NH2P-90 20F	20.0 x 300	9	$\geq 10,000$	2~13
F6830007		Asahipak NH2P-130 28F	28.0 x 300	13	$\geq 1,000$	2~13
F6710017		Asahipak NH2P-130G	7.5 x 50	13	guard	2~13
F6710100	Line filter	Asahipak NH2P-LF	8.0 x 75	5	line filter	2~13

Semi-micro (2.0, 1.0mmID) and micro column (0.8, 0.5, 0.3mmID) with 35, 50, 150, 250 mm length are also available.

1-2. Separation mechanism of NH2P-50

(a) Elution characteristics of amino columns

Amino columns, such as NH2P-50, are packed with material having high polarity, when compared with other partition/adsorption columns. (Fig. 1-2) 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.

(b) Ratio of non-protonated to protonated amino groups and theoretical plate number

As with conventional amino columns, columns of the NH2P-50 series are packed with ion exchange resin which terminate in anion exchange groups (amino groups) introduced in it. Due to the pH and ion composition of the eluent, there is equilibrium between the protonated and non-protonated amino groups. (Fig. 1-3) The ratio of non-protonated to protonated amino groups has a great influence on the elution characteristic of saccharides.

An NH2P-50 4E column was equilibrated using an aqueous ammonium acetate solution at three different pH values to produce different non-protonated/protonated ratios. Analysis was conducted with these columns holding all other conditions the same. The results showed the larger the non-protonated/protonated ratio, the smaller the elution volume for each saccharide and the sharper its peak. (Fig. 1-4, 1-5)

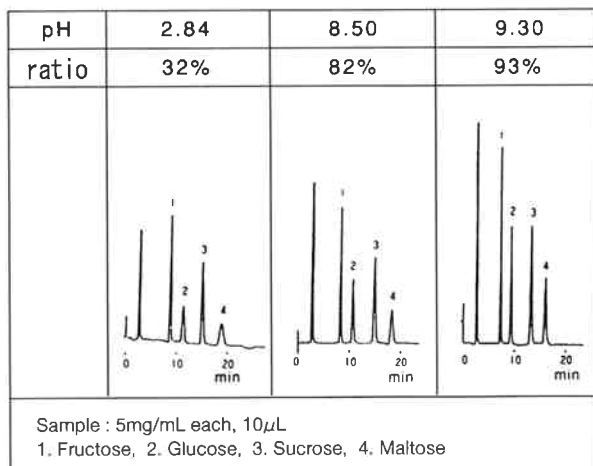


Fig. 1-4
Relationship between ratio of non-protonated to protonated amino groups and elution time (Equilibrium is achieved by passing a 100 mM aqueous ammonium acetate solution through the column.)

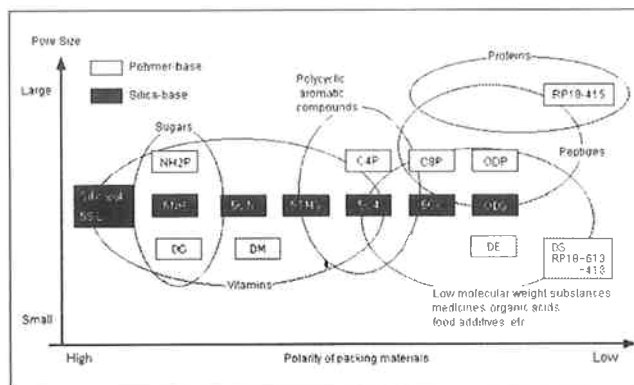


Fig. 1-2
Characteristics of packing material in partition/ adsorption columns and areas suited to use of these columns.

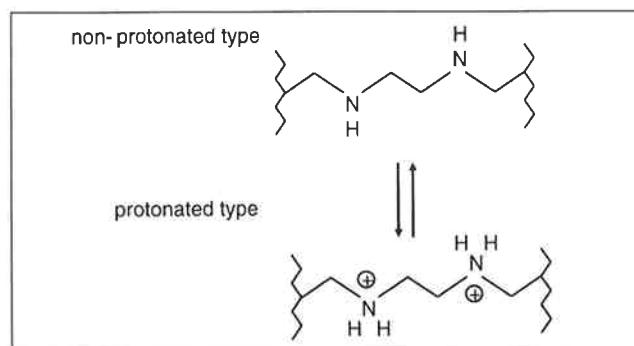


Fig. 1-3 Type of amino group

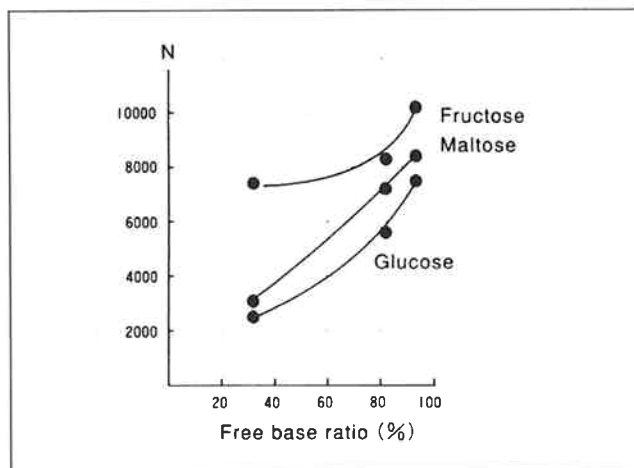


Fig. 1-5
Relationship between ratio of non-protonated to protonated amino groups and theoretical plate number of saccharides

Fig. 1-4 & 1-5 Column : Shodex Asahipak NH2P-50 4E(4.6 x 250mm)
Eluent : CH₃CN/H₂O=75/25
Flow rate : 1.0mL/min
Detector : Shodex RI
Column temp. : 30°C

1-3. Problem with anomer separation of saccharides

Reducing saccharides and saccharides with reducing terminals have α and β anomers. These anomers are in equilibrium in the solution. (Fig. 1-6)

Under the conditions in which the conversion rate between the anomers is low, α and β anomers are separated by the column causing the peak tops to split or widen. Measures to prevent these problems include the following:

- * Analysis at high temperature
- * Analysis under strong alkaline conditions

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.

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 temperature because the acrylamide group is not basic.

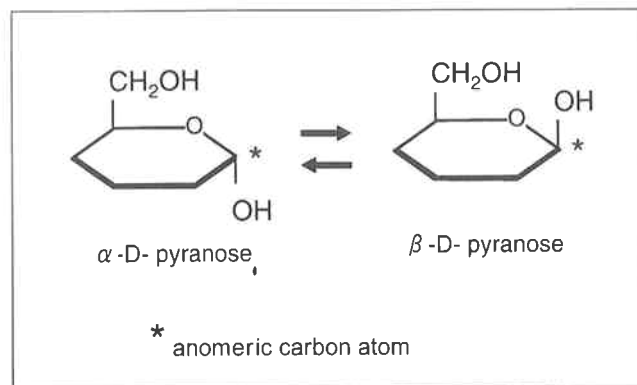


Fig. 1-6 Structural formula of α and β aldohexapyranose

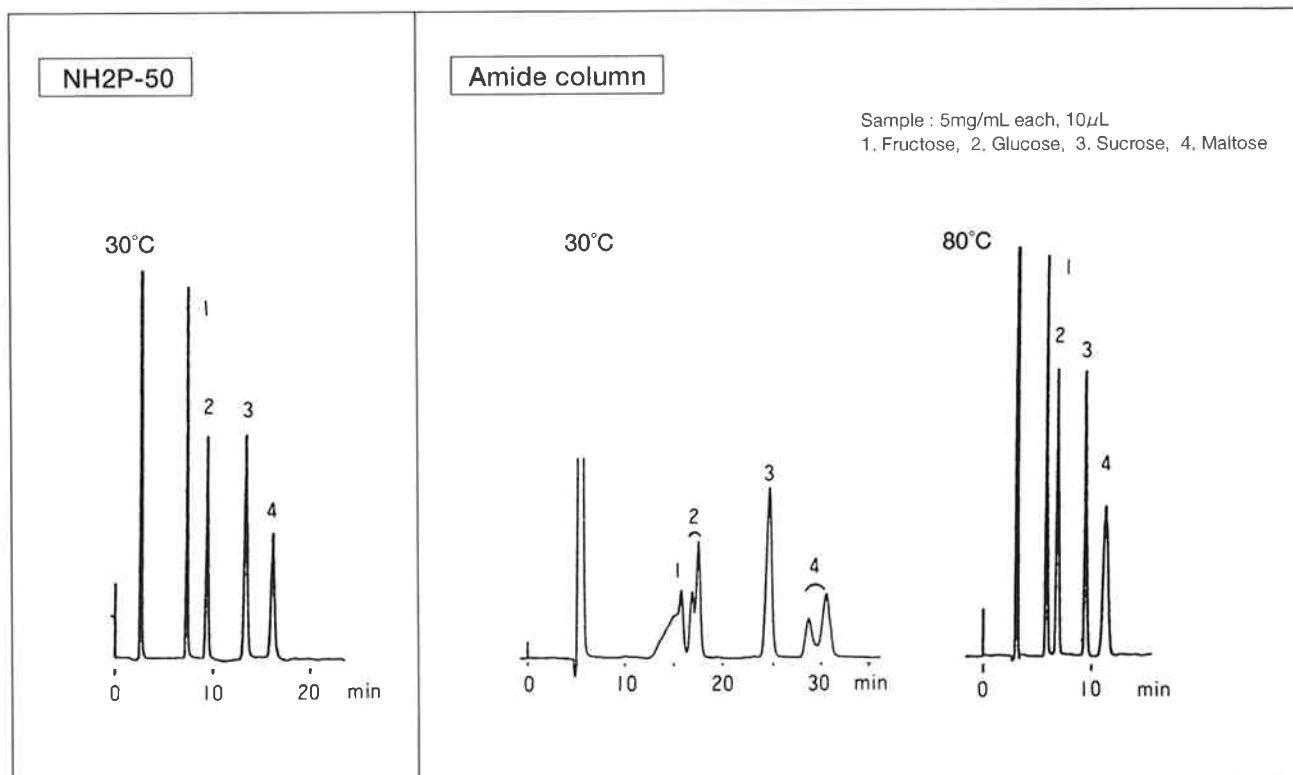


Fig. 1-7 Effects of column temperature on elution patterns (comparison with amide column)

Column : Shodex Asahipak NH2P-50 4E (4.6 x 250mm)
amide column from company-A (4.6 x 250mm)
Eluent : CH₃CN/H₂O=75/25
Flow rate : 1.0mL/min
Detector : Shodex RI