European Online Journal of Natural and Social Sciences 2017;
Vol.6, No 3 pp. 482-491
ISSN 1805-3602

Investigation of glucose and mannose separation by HPLC method

Hedieh Alavi Talab
Department of Chemistry, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran.
E-mail: halavi@iautmu.ac.ir .

Received for publication: 30 November 2016.
Accepted for publication: 07 August 2017.

Abstract

Glucose (Glc) and mannose (Man) are monosaccharides. Due to the six atoms of carbon and one aldehyde group both belong to the group of aldohexoses. Possible configurations of these monosaccharides are the D- or L-form, depending on configuration of fifth carbon atom in chain. D-forms mostly occur in nature. The aim of this work was to separate the mixture of glucose and mannose using cation exchanger in Na form. The data obtained from tests are used for simulation of SMB mannose isolation and separation efficiency guess estimation.

D-mannose and D-glucose were used for chromatographic separation on SMB unit and HPLC analysis Solution of 5 % glucose and 5 % of mannose has been prepared for this work. Separation was firstly realized on two columns which were subsequently temperate on 35.5 °C, 44.8 °C, 54.5 °C and 62.5 °C. The feed mixture has been injected for 3 min at flow rate 1.5 l h^{-1}. The fraction collecting started, when the signal generated by refractometer at the column output differs from its zero level base line. Fractions have been collected in time intervals of 2 to 3 min. The aim of this measurement was the searching of optimal temperature. In this case separation of binary mixture glucose and mannose is programmed with highest separation efficiency.

Comparing the obtained data from both views was found, that the separation efficiency in given range is the highest at temperature 62.5 °C. At this temperature the continuous chromatographic separation was performed using chromatographic separator KCHS-SMB-8-N. The achieved concentration profile of separated compounds shows, that the mixture did not ideally separated and the resulting course of compounds cannot verify the steady state. Work was done the separation performed again with wider set of operational parameters.

The possibility of continuous separation and the separation efficiency of column were verified firstly with mixture glucose and fructose using chromatographic separator on two serially connected columns at four different temperatures 35.5 °C, 44.8 °C, 54.5 °C and 62.5 °C . The maximum achieved purity and HETP were chosen as the criteria for evaluating the separation efficiency at given temperatures.

Keywords: chromatography, Glucose, mannose, separation.

Introduction

Glucose (Glc) and mannose (Man) are monosaccharides. Due to the six atoms of carbon and one aldehyde group belong both to the group of aldohexoses. Possible configurations of these monosaccharides are the D- or L-form, depending on configuration of fifth carbon atom in chain. D-forms mostly occur in nature. In the Fig. 1 you can see chemical formulas of D-mannose and D-glucose in the Fisher projection.

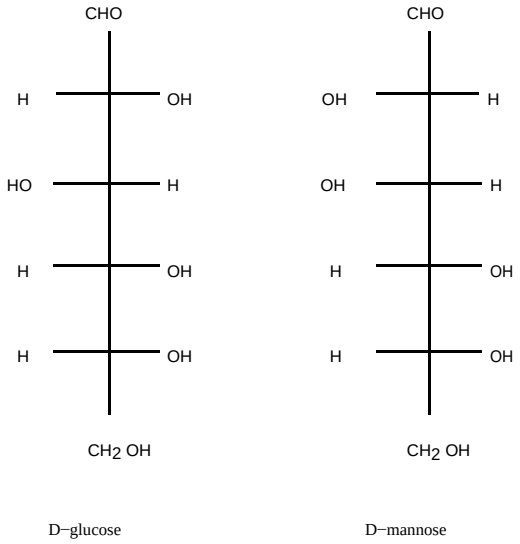

Figure 1: D-glucose and D-mannose in the Fisher projection

The one way of mannose production from naturally available sources is using the hydrolysis of polysaccharides containing mannose, e.g. hydrolysis of galactomannans. The second possible way to produce mannose is using of the enolisation in weak acidic or alkaline solution and followed reactions.

The enolisation in weak alkaline solution followed by isomeration and epimerisation is known since 1885 as Lobry-de Bruyn-van Ekenstein transformation. It is possible from glucose solution according to concentration, pH, solvent and temperature to obtain the mixture of glucose, fructose and mannose, but with a higher content of fructose. (Koeckritz *et al.*, 2008)

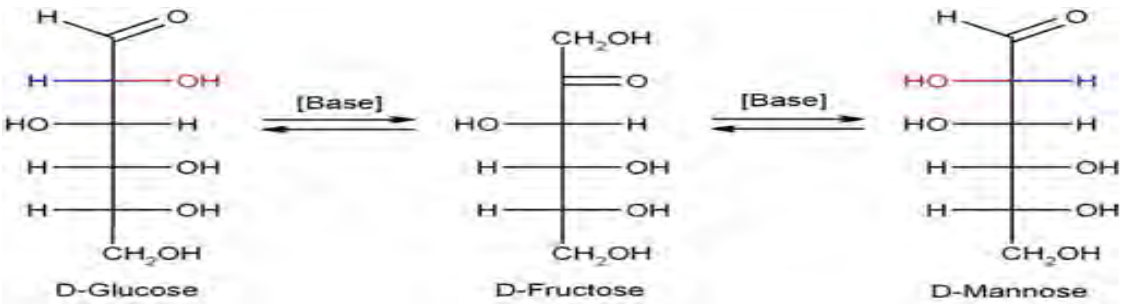

Figure 2: Lobry-de Bruyn-van Ekenstein transformation of D-glucose

Using the enolisation and epimerisation in weak acidic solution by presence of metal ions is possible to obtain mannose from glucose. Besides catalytic methods, which describe predominantly Ni and Ca ions as active species, the Bílik reaction (see Fig. 3) applying molybdate catalysts has gained in importance. Sodium molybdate (Na_2MoO_4), ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$), MoO_3, and further Mo sources are used as precursors for homogeneous catalysts over the years. Some literature is also available on heterogenisation of molybdate species onto anion exchanger resins. In conclusion they founded, that both sodium molybdate-based and molybdic acid-based

catalysts in batch reactions produced comparable yields of mannose, the molybdic acid-based catalyst C-IV showed superior performance in continuous runs. The reason for that behaviour, as supported by UV–vis spectra, may be the more rapid formation and the higher concentration of oligomer molybdate species on the support. (Koeckritz *et al.*, 2008)

Figure 3: The scheme of Bílik reaction (Koeckritz *et al.*, 2008)

Thomas and Lobel (1976) described optimum conditions for separating these sugars from each other and from other commonly occurring monosaccharides on cation-exchange resin in the K^+ and Na^+ forms. Sherman and Chao (1984) separated mannose from the mixture of monosaccharides like glucose, xylose, arabinose and galactose (plant tissue hydrolyzate) on zeolitic molecular sieves selected from the group consisting of BaX, BaY, SrY, NaY and CaY. Kulprathipanja (1989) separated mannose from mixture with glucose and other saccharides by adsorption on sulfonated polystyrene divinylbenzene crosslinked ion exchange resins in Ca^{2+}/ NH_4^+ (Dowex 99, Dow Chemical Co.) and desorbing the adsorbate with water. Glucose is removed from the adsorption process in the raffinate.

The aim of this work was to separate the mixture of glucose and mannose using cation exchanger in Na form. The data obtained from elution tests then consequently use for simulation of SMB mannose isolation and separation efficiency guess estimation. The feed mixture for chromatographic method was prepared from pure compounds - glucose and mannose. We have also prepared the mixture of glucose and mannose using epimerization with Mo-based catalyst. In our case we performed batch experiment with ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$) as homogenous catalyst in the presence of acetic acid (CH_3COOH). The produced mixture is supposed to test in future work as feed mixture for chromatographic separation on cation exchanger resin in Na form. This work is deal with separation of mixture of pure mannose and glucose.

Materials and methods

D-mannose and D-glucose were used for chromatographic separation on SMB unit and HPLC analysis. The continuous chromatographic separator KCHS-SMB-8-N (Henke *et al*., 2006) was used for chromatographic separation. The separator allows running both in discontinous and continuous modes of operation. It consists of eight serially connected identic columns packed with ion exchanger Lewatit MDS 1368 Na (Bayer, Germany) – strongly acidic catex in Na form used for separation of saccharides. The columns are 2 m high and their volume is 1 l . The mobile phase is deionized water using reverse osmosis. The continuous chromatographic station KCHS-SMB-8-N is equipped with two types of detectors – the conductometer and the refractometer on both output streams. The extract stream has refractometer Abbemat and conductometer Ahlborn (Ahlborn GmbH, Germany). On the raffinate is refractometer RFM340 (Bellingham+Stanley, great Britain) and conductometer Omega (Stamford, USA). Chromatographic separator, detectors and other

controlling and measuring elements are connected to programmable logic controller Simatic PCS7 (Siemens, Germany, which allows controlling the chromatographic system using personal computer and storing of measured values to the database.The HPLC system is used for analyzing of fractions collected from separation and it uses catex in Ca packed column Polymer IEX The mobile phase is demineralized water prepared using special station (Millipore, USA).

The aim of this measurement was the searching of optimal temperature. in this case searching the temperature, when the separation of binary mixture glucose and mannose is running with highest separation efficiency.

Solution of 5 % glucose and 5 % of mannose has been prepared for this work. Separation was firstly realized on two columns which were subsequently temperated on 35.5 °C, 44.8 °C, 54.5 °C and 62.5 °C. The feed mixture has been injected for 3 min at flow rate 1.5 l h^{-1} . The fraction collecting started, when the signal generated by refractometer at the column output differs from its zero level base line. Fractions have been collected in time intervals of 2 to 3 min.

Only one dry matter concentration profile occurs, when both monosaccharides in feed mixture (glucose and mannose) passed through columns no. 1 and 2 at every temperature in selected range. The main goal of this analysis is to obtain the elution concentration profile of each sugar. The glucose and mannose content in each fraction was analyzed using of HPLC method of extern standard - solutions: glucose 0.1379 g (100 ml)$^{-1}$, mannose 0.1508 g (100 ml) $^{-1}$. The mobile phase was degassed deionized ultrapure water (resistivity 19 MΩ.cm) prepared on Millipore (Millipore, USA) and filtrated on microfilter with 0.23 µm pore size The separation column was temperated at 65 °C, flow rate of mobile phase 0.5 ml min^{-1}, injected volume 30 µl.

The optimal temperature for SMB separation was chosen according discontinuous measurements. The operational parameters were obtained using special software (Henke *et al*., 2006) from chromatographic curves of discontinuous measurements at optimal temperature. The values of operational parameters were verified with simulation program (Henke *et al.,* 2006). The SMB system was started with predicted parameters and there were collected fractions in extract and raffinate during the steady state. Purity in each stream was calculated according the content of both substances in streams.

Results and discussion

The experiments were done at four different temperatures 35.5 °C, 44.8 °C, 54 °C, 62.5 °C (the thermostat was set at 40 °C, 50 °C, 60 °C, 70 °C) and the flow rate of mobile phase 1.5 l h^{-1}. During the separation the conduction and refraction (refractivity dry matter) were measured. All the values (feed injection period, elution time, conduction and refraction) have been store in the database.

There were collected together 48 fractions: 8 fractions (numbered 1C up to 8C) at 35.5 °C, 8 fractions (numbered 1B up to 8B) at 44.8 °C, 16 fractions at 54.5 °C (numbered 1 up to 16) and 16 fractions (numbered 1A up to 16A) at 62.5 °C. The samples have been analyzed without dilluting using the HPLC system at the same conditions (temperature, flow rate of mobile phase, injected volume, etc.)

The concentrations of glucose and mannose (in weight percents) are shown in Tables 1 up to 4. The purity of compound is expressed as its weigth percent content in dry matter.

Table 1: Content of collected fractions at 35.5 °C

Fraction number	Glucose content	Mannose content	Mannose purity
	wGlc (%)	wMan (%)	QMan (%)
1C	0.08	0.01	15.47
2C	0.35	0.13	26.62
3C	0.74	0.46	38.44
4C	0.86	0.82	48.91
5C	0.57	0.86	60.28
6C	0.28	0.62	68.85
7C	0.10	0.31	74.97
8C	0.04	0.13	75.26

Table 2: Content of collected fractions at 44.8 °C

Fraction number	Glucose content	Mannose content	Mannose purity
	wGlc (%)	wMan (%)	QMan (%)
1B	0.19	0.04	18.37
2B	0.60	0.26	30.35
3B	0.89	0.69	43.65
4B	0.77	0.94	55.10
5B	0.47	0.86	65.04
6B	0.21	0.53	72.26
7B	0.08	0.26	76.12
8B	0.05	0.14	75.82

Table 3: Content of collected fractions at 54.5 °C

Fraction number	Glucose content	Mannose content	Mannose purity
	wGlc (%)	wMan (%)	QMan (%)
1	0.00	0.00	0.00
2	0.00	0.00	0.00
3	0.00	0.00	9.70
4	0.08	0.01	13.66
5	0.40	0.13	24.31
6	0.71	0.40	36.26
7	0.93	0.76	44.96
8	0.78	1.02	56.67
9	0.46	0.90	65.94
10	0.28	0.71	71.58
11	0.11	0.31	74.79
12	0.04	0.11	71.17
13	0.02	0.05	70.01
14	0.02	0.03	61.03
15	0.02	0.02	58.80
16	0.02	0.02	58.42

Table 4: Content of collected fractions at 62.5 °C

Fraction number	Glucose content wGlc (%)	Mannose content wMan (%)	Mannose purity QMan (%)
1A	0.003	0.003	53.460
2A	0.003	0.003	52.141
3A	0.003	0.004	55.341
4A	0.003	0.004	52.435
5A	0.017	0.005	22.521
6A	0.188	0.026	12.206
7A	0.758	0.190	20.062
8A	1.109	0.472	29.880
9A	1.383	0.931	40.228
10A	1.294	1.325	50.593
11A	1.044	1.543	59.633
12A	0.724	1.567	68.405
13A	0.318	0.926	74.438
14A	0.163	0.562	77.485
15A	0.080	0.270	77.065
16A	0.037	0.086	69.926

The course of output concentrations is depicted in the Fig. 4-7. The calculated dry matter is the sum of both sugars weight percents in given fraction.

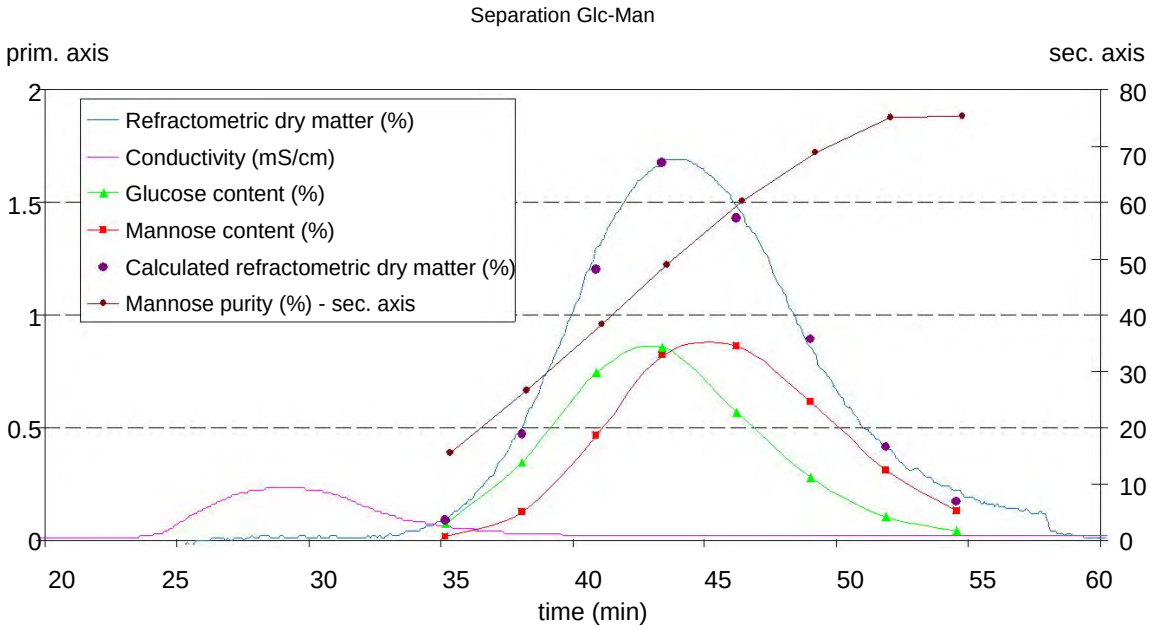

Figure 4: Separation of glucose-mannose mixture at 35.5 °C

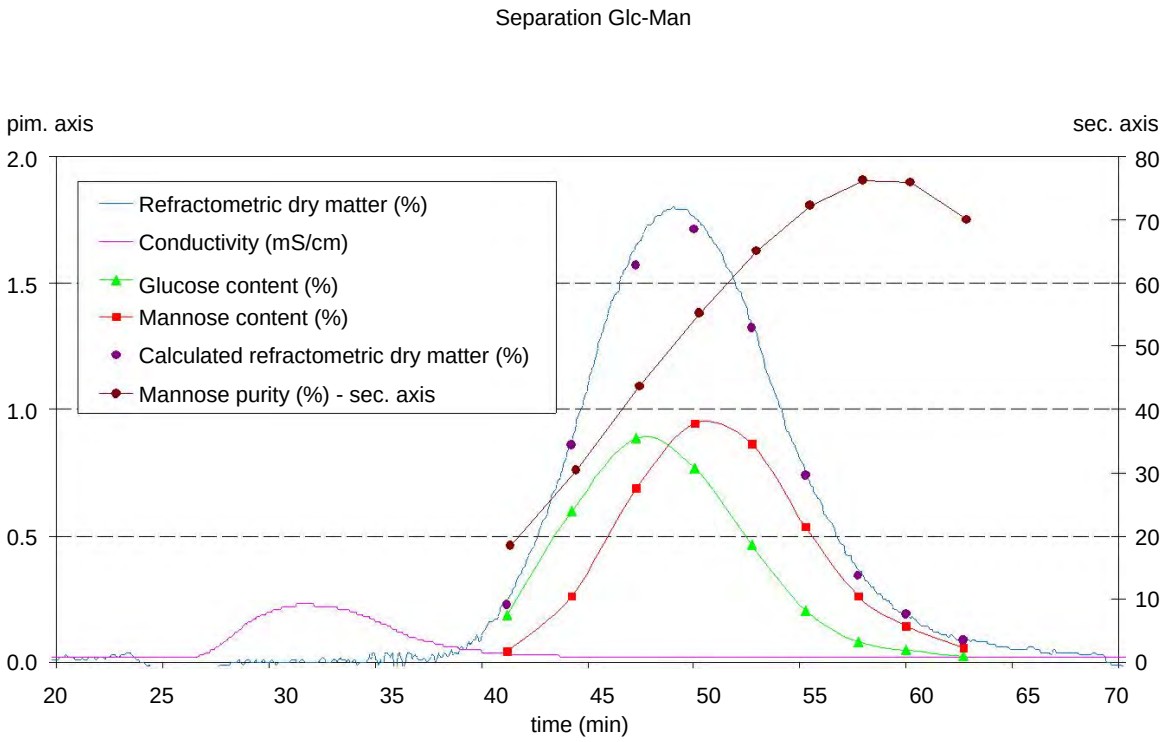

Figure 5: Separation of glucose-mannose mixture at 44.8 °C

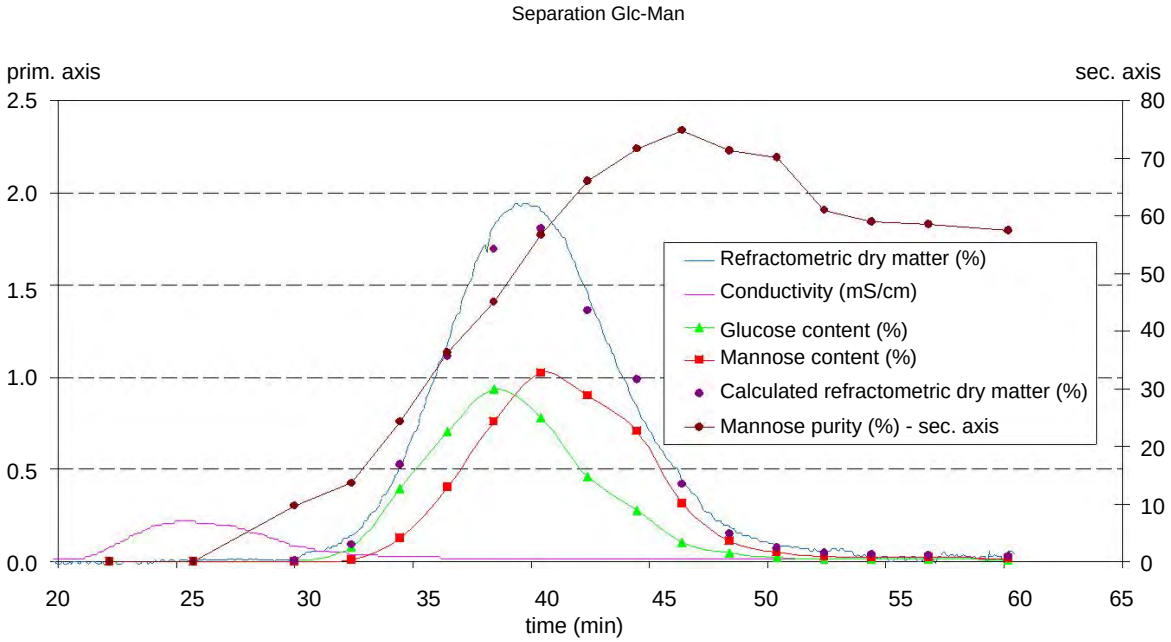

Figure 6: Separation of glucose-mannose mixture at 54.5 °C

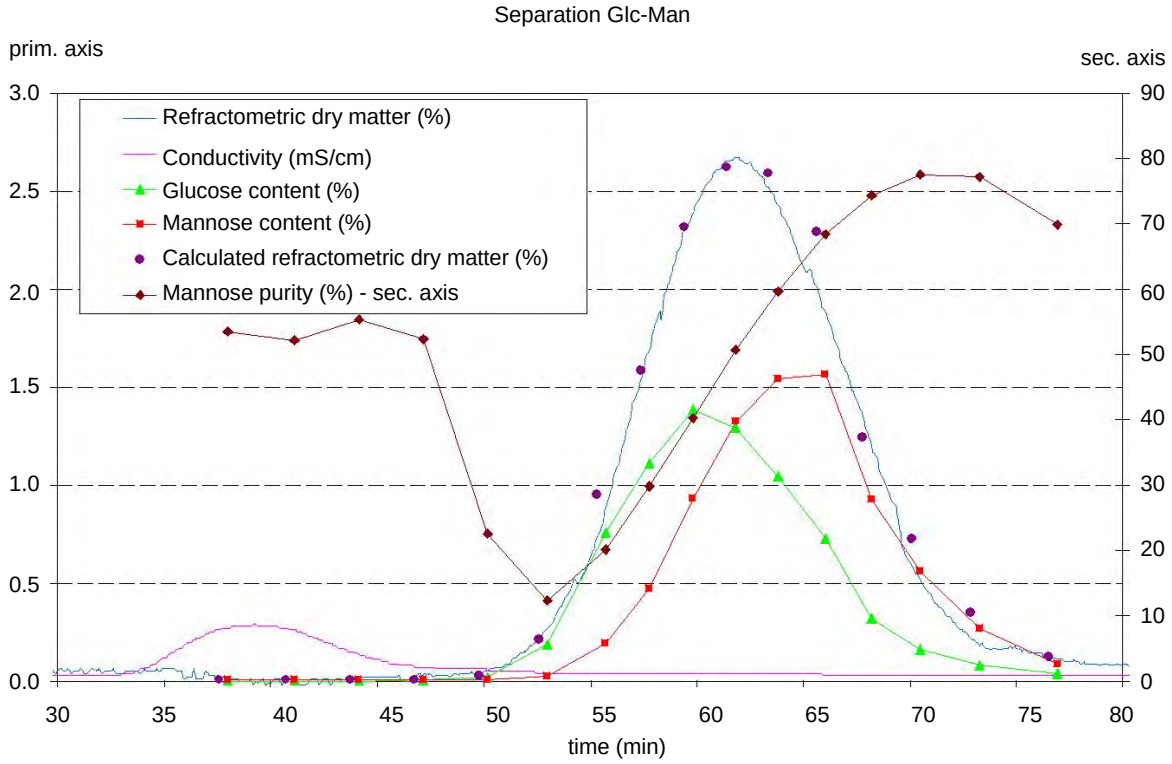

Figure 7: Separation of glucose-mannose mixture at 62.5 °C

Evaluating of separation efficiency of columns in KCHS-SMB-8-N separator is:

1- Evaluating according to purity of separated compounds

Table 5: Maximum purity of mannose (at correspondent temperature)

Temperature	Mannose purity
(°C)	maximum (%)
35.5	75.26
44.8	76.12
54.5	74.79
62.5	77.49

Comparing the maximum achieved mannose purities (see Tab. V) in the samples has been found, that the system separated with highest efficiency at 62.5 °C. On the other side purity is not sufficient condition for evaluating of separation efficiency of the separator. It is necessary to include also the concentration profile of mannose, so at which concentration the maximum of purity is achieved, and this can be the crucial impact on economic results of whole process.

2- Evaluating according HETP

On the base of graphs obtained there were evaluated parameters which are necessary to count of HETP evaluation and consequent HETP (see Table 6).

Table 6: Parameters for HETP evaluation from obtained graphs

Temperature (°C)	Glucose		Mannose	
	tR (min)	W1/2 (min)	tR (min)	W1/2 (min)
33.5	43.0	8.7	45.2	10.1
44.8	47.4	9.9	50.6	10.1
54.5	38.4	7.7	42.4	8.4
62.5	59.7	10.7	64.5	9.8

The count of HETP and HETP itself can be calculated from relations (1) and (2). Next - t_R is the retention time, t_P is the pipes time, t_N is inject time (t_N = 3 min), $W_{1/2}$ is peak width in a half of peak height, L is column length (L = 4 m) – two columns of 2 m of length were used.

$$N = 5{,}54\ t_R - t_P - \frac{t_N}{2}\frac{1}{W_{1/2}}^2 \quad (1)$$

$$H = 100\ \frac{L}{N} \quad (2)$$

The results are summarized in Table 7.

Table 7. The values of calculated parameters of the column

Temperature	Glucose		Mannose	
(°C)	N	HETP (cm)	N	HETP (cm)
33,5	115	3,50	96	4,21
44,8	110	3,66	121	3,31
54,5	116	3,47	120	3,34
62,5	155	2,59	217	1,85

In examined range of temperature the HETP was lowest at 62.5 °C, so this temperature is the optimal temperature for glucose-mannose separation.
Firstly the operational parameters have been calculated, so the flow rates of inlet and outlet streams and the switch period of multifunctional valve with help of special software SMBmodel (Henke *et al.*, 2006), consequently these parameters have been passed to the simulation program SMBmodel (Henke *et al.*, 2006) and the time dependence of concentration profiles for both substances was obtained. These operational parameters have been used for continuous separation. Calculation of operational parameters at optimal temperature is too:

The values of operational parameters are calculated on the base of retention time values at temperature 62.5 °C. The set of algorithms included in software SMBmodel (Henke *et al.*, 2006) has been used for the calculation. The calculated parameters are in Table 8.

Table 8: Table of calculated operational parameters

Eluent	15.52	ml min^{-1}
Extract	8.03	ml min^{-1}
Feed	1.11	ml min^{-1}
Raffinate	8.60	ml min^{-1}
Recycle	17.81	ml min^{-1}
Switch	27.83	min

Nomenclature

Glc	glucose	
Man	mannose	
SMB	Simulated Moving Bed	
H, HETP	height equivalent to theoretical plate	cm
N	number of HETP	1
t_R	retention time	min
t_P	pipes time	min
t_N	inject time	min
w	weight percent	%
$W_{1/2}$	peak width in half of height	min
L	column length	m
S	refractometric dry matter	%

References

Henke, S., Bubník, Z., Kubát, M. (2006): Modelling, simulation and design of SMB apparatus. 2nd Technical Syposium of CIGR Section VI future of Food Engineering, April 26-28, Warszawa, Poland, ISBN 88-900775-8-1.

Koeckritz, A., Kant, M., Walter, M. and Martin, A. (2008): Rearrangement of glucose to mannose catalysed by polymer -supported Mo catalysts in the liquid phase. *Applied Catalysis A: General*, **334**(1-2): 112-118.

Kulprathipanja, S. (1989): Process for separating glucose and mannose with CA/NH4-exchanged ion exchange resins. US Patent 4 837 315.

Sherman, J. D. and Chao, C. C. (1984): Separation of mannose by selective adsorption on zeolitic molecular sieves. US Patent 4 471 114.

Thomas, J. and Lobel, L. H. (1976): Chromatographic separation of glucose and mannose on cation-exchange resin. *Analytical Biochemistry*, **73**(1): 222-226.