APPLICATION OF BINARY PACKING FOR STARCH SEPARATION BY HYDRODYNAMIC CHROMATOGRAPHY

M. Mota[1], J.A. Teixeira[1], R. Dias[2] and A. Yelshin[1]
[1] IBB,Centro de Eng. Biológica, University of Minho, 4710-057 Braga, Portugal
[2] ESTiG, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 134, 5301-857 Bragança, Portugal

ABSTRACT

Columns packed with commercial glass beads of 5 and 19 microns average diameter and binary mixtures with finest fraction of 5 micron (30 % volume fraction of the mixture) were used to analyse starch by hydrodynamic chromatography (HDC). Experiments were carried out at 3 and 15 ºC. The observed resolution increased with the application of binary packing as compared with single-size packing. The best results were obtained at starch's amylopectin and amylose separation with a glass beads mixture (5 + 19 micron) at 3ºC. In what concerns amylopectin and amylose separation, a lower pressure drop were obtained for the mixed binary packing when compared with the packing containing uniform 5 micron glass beads. For the Hylon VII starch RRT were 0.777 and 0.964 for amylopectin (AP) and amylose (AM), respectively, while for the Tapioca starch the obtained RRTs were 0.799 and 0.923. Application of unbound glass beads as column packing might reduce equipment and running costs in preparative scale separations.

KEYWORDS

Starch, Chromatography, Glass Beads, Macromolecules, HDC

1. Introduction

The increasing volume and range of polysaccharide applications, in particular dextran, pullulan and starch, in biotechnology, food, medicine and industry calls for a reduction on equipment and running costs in preparative scale separations.
Investigations of hydrodynamic chromatography (HDC) separations of dextran and polyethylene glycol of different molecular mass were performed previously, using a monosize and binary packed columns of glass beads of industrial grade (size ratio up to ~ 10) and HDC of micro-spheres, bacillus and yeast cells [1, 2]. Obtained results show the advantage of using binary packed columns formed by fine and coarse particles instead of a monosize packing of fine particles.
In the case of a binary packing bed, shaped micro-particles display a different behaviour than the one exhibited by spherical particles of the same scale. This might be explained by the interaction between shaped and the bed's pore topology. The importance of pore channels tortuosity effect on the separation of shaped micro-particles using a binary packing was demonstrated [2].
In the region of minimum porosity of particulate binary mixtures, heat exchange and permeability were found to be higher than the ones obtained with a mono-size packing built with the same small size particles used in the binary packing. This effect was observed for the range of the particles size ratio larger than 0.1. The obtained

improvement on thermal performance was related with binary packing structure. Permeability can increase by a factor of two, if the size ratio between small and large spheres of a loose packing stays in the range 0.3 – 0.5 [3].
The feasibility of the separation of different starch fractions using binary mixtures was studied in this work.

2. Experimental background

Analysis of starch components amylose and amylopectin is conventionally carried out by high-performance size-exclusion chromatography (HPSEC) [4, 5]. Working temperature normally varies between room temperature and around 60 ^{o}C. Amylopectin is eluted at the void volume of the column, whereas amylose is eluted later on, due to partitioning between the gel particles. The limitation of SEC is the high cost of the column packing especially at preparative scale processes [6].
Equipment costs can be substantially reduced with the application of hydrodynamic chromatography. Klavons et al. [7] used 25- x 1-cm columns filled with solid particles of 5 – 15 microns diameter (average diameter was estimated to be 10 micron). Columns were maintained at 60°C and the flow rates stayed in the range 0.1– 0.5 mL/min.
Hydrodynamic chromatography RRT depends on the ratio between the macromolecule size and the pore size. In turn, the macromolecule size is related to its conformational state that depends on the solute temperature [8]. Based on this behaviour, it is possible to control macromolecular properties by controlling temperature. When the starch solution temperature is decreased, the amylopectin macromolecules flexibility is reduced while the amylose keeps its flexible macromolecular structure [9, 10]. If the solution cooling time is short (i.e. the residence time of the sample in the chromatographic column is in the range 2 – 3 minutes), decreasing in temperature will affected AP, whereas random coil AM molecules still less sensitive to the temperature reduction, simultaneously, a retrogradation effect will be avoided [11].

3. Experimental conditions

The chromatographic system consists of an HPLC pump PU-1580 from *Jasco* (Tokyo, Japan), column, and a refractive index detector RI-2031 from *Jasco* including a PID temperature control and a cell of capacity 10 µl. The detector signal was monitored using a recorder PeakSimple 203 and integrator PeakSimple 2000 from *SRI Instruments* (CA, U.S.A.).
Stainless steel columns 200 × 4.6 mm I.D. (*Grom Analytik*) packed with soda lime non-porous glass beads of industrial grade from *Potters Ballotini* were used in the form of monosize packing as well as binary mixture packing. The mixture composition was prepared according to [12] and contains 70% of coarse and 30% of finest beads. Glass beads of 5 and 19 microns average diameter were used. In binary mixtures the smaller particles were 5 micron diameter. Experiments were carried out at temperatures of 3 and 15 ºC. High purity water was used as the mobile phase and solvent.
To achieve a column temperature of 3 ºC the column was immersed and stabilised in a water bath containing crushed ice. The sample temperature in both cases was 15 ºC. The column isothermal condition is attained at the region less of 10% of packing length.

The following starch solutes were used: Novation 2600 (~ 99% amylopectin), Tapioca starch (~ 22% amylose), and Hylon VII (~68% amylose). Sucrose was used as the HDC inert marker.

4. Results and discussion

Starch separation was performed using mono and binary packing columns. Examples of obtained chromatograms are shown in Figs. 1 and 2, where the arrow represents the retention time corresponding to the maximum value of the sucrose peak used as non-retained solute. The first peak corresponds to amylopectin and the second to amylose.

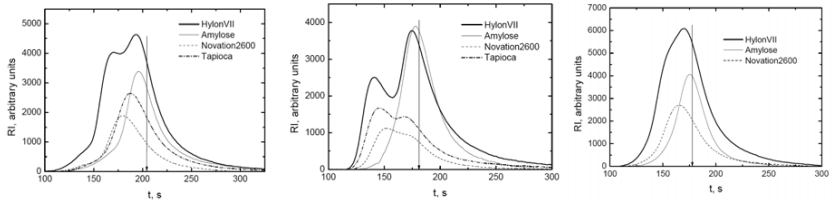

Figure 1. Chromatograms obtained with the column temperature 3 ^{o}C and flow rate 0.5 ml/min: (a) – 5 micron mono-packing; (b) – binary packing (5+19 microns); (c) – 19 micron mono-packing.

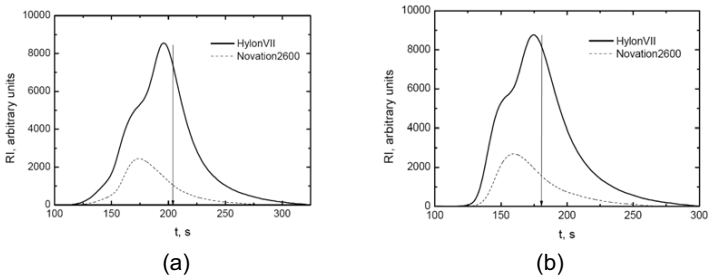

Figure 2. Chromatograms obtained at a column temperature of 15 ^{o}C and a flow rate 0.5 ml/min: (a) – 5 micron mono-packing; (b) – binary packing (5 + 19 microns).

The best results were achieved with the mixed binary packing (Fig. 1b), where, for Hylon VII, the relative retention times for amylopectin and amylose were relative retention time (RRT) = 0.777 and 0.964, respectively. Moreover, the mixed column was able to separate amylose and amylopectin in Tapioca starch, giving an amylose amount of 22.7%. For Tapioca starch the relative retention times in the mixed column, for amylopectin and amylose were determined, respectively, as RRT = 0.799 and 0.923. The resolution observed with Hylon VII was higher than with Tapioca starch (Fig. 1b), due to the difference in average sizes of amylose and amylopectin in starches tested. The observed dispersion of Novation 2600 (containing ~ 99%

amylopectin) peak (Fig. 1b) may be attributed to the variety of amylopectin polymerisation degree yielding consequently different responses to the cooled column. The HDC efficiency decreases when large size particles (19 microns) (Fig. 1c) and a column temperature of 15 ^{o}C, are used both for monosize (Fig. 2a) and for mixed packing (Fig. 2b).
The obtained results confirm the above assumption on the temperature effect on starch separation. It must be pointed out that the amylose – amylopectin peak resolution in the cases of Figs. 2a and 2b is at the level of size exclusion chromatographic mode. The backpressure ratio of mono- (5 μm) and binary packing column was ~ 1.6 that may also be considered as an additional benefit of this new methodology.

5. Conclusions

Presented results show that HDC in a binary packed bed formed by commercial grade glass beads has the ability to separate starch's amylose and amylopectin at a column temperature of 3º C. The mixed packing (5+19 microns particles) provides a quicker cooling of the mobile phase, a better resolution and a lower pressure drop, when compared with the packing containing glass beads with average diameter 5 microns. The use of unbound glass beads as column packing reduces the equipment cost and may be useful to monitor the amylose and amylopectin content of starch samples from different sources. The potential application of this method to produce highly purified starch fractions is quite high.

Acknowledgement

The authors wish to thank FCT for the grant provided to Dr. Alexander Yelshin (Yelshyn). This work was developed under the framework of the project POCI_EQU_58337/2004, partially funded by FEDER.

References

[1] M. Mota, J.A. Teixeira, A. Yelshin, R. Dias and S. Cortez, Application of binary packing for chromatographic separation, Proceedings of International Conference CHEMPOR 2005, September 21 - 23, 2005, Coimbra, Portugal (ISBN: 972-8055-13-7). Paper ESF-002, 1-6.

[2] M. Mota, J. A. Teixeira, A. Yelshin and S. Cortez, Utilisation of controlled pore topology for the separation of bioparticles in a mixed-glass beads column, Journal of Chromatography B, 843 (2006) 63-72.

[3] R. Dias, C. S. Fernandes, M. Mota, J. A. Teixeira and A. Yelshin, Permeability and effective thermal conductivity of bisized porous media, Int. J. Heat Mass Transfer, 50 (2007) 1295-1301.

[4] S. Kobayashi, S. J. Schwartz and D. R. Lineback, Rapid analysis of starch, amylose and amylopectin by high-performance size-exclusion chromatography, Journal of Chromatography A, 319 (1985) 205-214.

[5] A. Henshall, High-performance liquid chromatography techniques used in dietary fiber and complex carbohydrates analysis, in S.S. Cho and M.L. Dreher (Eds.), Handbook of Dietary Fiber, Marcel Dekker, 2001.

[6] W. Vorwerg, S. Radosta and E. Leibnitz, Study of a preparative-scale process for the production of amylose, Carbohydrate Polymers, 47 (2002) 181-189.

[7] J. A. Klavons, F. R. Dintzis and M. M. Millard, Hydrodynamic chromatography of waxy maize starch, Cereal Chem., 74 (1997) 832-836.

[8] W. M. Jones, The flow of dilute aqueous solutions of macromolecules in various geometry: VI. Properties of the solutions, J. Phys. D: Appl. Phys., 12 (1979) 369-382.

[9] G. Galinsky and W. Burchard, Starch fractions as examples for nonrandomly branched macromolecules. 4. Angular dependence in dynamic light scattering, Macromolecules, 30 (1997) 6966-6973.

[10] L.S. Collado and H. Corke, Starch properties and functionalities, in G. Kaletunç and K.J, Breslauer (Eds.), Characterization of cereals and flours: properties, analysis, and applications, Marcel Dekker, New York, 2003.

[11] I.A. Farhat and J.M.V. Blanshard, Modeling the kinetics of starch retrogradation, in P. Chinachoti and Y. Vodovotz (Eds.), Bread Staling, Contemporary Food Science Series, CRC Press LLC, Boca Raton, 2001.

[12] R. Dias, J. A. Teixeira, M. Mota and A. Yelshin, Preparation of controlled particulate mixtures with glass beads of different sizes, Separation and Purification Technology, 37 (2004) 69-80.