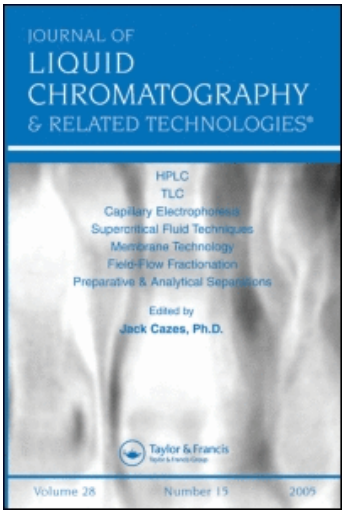

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Immobilization of Amylases on Silica Support to Study Breakdown Products of Potato Starch by HPLC

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To cite this Article Khurana, Amrik L. and Ho, Chi-Tang(1989) 'Immobilization of Amylases on Silica Support to Study Breakdown Products of Potato Starch by HPLC', Journal of Liquid Chromatography & Related Technologies, 12: 9, 1669 — 1677
To link to this Article: DOI: 10.1080/01483918908049533
URL: http://dx.doi.org/10.1080/01483918908049533

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(9), 1669-1677 (1989)

IMMOBILIZATION OF AMYLASES ON SILICA SUPPORT TO STUDY BREAKDOWN PRODUCTS OF POTATO STARCH BY HPLC

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ABSTRACT

Immobilization of α- and β-amylases on epoxypropylsilanized PartiSphere-5 was achieved. Hydrolysis of 2% potato starch solution yielded limit dextrin on α-amylase bound column while a mixture of limit dextrin, maltose and glucose was obtained from β-amylase bound column. The β-amylase bound column converted limit dextrin from α-amylase column into glucose.

INTRODUCTION

Applications of amylases to food processing and starch conversion have been reviewed by Reed (7). Use of immobilized amylases is feasible as the conversion of starch to lower molecular weight saccharides is carried out in fluid systems. Grubhover and Schieth (5) covalently attached α-amylase to diazotized polyaminostyrene. Later on Epton et al (4)

immobilized the same with crosslinked polyacrylamide derivatives but the immobilized enzyme was found to be less heat stable than the native enzyme. In the present investigation, the immobilization of α- and β-amylases has been achieved on epoxypropylsilanized PartiSphere-5.

EXPERIMENTAL

Materials

α-Amylase from Bacillus species, β-amylase from barley and potato starch were bought from Sigma Chemical Co. (St. Louis, MO). Maltose, glucose, limit dextrin, methanol, sodium chloride, dinitrosalicylic acid, potassium sodium tartrate, sodium hydroxide, sodium acetate, glycerine and potassium monobasic and dibasic phosphate were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). The soluble starch for assay was bought from E. Merck Chemicals (Cherryhill, NJ).

Packing Material

Epoxypropylsilanized PartiSphere-5 and PartiSphere-5 PAC phases were obtained from Whatman Inc. (Clifton, NJ)

Sample Preparation

Solution of potato starch was prepared by dissolving 2 g of the same in 100 ml of water. The reagent solutions for enzyme assays were prepared as follows: 1% soluble starch in 0.016M sodium acetate (pH 4.8), 1 g of 3,5-dinitrosalicylic acid in 20 ml of 2N sodium hydroxide and 50 ml of water and 30 g of potassium tartarate in 100 ml water.

Preparation of Packing Material

α- And β-amylases were immobilized by reacting 10 g of each with 10 g of epoxypropylsilanized PartiSphere-5 in phosphate buffer (0.05 M solution of KH_2PO_4 and K_2HPO_4 in equal amount with the addition of 0.01 N KOH to bring the pH to 7) at 35°C. The columns were packed by slurrying enzyme immobilized bonded phases in methanol and applying a pressure of 6,000 psi. The unreacted epoxy groups were deactivated by treating the columns with 2% aqueous solution of glycerol.

Assay of Amylase Activity

The amylase bonded phases were assayed with soluble starch as substrate according to the method described by Bernfeld (3).

HPLC Analysis

HPLC was performed using a variable wavelength UV detector, Spectroflow monitor SF-770 (Kratos Analytical, Ramsey, NJ); a programmable solvent delivery system, Series 3B (Perkin-Elmer Corp., Norwalk, Conn.); a manual injection valve, with 50 µl loop (Valco Instruments Co., Houston, TX) and a chart recorder (Laboratory Data Control, Riviera Beach, FL).

0.05M phosphate buffer (pH 7) was used as a mobile phase on α-amylase and β-amylase bound epoxypropylsilanized PartiSphere-5 phases. A mixture of acetonitrile and water (50:50, v/v) containing 1% ammonium hydroxide was used as a mobile phase on PartiSphere-5 PAC column.

RESULTS AND DISCUSSION

α- And β-amylases have been immobilized on epoxypropyl-silanized PartiSphere-5 phase. The performance of these phases was demonstrated by hydrolysis of 2% soluble potato starch solution. Figure 1 shows the hydrolysis of potato starch solution on α-amylase bound column. The hydrolyzed solution was collected from this column and identified as limit dextrin on PartiSphere-5 PAC column (Figure 2). This hydrolyzed solution was further converted into glucose when injected on the β-amylase bound column (Figure 3).

Figure 4 exhibits the hydrolysis of the potato starch solution on β-amylase bound column. Components such as limit dextrin, maltose and glucose were identified as the hydrolyzed products which were further confirmed and quantified on PartiSphere-5 PAC column (Figure 5A and 5B).

An assay run on the α- and β-amylase bound phases exhibited 75,000 and 8,350 units/g of activities respectively. These

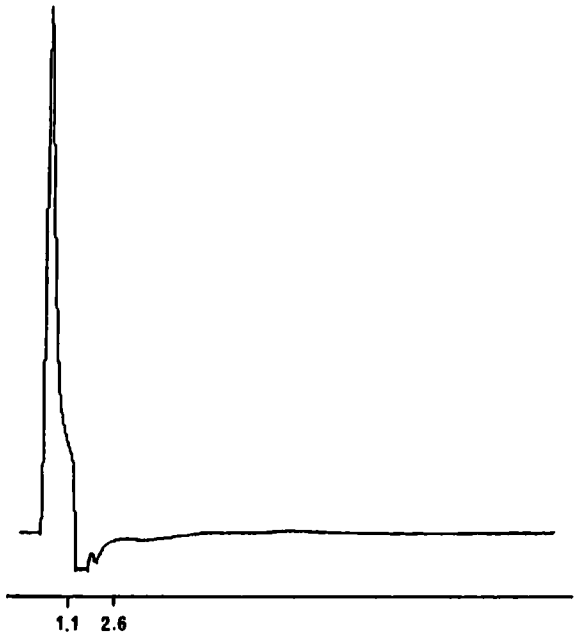

Fig. 1. Hydrolysis of 2% soluble potato starch solution on α-amylase bound epoxypropylsilanized PartiSphere-5 column (20 cm x 4.6 mm, I.D.). Mobile phase: 0.05M phosphate buffer (pH 7); flow rate: 0.9 ml/min; sample size: 15 µl; detector: refractive index.

phases as such were found to be less active as compared to HPLC packed columns. This was concluded by comparing the amounts of various hydrolyzed products such as limit dextrin, glucose and maltose produced during the same time.

No difference in reactivity of the packed columns was found when run at various temperatures such as 25°, 40° and 50°C. The different flow rates of the mobile phase such as from 0.2 to 2 ml/min had no effect on the rate of hydrolysis. No loss in activity of enzyme immobilized columns was observed during 30 days of continuous operation. The immobilized enzymes showed

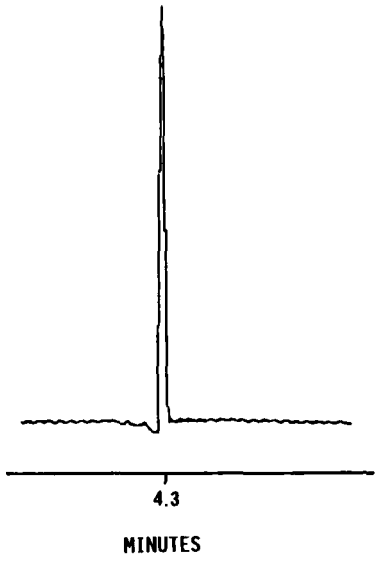

Fig. 2. Identification of limit dextrin as collected from α-amylase bound epoxypropylsilanized PartiSphere-5 column (Figure 1) on PartiSphere-5 PAC column (20 cm x 4.6 mm, I.D.). Flow rate: 0.5 ml/min; sample: 50 ml of potato starch solution as collected from α-amylase bound column (Figure 1) after injecting 200 µl of starch solution each time; sample volumn injected on PartiSphere-5 PAC column: 15 µl; ; detector: refractive index; mobile phase: acetonitrile and water (50:50, v/v) containing 1% ammonium hydroxide..

good storage stability. No significant loss in activity was noticed during five months of storage of either packed columns or the enzyme bound phase as such. Earlier, Barker and Epton (2) observed that covalent binding of α-amylase to crosslinked polyacrylamide derivative affected heat and storage stabilities of the enzyme. The β-amylase was found to be less heat stable when immobilized on Enzacryl derivatives (1).

Specificity of immobilized amylases changes upon attachment to insoluble support. Ledingham and Hornby (6) observed marked increase in multiplicity of attack on starch as compared to

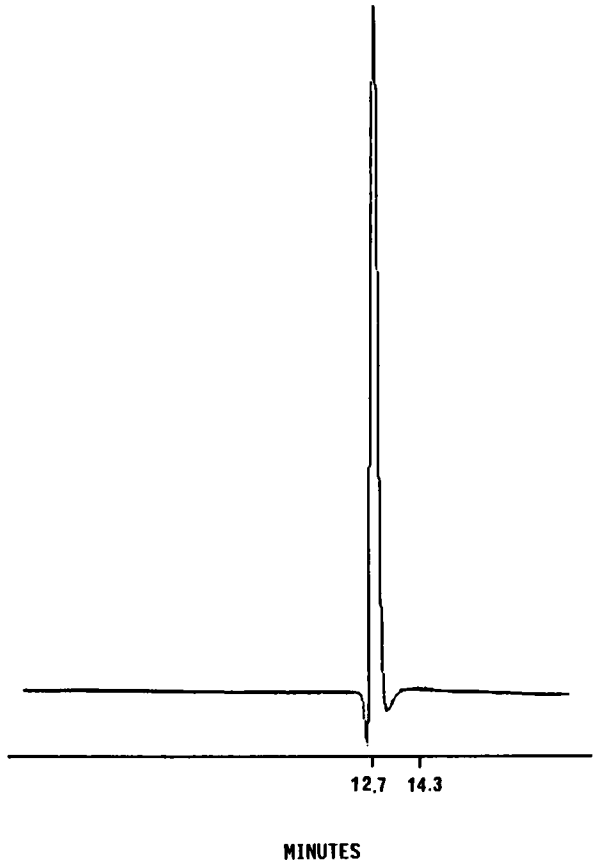

Fig. 3. Conversion of 50 ml limit dextrin solution as collected from α-amylase bound epoxypropylsilanized PartiSphere-5 column (20 cm x 4.6 mm, I.D.) into glucose on β-amylase bound epoxypropylsilanized PartiSphere-5 column (20 cm x 4.6 mm, I.D.). Mobile phase: 0.05M phosphate buffer (pH 7); flow rate: 0.2 ml/min; sample size: 15 µl; detector: refractive index.

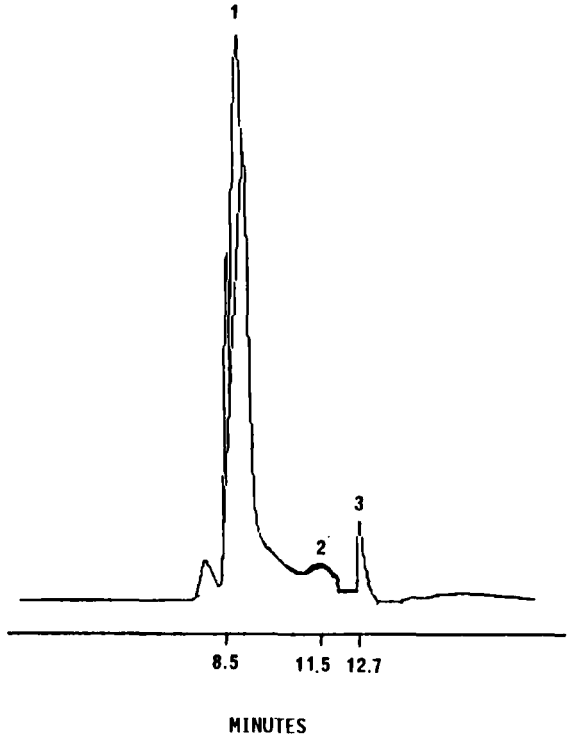

Fig. 4. Hydrolysis of 2% soluble potato starch solution on β-amylase bound epoxypropylsilanized PartiSphere-5 column (20 cm x 4.6 mm, I.D.). 1. limit dextrin, 2. maltose, 3. glucose. Mobile phase: 0.05M phosphate buffer (pH 7); flow rate: 0.2 ml/min; sample size: 15 µl; detector: refractive index.

native enzyme when α-amylase was immobilized on polystyrene. However, the current approach of immobilizing amylases on silica support offers an opportunity to produce glucose from a starch solution.

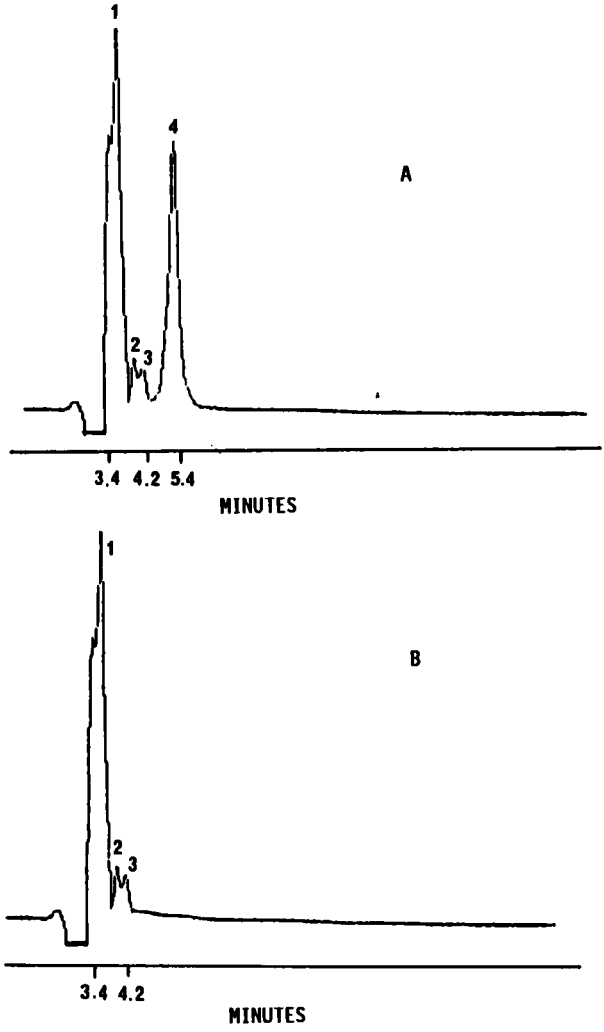

Fig. 5A and 5 B.
Analysis of 50 ml of potato starch solution as collected from α-amylase bound column (Figure 4) after injecting 200 µl of starch solution each time on PartiSphere-5 PAC column (20 cm x 4.6 mm, I.D.). Mobile phase: acetonitrile and water mixture (50:50, v/v) containing 1% ammonium hydroxide; flow rate: 0.7 ml/min; sample size: 15 µl; detector: refractive index.
Fig. 5A: 1. limit dextrin, 2. maltose, 3. glucose.
Fig. 5B: 1. limit dextrin (94%), 2. maltose (3.4%), 3. glucose (2.6%), 4. maltpentose as internal standard.

ACKNOWLEDGEMENT

New Jersey Agricultural Experiment Station Publication No. D-10205-1-89 supported by State Funds and Hatch Regional Project NE-116.

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