



HAL
open science

Characterisation by SEC and High Field NMR of Limit-Dextrins from Enzymatically Degraded Cationic Starches

Ali Ayoub, Christophe Bliard

► **To cite this version:**

Ali Ayoub, Christophe Bliard. Characterisation by SEC and High Field NMR of Limit-Dextrins from Enzymatically Degraded Cationic Starches. 12th European Carbohydrate Symposium, Jul 2003, Grenoble, France. , 258, pp.115 - 121, 2003. hal-02278861

HAL Id: hal-02278861

<https://hal.science/hal-02278861>

Submitted on 4 Sep 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Characterisation by SEC and High Field NMR of Limit-Dextrins from Enzymatically Degraded Cationic Starches

A.Ayoub* & C. Bliard

Laboratoire de Pharmacognosie UMR C.N.R.S. 6013 Bat 18 Moulin de la Housse
Université de Reims Champagne Ardenne 51687 REIMS cedex 2 France
ali.ayoub@univ-reims.fr

Chemically modified starches are widely used derivatives in fields such as paper-making and textile industries.

The physicochemical macroscopic properties of these products are closely linked to their structural organisation at the molecular level. There is a wide range of possible modification pattern on macromolecules such as amylopectins. Different reactions may lead to a variety of structures. In an earlier work we observed that high acetylated starches of the same DS, but synthesised via two different processes, had different structures as seen from their NMR spectra [1]. Woorntman and Steeneken [2] reported that granular starch was methylated in a more block-wise manner whereas when reacted in solution it was modified in a random fashion.

Based on the results of enzymatic degradation Kavitha and Bemiller [3] showed that a commercial hydroxypropyl starch seemed to be more modified in areas near to the branching zones. Commercial cationic starches are synthesised either in solution or more likely by dry process, grafting ammonium groups ether bonds [4]. In a recent work we describe an alternative synthetic route to cationic starches using a glycerol-water plasticised molten medium to achieve the reaction [5]. The products of the β -amylolytic degradation are separated by SEC and analysed by NMR Spectroscopy. The results of structural characterisation are presented.

- [1] B. Laiguel, C. Bliard, G. Massiot and J.M. Nuzillard, Carbohydr. Res., 298 (1997) 251-260
- [2] P.A.M Steeneken, A.J.JL Wortman, Carbohydr. Res., 258 (1994) 207-221
- [3] R. Kavitha, J.N. BeMiller, Carbohydr. Polym., 37 (1998) 115-121
- [4] D.B. Solarek, O.D. Wurzburg ed. Modified Starches : properties and use. CRC Press, Boca Raton, FL,USA, 1986.
- [5] A. Ayoub, C. Bliard, Starch/Stärke, (2003) (accepted)

Introduction

Hydroxypropyltrimethylammonium starches used in paper industries are synthesised by reaction of either **3-chloro-2-hydroxypropyltrimethylammonium chloride (1)** or **2-epoxypropyltrimethylammoniumchloride (2)** catalysed with sodium hydroxide. The cationisation was performed in a glycerol-water molten plasticised starch mixture, using a reactive extrusion system (Fig.1). β -amylolysis, size exclusion chromatography (SEC) and NMR spectroscopy was used in this study to examine the molecular architecture of these modified polysaccharides.

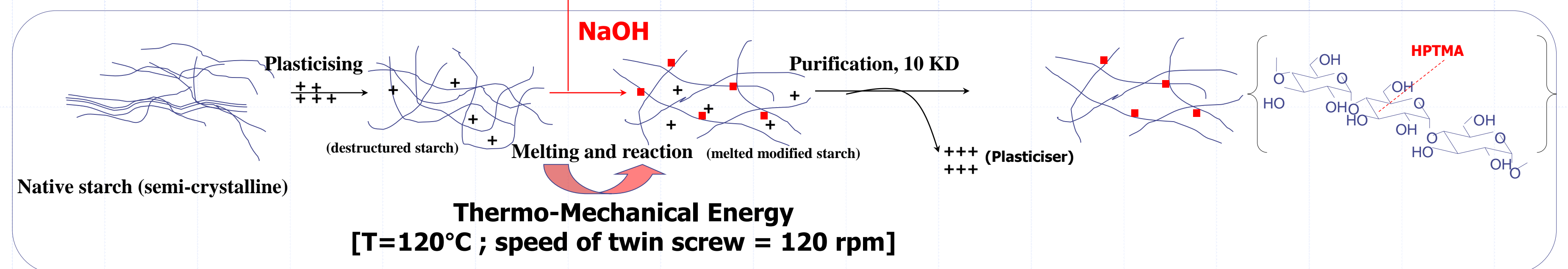
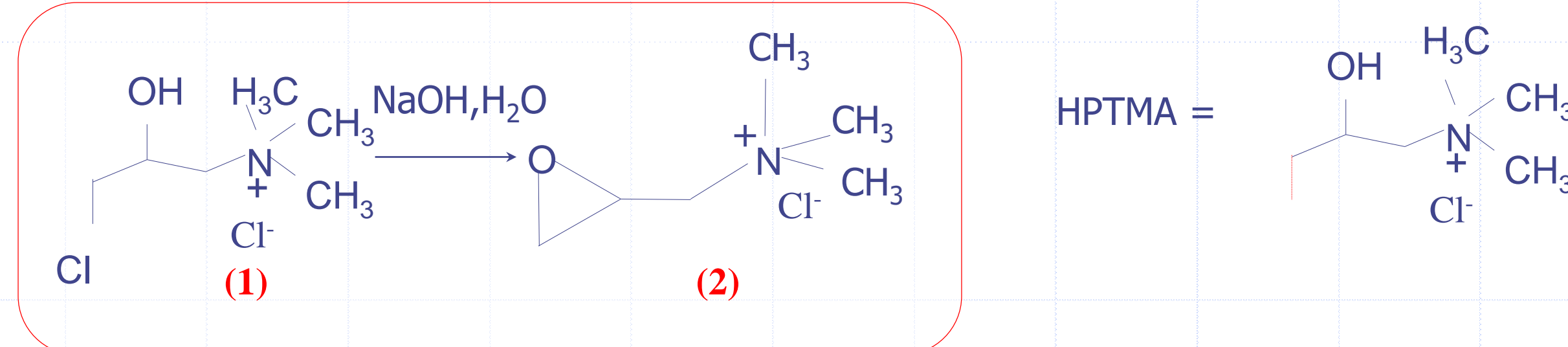


Fig 1 : Starch Cationisation in molten medium

Results and discussion

A) Size-exclusion Chromatography (SEC)

A.1) Fractionation of wheat starches

Amylose and amylopectin fractions of unmodified and HPTMA-modified starches were analysed using *Size-Exclusion Chromatography* (SEC) on a **Sepharose® CL-2B** Column (Pharmacia). The void and total volume of the column were determined using *Blue dextran* (MW 2,000,000) and *glucose* respectively. An aliquot of native, molten and chemically modified wheat starches (10mg / 1ml, dry starch basis) was taken and 200ul of NaOH 1N was added. The sample was dissolved by heating at 60°C and stirring for 20 min. The solution was then filtered at 5um. The filtered sample was placed on the Sepharose® column (25 x 680 mm) and eluted with a 50mM NaCl / 50mM NaOH aqueous solution stabilised with 0.01% NaN₃, at a flow rate of 1.68 ml/min.

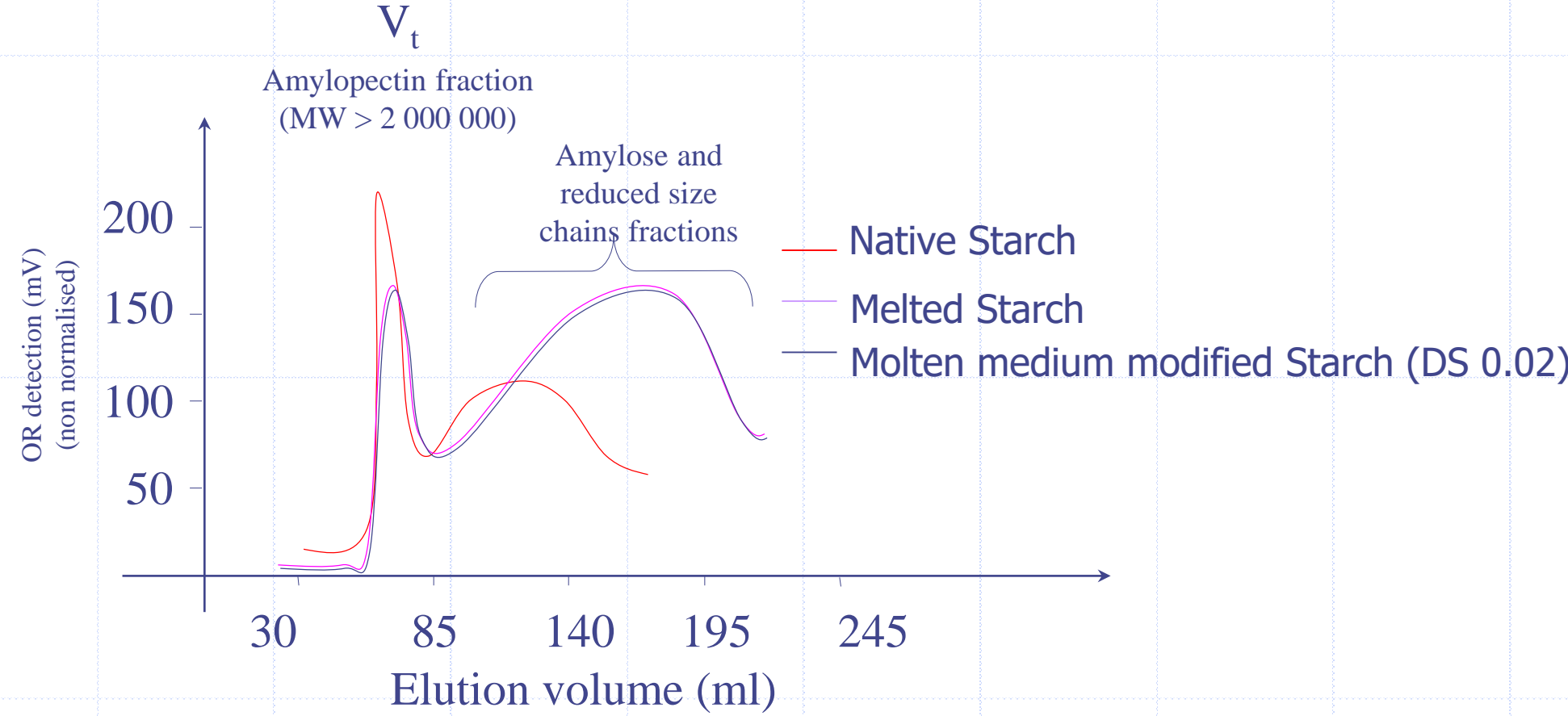
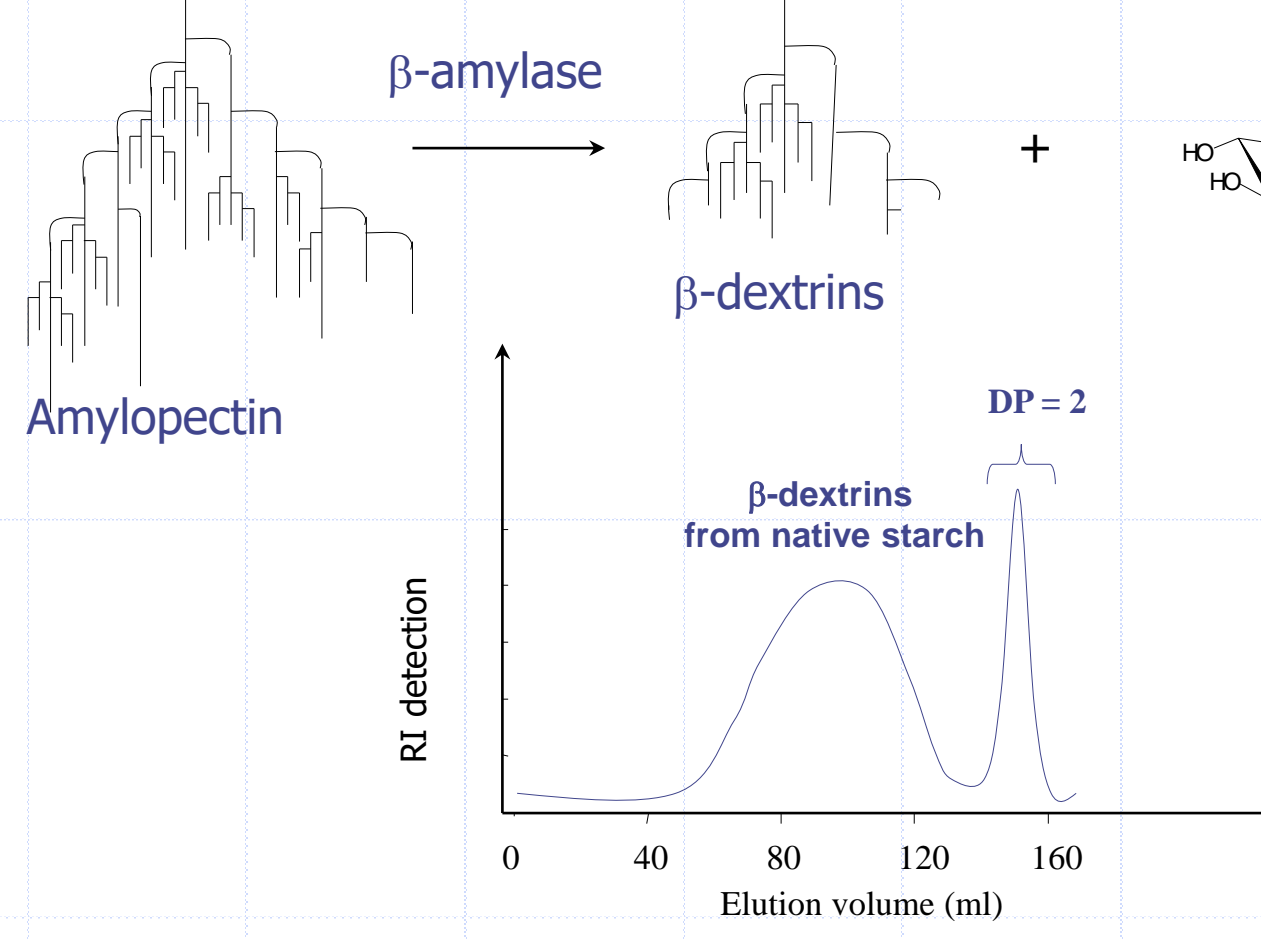


Fig 2 : Native, extruded unmodified and modified starches profiles by SEC (Sephacose® CL-2B)

A decrease of the excluded fraction [MW > 2 000 000 between (40) and (85) ml] corresponding to the amylopectin fraction was observed for both melted samples (Fig 2). This chain length reduction was attributed to the thermo-mechanical energy action. A shift towards the shorter chains was observed with the melted samples, but the cationisation treatment did not cause further macromolecular reduction.

A.2) Fractionation of wheat starch β -limit dextrins

Wheat starches (unmodified and modified, 5 mg) were dispersed in 2 ml of 50mM sodium acetate buffer, pH 4.8; 2 units of β -amylase (Sigma, A 7005) were added, and the mixture was incubated for 24 h at 20°C. The enzyme was inactivated by placing the tube in a boiling water bath for 10 minutes. The digest was centrifuged to remove the precipitated enzyme. The supernatant was fractionated on **Biogel® P-10** to separate two fractions - β -dextrins and maltose. The carbohydrate contents of each fraction was estimated by the phenol-sulphuric acid method (Dubois et al., 1956). Appropriate fractions were pooled to obtain β -dextrins and the solutions were dialyzed and freeze-dried. The resulting fractions were placed on a Sepharose column CL-2B.



Maltose and β -dextrins separated by SEC (Biogel® P-10 column)

The quantity of liberated maltose depends on the chemical modification of starches

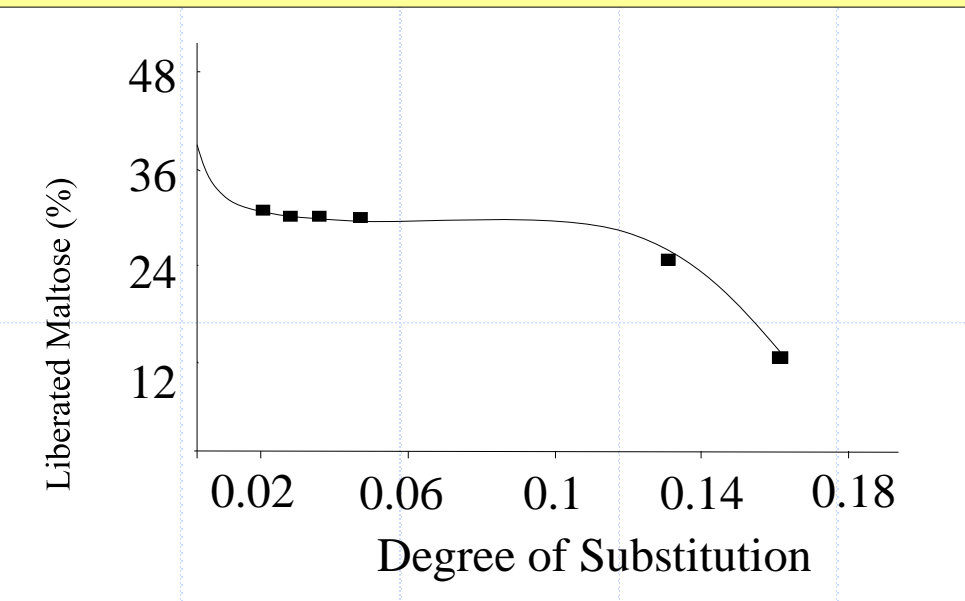


Fig 2 : % of liberated maltose vs. DS

The results of the enzymatic degradation suggests that some of the chemical grafting happens on external α (1-4) chains. However the enzymatic action was not affected when the number of cationic groups were increased up to DS 0.13, suggesting that the subsequent modifications occur almost exclusively on internal chains. (Fig 3)

A.3) Fractionation of β -dextrins separated after β -amylase enzyme on modified and non modified wheat starches

Unmodified and HPTMA-modified β -dextrins were partitioned using SEC on the **Sepharose® CL-2B** column. An aliquot of each dextrin (from native, melted or chemically modified starch in molten medium at DS 0.13) was dissolved in 1 ml water, 200ul of 1M NaOH was added. The solution was filtered at 5um. The solution was placed on the **Sepharose®** column and eluted in a 50mM (NaCl)/50mM (NaOH).

A relative increase of the amylopectin peak between 55-85 ml, and the disappearance of the amylose peak (linear chains), was observed in native starch β -dextrins (Fig 4). But with modified and non-modified melted starch a different behaviour was observed (Fig 5). After the action of thermo-mechanical energy and β -amylolysis, the excluded amylopectin peak almost disappears in melted starch limit dextrin. Whereas after the cationisation treatment, this excluded peak was partly retained, showing an inhibiting effect of the grafted groups on the enzymatic action.

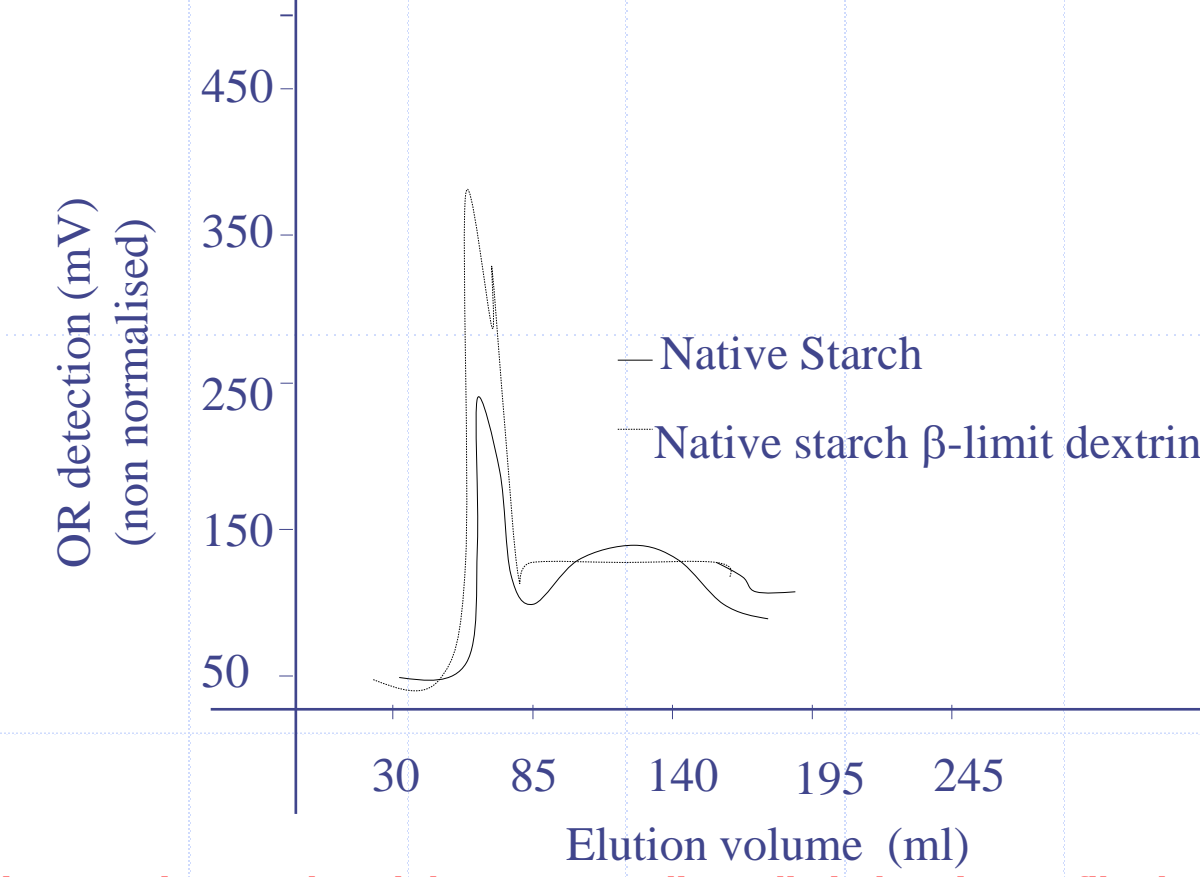


Fig 4 : Native starch and the corresponding β -limit dextrins profiles by SEC (Sephacose® CL-2B)

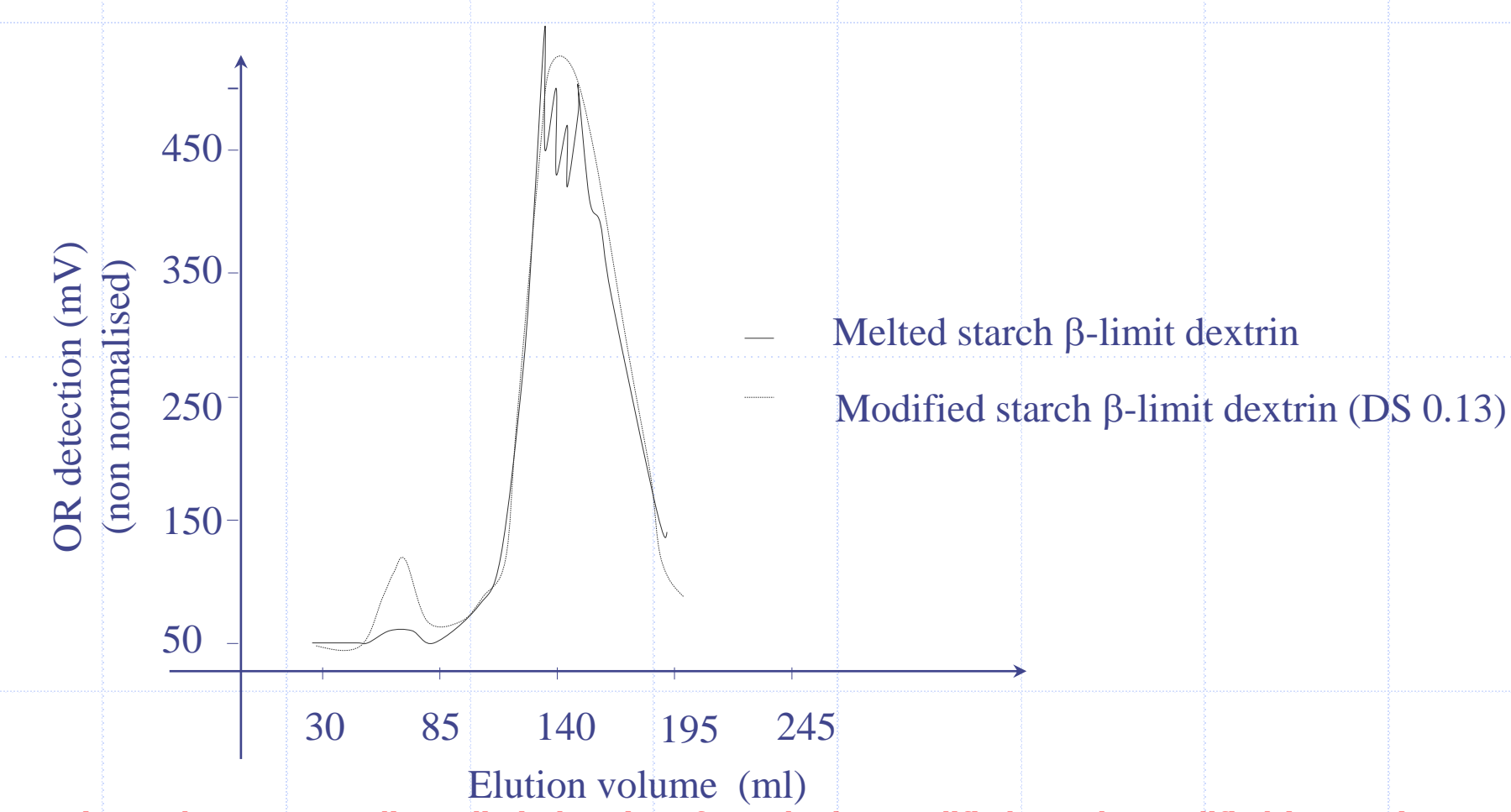


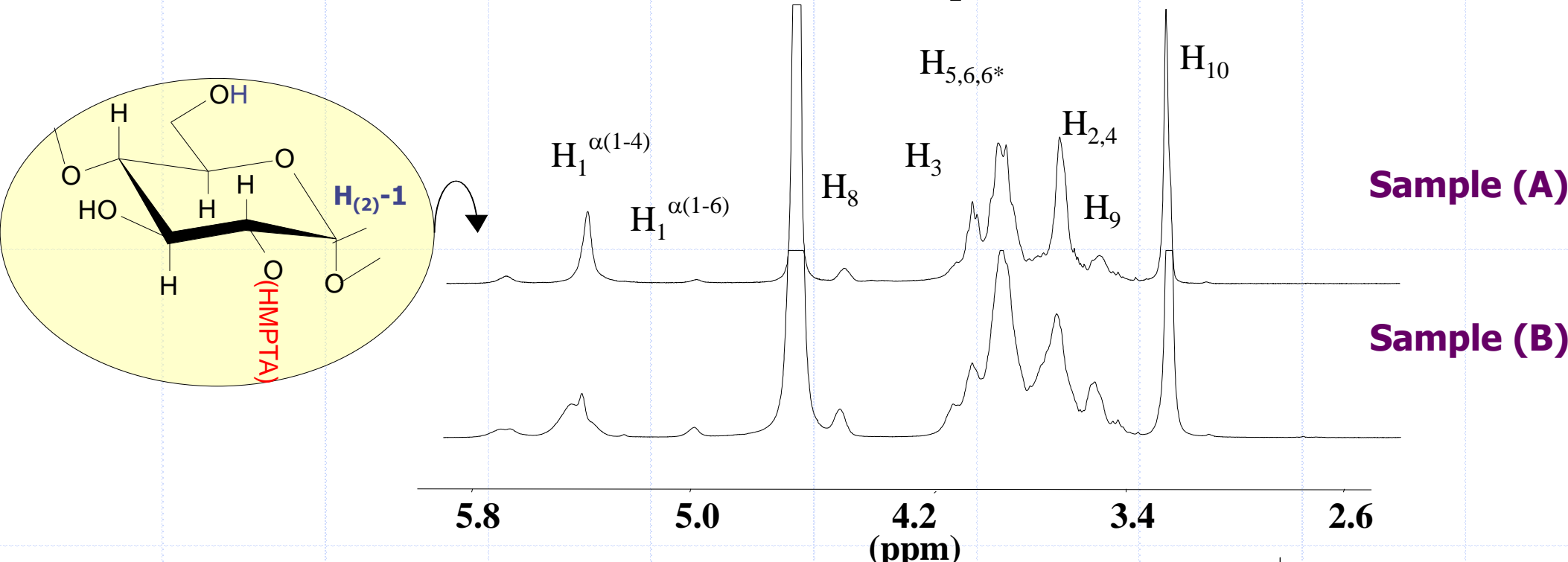
Fig 5 : The corresponding β -limit dextrins of « Melted unmodified » and « modified (DS0.13) » starches profiles by SEC (Sephacose® CL-2B)

B) NMR Spectroscopy

Two samples: A) cationic starch synthesised in molten medium with DS 0.13 and B) the corresponding β -limit-dextrin (DS 0.175), were studied by NMR multidimensional spectroscopy

B.1 NMR 1D - Samples A & B

Proton spectra [500MHz, T =298K, Solvent : D₂O (400ul+ 100ul NaOD)]

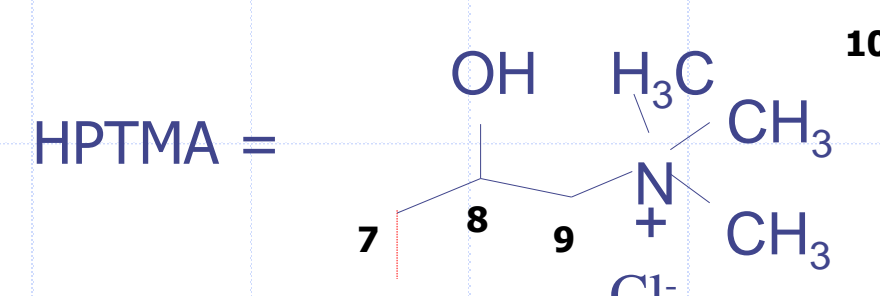


3 possible positions of grafting (HPTMA) groups via ether bonds on the anhydroglucose macromolecules

Modification on position 2 :

Modification on position 3 :

Modification on position 6 :



B.2 NMR 2D COSY - Sample B

