Host-guest molecular interactions in vanillin/amylose inclusion complexes

Silvio D. Rodríguez [a] and Delia L. Bernik [a,*]

a Instituto de Química Física de Materiales, Ambiente y Energía (INQUIMAE) Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA, Buenos Aires, Argentina.

* To whom correspondence should be addressed
Ciudad Universitaria, Pabellón II, INQUIMAE. C1428EGA, Buenos Aires, Argentina.
e-mail: dbernik@qi.fcen.uba.ar TE: +54-11-4576-3378 FAX: +54-11-4576-3341

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Abstract

The interaction of 4-hydroxy-3-methoxybenzaldehyde (vanillin) and Hylon VII due to the formation of an inclusion complex is studied by Fourier transformed infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and circular dichroism (CD). The results confirm the close interaction among the different functional groups of vanillin and its host. In addition, a second case study was carried out with an amylose from a different source (100% amylose, APTIII). As a result, remarkable differences

were found in the vanillin complexation capability of this amylose, which is only evidenced in solution by circular dichroism spectroscopy studies through a clear Cotton effect. This finding confirm the value of using CD studies, which allow finding that, depending of the amylose source, inclusion complexes can be found only in solution, or both in solution and the coexisting precipitates, when this is evidenced by other techniques such as X-ray diffraction (XRD) or Differential Scanning Calorimetry (DSC). Moreover, solubility assays and complexation of both starches with iodine and subsequent absorption spectroscopy studies gives more information regarding the possible source of the starch encapsulation capability. Thus, Hylon VII evidences higher capacity as vanillin encapsulant than APTIII, showing the formation of inclusion complexes both in solution and solid phase, whereas APTIII complexes are only perceivable in solution.

Introduction

Cereal grains like wheat, rice, corn, oats, barley and also tubers like potatoes are rich in starch, a glucose polysaccharide used as reservoir of energy. Natural starches are mixes of two polymers: the linear amylose (~10-25%) and the branched amylopectin (~75-90%), both made of α-1,4 linkages with a small proportion of α-1,6 bonds. About 5% of the glucose units are linked in amylopectin in the form α-1,6, and 1% in the case of amylose, explaining the branched or the linear conformations respectively.[1] Amylose adopts a helical conformation known as V-amylose, giving the possibility to host molecules such as flavor compounds inside the cavity formed by the helix.[2,3] These structures, called inclusion complexes, provide protection to the guest molecules against degradation processes.[4,5] Useful techniques to investigate amylose inclusion complexes in solid phase are differential scanning calorimetry (DSC) and X-ray diffractometry (XRD), while circular dichroism (CD) has proved to be a valuable technique when studying inclusion complexes in solution.[6,7]

Vanillin (4-hydroxy-3-methoxybenzaldehyde), the main component of natural vanilla extracts, is one of the most used compounds in food and pharmaceutical industries as flavoring, antioxidant and masking agent.[8] In a work done previously,[9] demonstrated the ability of the amylose-rich commercial starch Hylon VII (70% amylose) to form an inclusion complex with vanillin molecule. In that work, the formation of the complex was addressed for the first time both in the soluble fraction as well as in the precipitate obtained, using CD and XRD. Moreover, the results obtained by CD were corroborated by theoretical simulations using quantum mechanical hybrid approaches, which showed the energetic stability of the complex and suggested that the chiro-optical changes observed arises from the geometric distortion undergone by the vanillin molecule when included in the amylose helical cavity.

In this work, a detailed description of the molecular interaction between vanillin and Hylon VII is given on the basis of the results obtained by Fourier transformed infrared spectroscopy (FTIR) and DSC. The results confirm the existence of the inclusion complex and remark the close interaction among the different functional groups of vanillin and its host. In addition, a second case study was carried out with an amylose from a different source (100% amylose, APTIII); as a result, remarkable differences were found in the vanillin complexation capability of this amylose, which is able to form inclusion complex with vanillin in solution as revealed by a clear Cotton effect observed by circular dichroism, but do not show complex signals in either DSC and XRD analysis, indicating that no complex is present in the solid phase. In order to study the possible structural differences that cause this disparity in the encapsulant capability, limiting solubility studies and complexation of both starches with iodine and subsequent absorption spectroscopy studies were assessed, confirming that the complexing ability of an amylose containing starch to form inclusion complexes with vanillin is strongly dependent on the amylose type and source.

Materials and methods

Materials

High amylose (70%) maize starch Hylon VII was provided by the National Starch & Chemical Company (Bridgewater, NJ, U.S.A.). Pure 100% amylose (APTIII) and 4-hydroxy-3-methoxybenzaldehyde (vanillin, 99% purity) were purchased from Sigma-Aldrich. All the other reagents were of analytical degree and used as received.

Experimental

Preparation of the Hylon VII and pure amylose dispersions

1.00 g of Hylon VII was dispersed in 150ml of Milli-Q water and heated at 130ºC for 90 min in a flask with a screw cap. The suspension was then left to cool down to 50ºC, at this temperature vanillin was added as indicated in each case. The same procedure was followed when using APTIII.

Inclusion Complexes: sample preparation

1.20 g of vanillin (54.5% w/w vanillin/starch) dissolved in 1.50 ml of ethanol was added to 150 ml of the starch dispersion. The mixture was allowed to rest at room temperature for 24 h. The precipitated obtained was filtered, washed, centrifuged, and intensively dried. The supernatant was used for the CD studies.

Solubility determination

The limiting (saturated) solubility of each starch was calculated by heating an excess of starch in a given volume of Milli-Q water at heated at 130ºC for 90 min in a flask with a screw cap, with agitation. After that, the dispersion was cool down at room temperature (20ºC) several hours, centrifuging the suspension at 9000 g and discarding the pellet. The supernatant (a completely translucent solution) was separated, 8700 g during 30 minutes at 20ºC, and an aliquot of this solution was freeze-dried and weighted, allowing calculating the amount of dissolved starch in each case.

FTIR spectra

About 5 milligrams of solid were mortared and mixed with 200 mg of KBr (IR grade) to obtain the pellet. The Fourier transform infrared spectra (FTIR) were recorded on a Thermo Nicolet 8700 spectrometer, in the range of 660-4000 cm-1. Thirty two scans were accumulated for each measurement. As a control, a mechanical mixture was

prepared by mixing the amounts of starch and vanillin, matching the relative amounts of each component to the one used in the inclusion complex preparation.

DSC measurements: experimental conditions

The dried material obtained (see Inclusion Complexes: sample preparation) was used to carry out the high pressure differential scanning calorimetry (HP-DSC, *Shimadzu DSC-50*) using sealed aluminum capsules. 1 mg of dried sample was placed into a high pressure DSC pan adding 5 µl of Milli-Q water to ensure an adequate moisture amount. The DSC pan was closed and leave to rest 1 hour until the measurement. The heating rate was 5ºC/min from 30 ºC until 190ºC, and the stage of cooling (heat dissipation) of each sample was also recorded.

Regular DSC scans were performed with the same equipments under a rate of 10 ºC /min using standard aluminum sealed capsules. Pans are crimped, but not hermetically sealed, supporting a maximum pressure of 0.3 MPa (Shimadzu, #201-53090).

Circular dichroism measurement of APTII complex

An appropriate dilution (0.15 ml into 3.00 ml of Milli-Q water) of the supernatant obtained (see Inclusion Complexes: sample preparation) was used to determine the CD and UV spectra, recorded using a Jasco-815 spectrometer under a 5 L/min flow of nitrogen (99.998%). The scans were done at 20 nm/min with a response of 8 seconds, using a band width of 1 nm and averaging 5 accumulated spectra for each measurement.

X-Ray diffraction measurement of complexes

X-ray diffraction patterns of the powders obtained (see Inclusion Complexes: sample preparation) were recorded in a Siemmens D5000 diffractometer using a Cu Kα radiation. The operating conditions were a 0.154 nm radiation wavelength, a voltage of 40 kV and a current of 30 mA. Diffractograms were scanned over the 2θ range of 3.5 - 35º, with a scan rate of 0.022º/s.

Iodine complexation of Hylon VII and APTII

In order to measure the UV/VIS spectrum of the amylose/iodine complex different aliquots of the supernatant solutions obtained for Hylon VII and APTIII (see Solubility determination) were added to 3.00 ml of a 2×10^{-3} M KI/5.2×10^{-5} M I_2 solution, obtaining the typical blue coloration. The spectra were recorded from 700 to 300 nm with a PG T60 spectrometer.

Results

FTIR studies of Hylon-vanillin inclusion complexes

FTIR spectra were recorded for samples of the starch Hylon VII, pure vanillin (4-Hydroxy-3-methoxybenzaldehyde), the vanillin-starch inclusion complex and the mechanical mixture of starch and vanillin, matching in this last sample the relative amounts of each component used in the complex preparation (Figure 1). For clarity, the figure was divided in part A, which shows the spectra of the starch Hylon VII (black line) and the mechanical mixture starch+vanillin (green line). Figure 1B shows the spectra of pure vanillin (red line) and the one of the inclusion complex (blue line).

In part A, the spectrum of the pure starch shows the typical absorptions of the polysaccharides. The region of frequencies around 1000 cm^{-1}, attributed to different types of transitions: two of the most intense are assigned to the C–C stretching

vibrations from 1200 to 1103 cm $^{-1}$, and to the C–O bending vibrations from 1047 to 994 cm^{-1}; at longer wavenumbers, the noticeable O-H stretching vibration band centered at 3402 cm^{-1}.[10] The mechanical mixture displays high absorption intensity in the whole fingerprint region between 700 and 1700 cm^{-1}, and a broad O-H band between 3100 and 3600 cm^{-1}. The overall spectrum and in particular this very broad O-H stretching vibration band represents, as expected, the sum of the individual spectra of the starch and vanillin, as both components were mixed just before running the spectrum.

In Figure 1B, the pure vanillin spectrum displays a wide fingerprint region between 700 and 1700 cm^{-1} with several peaks of high absorption intensity, and the phenol O-H stretching vibration band centered at 3170 cm^{-1}. For the Hylon VII-vanillin inclusion complex, remarkable differences can be observed in the spectrum. First, the vanillin's O-H band at 3170 cm^{-1} is not observed, appearing only a unique band centered at 3415 cm^{-1}. In addition, several intense bands of the vanillin spectrum originally found between 700 and 1700 cm^{-1} are in the complex depleted or their absorption intensity significantly diminished.

To better describe the numerous changes observed in the fingerprint region of the inclusion complex, the Figure 2 plots exclusively this region showing simultaneously the bands of the four samples studied, and each band is particularly depicted. At first glance, it is noticeable that some bands (pointed out with black dotted arrows) observed in the vanillin spectrum vanish in the spectrum of the inclusion complex (compare the red and blue curves, respectively).

The bands at 1465 cm^{-1} and 1429 cm^{-1} are assigned to the asymetric deformation of the methyl group belonging to the –OCH_3 substituent.[11,12] The two strong bands at 1265 and 732 cm^{-1} correspond to the C-OCH_3 stretching mode.[13] Additionally, the bands at 812 cm^{-1} and 856 cm^{-1} assigned to C-H out-of-plane bending

vibrations, also vanished.[14] It is worth to recall that many of these bands belonging to the vanillin molecule fall in regions where no absorption bands are present in the pure starch spectrum, which facilitates the comparison of and allows seeing which of these remain in the starch-vanillin mechanical mixture and which disappear after obtaining the inclusion complex.

The vanillin's bands which still remain, although significantly lowered in their intensity and some of them slightly shifted, are those bands centered originally at: 1300 cm^{-1} (shifted to 1292 cm^{-1}, assigned to the phenolic OH bending), 1510 cm^{-1}(shifted to 1516 cm^{-1}) and 1589 cm^{-1} (shifted to 1593 cm^{-1}) both assigned to C=C and C-C stretching mode vibrations of the benzene ring; and 1664 cm^{-1} (shifted to 1672cm^{-1}, corresponding to the aldehyde C=O stretching mode).

DSC studies of Hylon-vanillin inclusion complexes

Figure 3 (main frame) shows two HP-DSC thermograms obtained by scanning the temperature from 30°C to 190°C for the Hylon VII-vanillin inclusion complex sample (full black line) and the Hylon VII control sample (dashed red line). The thermogram obtained for Hylon VII-vanillin shows a unique well-defined endothermic peak at 108°C, which is assigned to the dissociation of the inclusion complex. In fact, 90°C-110°C is the range of temperatures reported for the "melting" peaks of most of the starch inclusion complexes with different ligands, which are listed in Table I, incorporating the present case.

Instead, the thermogram obtained with Hylon VII control sample (dashed red line) presents a well defined peak at 177°C. Such transitions at elevated temperatures (above 140°C) have been previously assigned to changes in the amylose fraction of the starch due to endothermic transitions of amylose crystals,[15] formed in our case probably

during the experimental procedure followed to obtain the complex (starch gelatinization, then subsequent precipitation by slow cooling). During cooling processes no signals were observed, either for the Hylon VII control or the Hylon VII-vanillin samples, indicating that the melting process of the amylose-vanillin complex is not reversible; and either the transition originally found for the control sample, at least under the scanning conditions assayed. A pure vanillin control sample presented no signals by HP-DSC during the whole heating and cooling cycle, indicating that vanillin is dissolved in the water and chemically stable in the range of temperatures tested. These transitions were observed in thermograms obtained in high pressure aluminum capsules with an excess of water added to the solid sample. However, some works have also been reported in which the formation of inclusion complexes are studied analyzing the dried solid samples obtained, focusing on the transitions (melting) occurring to the guest ligands before and after complexation. Therefore we also performed a DSC using regular crimped aluminum capsules and run scans of dried samples of pure vanillin crystals (blue solid line) and the Hylon VII-vanillin inclusion complex (blue dotted line), which are displayed in the inset of Figure 3. In both cases the amount of vanillin introduced in the samples was the same. The first run shows a strong endothermic peak assigned to the melting at 82°C of pure vanillin crystals,[16] and the second run, in contrast, remarks the disappearance of the peak at 82°C, leaving a remaining small and broad peak at lower temperatures.

Characterization of a vanillin inclusion complex obtained with another amylose source

To evaluate the complexing ability of amylose obtained from a different source we assayed the amylose APTIII (from Sigma-Aldrich, see materials). The APTIII-

vanillin inclusion complex was prepared with exactly the same methodology and host/guest ratios used in the case of the starch Hylon VII.

The first study was to record the circular dichroism spectra of the solution after centrifugation and separation of the precipitate. The experimental procedure is clearly detailed in methods (see Inclusion Complexes: sample preparation) and in a previous work.[9] The obtained absorption and associated circular dichroism spectra of the APTIII-vanillin solution are shown in Figure 4; being similar to those obtained for the Hylon VII-vanillin inclusion complex. The CD spectrum shows the same two negative bands at 205 and 230 nm, centered at the same wavelengths observed in UV spectrum. Notice that the vanillin molecule has no chiral centers, thus, a pure aqueous vanillin solution does not show any signal in CD, and the water soluble fraction of pure amylose have no absorption above 180 nm.

As in the case study with Hylon VII, X-Ray diffraction studies were performed with the solid isolated and dried after obtaining the APTIII-vanillin complex. However, no differences between the complex and the control samples were found in the x-ray pattern. Moreover, HP-DSC measurements showed no peaks in heating-cooling cycles, confirming that no APTIII-vanillin complex was present in the solid phase. CD, XRD and HP-DSC experiments were repeated with different batches, arriving to the same results, that is, CD yielded evidence of complex formation in solution, but lack of signals in XRD and DSC, indicating that no complex is found in the solid phase.

Comparison of the ability of Hylon VII and APTIII to form inclusion complexes studied by titration of an iodine solution.

With the aim of characterizing the capability of inclusion complex formation of both amylose sources, both Hylon VII and APTIII were complexed with iodine

solutions according to the method developed by Gilbert and Spragg.[17] First, saturated solutions at room temperature (20ºC) of both starches were obtained, and the limiting solubility of each starch in water was determined (see Solubility determination). The results showed that the limiting solubility in pure water was 172 mg/ml for Hylon VII and 226 mg/ml for APTIII amylose.

Two well measured volumes of iodine solutions from the same batch (see Iodine complexation of Hylon VII and APTII) were titrated separately by adding successive aliquots of the Hylon VII and APTIII saturated solutions. As a result, a linear increasing formation of the iodine-amylose complex is clearly observed with both starches. In Figure 6 the absorption at the maximum absorption wavelength ($\lambda_{\max}^{abs}$) of the iodine complexes with Hylon VII and APTIII is plotted as a function of polysaccharide concentration. Two straight curves with excellent linear correlation coefficients (higher than 0.99) were obtained. It is remarkable that the $\lambda_{\max}^{abs}$ was invariant, being always 602-603 nm irrespective of the starch identity and concentration.

Noticeable, a pronounced difference among both starches is observed in the slope of the linear regressions, indicating that for a similar concentration of starch, the absorbance of the Hylon complex is about 10 times higher than the absorbance of the APTIII iodine complex. This difference is observed for polysaccharides concentrations well below the limiting solubility of both starches.

A valuable parameter for the characterization of a starch ability to form inclusion complexes with iodine is the so called "blue value",[17] which is a calculation based on the absorption in the far red side (680 nm) of the iodine complex spectrum, which is directly proportional to the concentration of said complex,

$$BV = \frac{0.4 \times Abs_{680nm}}{C_{starch}(mg/ml)} \qquad \text{Eq. 1}$$

To make a reliable calculation of BV is relevant performing calibration curves as a function of the concentration of the tested starches, as shown in Figure 5. The results described in Table II shows a very good reproducibility in the BV value obtained, which for Hylon VII is 12 times larger than for APTIII.

Discussion

The molecular structure of the amylose, a linear polysaccharide having 99% of α-(1 → 4) links among glucose monomers with almost no branching, gives the possibility of forming different types of helices, such as the double helices observed in the diffraction patterns A and B in the starch granules, and also a single helical conformation, characteristic in the case of inclusion complex formation, with a diffraction pattern called type V.[18]

The amylose molecule builds an helical internal cavity which, when hosting the vanillin molecules forms an inclusion complex and shrinks wrapping around the guest, as depicted in the sequence depicted in Figure 6 parts a and b, thus decreasing the interatomic distances favoring the interaction among vanillin and amylose functional groups.[9] This close interaction becomes evident in the changes observed in the FTIR spectrum of the complex, when comparing with the spectra obtained with pure vanillin, amylose, or a simple mechanical mixture of both.

In general, in Figures 1 and 2, we can observe that the host-guest interaction significantly reduce the absorption intensity of the vanillin, the guest in this case. First, the vanillin's O-H band originally at 3170 cm^{-1} is not observed, appearing only a unique band centered at 3415 cm^{-1}. This suggests a close interaction of this functional group with the amylose in the complex, leading to a noticeable shift to higher frequencies (a faint shoulder can be seen at 3260 cm^{-1}), being overlapped by the O-H stretching band

319 of the starch, which was in fact also shifted from 3350 to 3415 cm^{-1}.[21] This is supported
320 by a previous theoretical approach, which also allowed understanding the circular
321 dichroism spectrum of the amylose-vanillin inclusion complex. That study mentions the
322 hydroxyl phenolic group among the chemical substituents of vanillin which experience
323 the shortest interatomic distances (<3Å) with the atoms of the amylose (see Table II and
324 Figure 5 in Rodríguez et al.[9]) Similar changes were observed in the obtaining of the
325 inclusion complex of vanillin with cyclodextrin, as reported by Rajendiran &
326 Balasubramanian,[19] who also attributed this change to a more internal and compromised
327 position of the OH group within the helical cavity.

328 Among the other differences observed in the FTIR spectra, the bands at 1465
329 cm^{-1} and 1429 cm^{-1} (asymetric deformation of the $–OCH_3$ methyl group) and the two
330 bands at 1265 and 732 cm^{-1} (C-OCH_3 stretching mode) disappear in the inclusion
331 complex spectrum. This is also consistent with the changes induced by the host when
332 including the guest, suggested by theoretical modeling: the Figure 6 c shows a torsion
333 out of plane of the $–OCH_3$ methyl group which, by simulation, gives place to a
334 theoretical CD spectrum similar to the experimentally found, supporting the proposed
335 structure. The bands observed in the CD spectra are then assigned to the quirality
336 induced by the geometrical distortion of the vanillin molecule after entrapment into the
337 amylose helix, leading to a new chiral conformation and producing the signals in the
338 CD spectra (Rodríguez et al., 2011,[9] and this work). This represents a clear Cotton
339 effect, which, in the case of this complex is induced by the molecular distortion of the
340 vanillin when included in the amylose cavity.

341 The formation of the complex is also undoubtedly ascertained by using the
342 thermoanalytical DSC analysis, one of the most widespread techniques to study the
343 formation of amylose inclusion complexes. When applied to polymers, DSC detects

transformations that can be primarily ascribed to fusion, inter-conversion between different crystalline states, and sub-Tg transitions of glassy or crystalline polymers. In the case of HP-DSC applied to starches and their complexes with diverse guest molecules, this technique reveals endothermic transitions assigned to the dissociation of the complex when the solid is subjected to heating in the presence of water, a process commonly called "melting" of the inclusion complex.[18] The peak at 108 °C found by HP-DSC and the disappearance of the melting peak of the pure ligand (Figure 3) are unequivocal evidences of the amylose-vanillin inclusion complex existence. Similar findings were reported in the cases of β-cyclodextrin/vanillin and β-cyclodextrin/flavonoid inclusion complexes.[20,21] On the other hand, the transitions observed on pure starches at elevated temperatures (above 140 °C) have been assigned to changes in the amylose fraction of the starch, probably due to an endothermic transition of amylose crystals, in our case formed during the experimental procedure to obtain the complex (starch gelatinization, then vanillin addition and subsequent precipitation by slow cooling). We found such a transition at 177 °C (see Figure 3, red dashed line), a similar to the one observed by Heussen et al.[15]

The examples of inclusion complexes and their melting points shown in Table I remark that melting temperatures fluctuate depending on factors such as the source of amylose used, its native or processed nature, and the guest molecule incorporated in the helical structure. Factors depending particularly on the amylose source are evidenced by the inclusion complexes obtained with geraniol and fenchone, whose reported melting temperatures are 91°C/107°C and 92°C/114°C, for the case of complexation using native potato starch and using potato amylose, respectively (see Table I; Nuessli et al.[22]; Nuessli et al.[6]). Another example is the melting temperatures recorded when using native and pre-processed starch, like in the complexes obtained with Hylon VII by

Lay Ma et al.[23]. The authors observed differences in the temperatures of dissociation of the complexes formed among Hylon VII and three different fatty acid esters (ascorbyl palmitate, retinyl palmitate and phytosterol esters), when the starch is used in its native form or after lipid extraction, informing temperatures of 100°C/ 98°C, 102°C/ 80°C and 126°C/ 98°C, respectively.

Thus, the key influence of the starch source, its composition and pre-treatment before complexation with a flavor ligand help to explain why in this work we can show that Hylon VII forms an inclusion complex both in the solid, as in the solution obtained by centrifugation and filtration; whereas pure APTIII amylose evidences the formation of inclusion complex with vanillin only in solution. To better understand starch properties which may account for these differences, we tested the complexing ability of the starches by obtaining iodine inclusion complexes, a well known spectroscopic tool.

Remarkable differences were found between both Hylon VII and APTIII starches. The lowest complexation capacity displayed by APTIII, besides the fact that the solubility of APTIII is greater than the one observed for Hylon VII, suggest that APTIII has higher proportion of short chains, which do not form iodide complex, together with a lower proportion of soluble chains with a length similar to those found in the Hylon VII solutions. This last statement is suggested by the fact that the maximum absorption wavelength ($\lambda_{\max}^{abs}$) of the complexes with iodine is the same for both Hylon VII and APTIII. According to Wulff et al.[24] (and references therein), the $\lambda_{\max}^{abs}$ shifts to longer values as the average chain length of the amylose increases. In addition, the finding of a "blue value" for Hylon VII twelve times higher than the one obtained for APTIII further supports all the previous statements.

Conclusions

Summarizing, Hylon VII evidences higher capacity as vanillin encapsulant than APTIII, showing the formation of inclusion complexes in the solid phase whereas APTIII complexes are only appreciable in solution by circular dichroism. These conclusions are reached on the basis of a joint analysis of the spectroscopic evidences provided by Circular Dichroism, FTIR, XRD analysis and iodine complexation, as well as thermoanalytical DSC analysis and the previous support shown by theoretical modeling. These studies confirm the ability of the amylose to form complexes with vanillin and the close interaction of the corresponding host/guest functional groups.

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Figure Captions:

Figure 1: FTIR spectra of A) Hylon VII (black solid line) and the mechanical mixture of Hylon VII with vanillin (green solid line). B) Inclusion complex between Hylon VII and vanillin (blue solid line) and pure vanillin (red solid line).

Figure 2: FTIR spectra of the Figure 1 showing a selected region between 700-1800 cm^{-1}, for Hylon VII (black solid line), mechanical mixture of Hylon VII with vanillin (green solid line), inclusion complex between Hylon VII and vanillin (blue solid line) and pure vanillin (red solid line). The vanillin bands which disappear in the complex (and their wavenumbers) are indicated with dashed arrows, whereas the bands which have a significant lowered intensity are indicated by solid arrows. The assignations of the bands are described in the text.

Figure 3: High pressure DSC thermograms of the inclusion complex between Hylon VII and vanillin (black solid line) and Hylon VII as control sample (red dashed line) in presence of water. Insert : DSC thermograms of the sample containing the solid inclusion complex (blue dashed line) and pure vanillin crystals (blue solid line).

Figure 4: CD and UV spectra of the inclusion complex between ATPIII and vanillin in solution. For scanning conditions details, see Circular dichroism measurement of APTII complex.

Figure 5: Scatter plot of the absorbances measured at the maximum absorption wavelength $\lambda_{\max}^{abs}$ of iodine complexes formed with increasing concentrations of Hylon VII (green triangles) and APT III (red squares).

Figure 6: a) top view of a 10 units amylose helix alone ; b) top view of the inclusion complex of the helix and vanillin, and c) represantation of the molecular distortion of vanillin upon inclusion, which gives place to CD spectrum.

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556 Table I: phase transition temperatures (melting) of inclusion complexes between
557 amylose and various guest compounds.

Guest molecule	**Amylose source**	**Transition temperatures (°C)**	**Reference**
Hexanal	native potato starch	107	Jouquand et al.[25], 2006
γ-hepta-lactone		90	Heinemann et al.[26], 2001
γ-nona-lactone		91	
γ-deca-lactone		95	
δ-deca-lactone		105	
δ-dodeca-lactone		115	
Decanal		90	Nuessli et al.[6], 1997
(-) fenchone		107	
(-) carvone		91	
Geraniol		91	
(+) campher		76	
Thymol		105	
1-naphthol		109	
Fenchone	potato amylose	114	Nuessli et al.[22], 2003
Geraniol		92	
Menthone		114	
ácido láurico	Hylon VII	109	Zhang et al.[27], 2012
vanillin		**108**	**This work**
ascorbyl palmitate		100 y 98*	Lay Ma et al.[23], 2011
retinyl palmitate		102 y 80*	
phytosterol esther		126 y 98*	

558 *Two temperatures reported for complexes obtained with native and lipid-free starch,
559 respectively.
560
561
562
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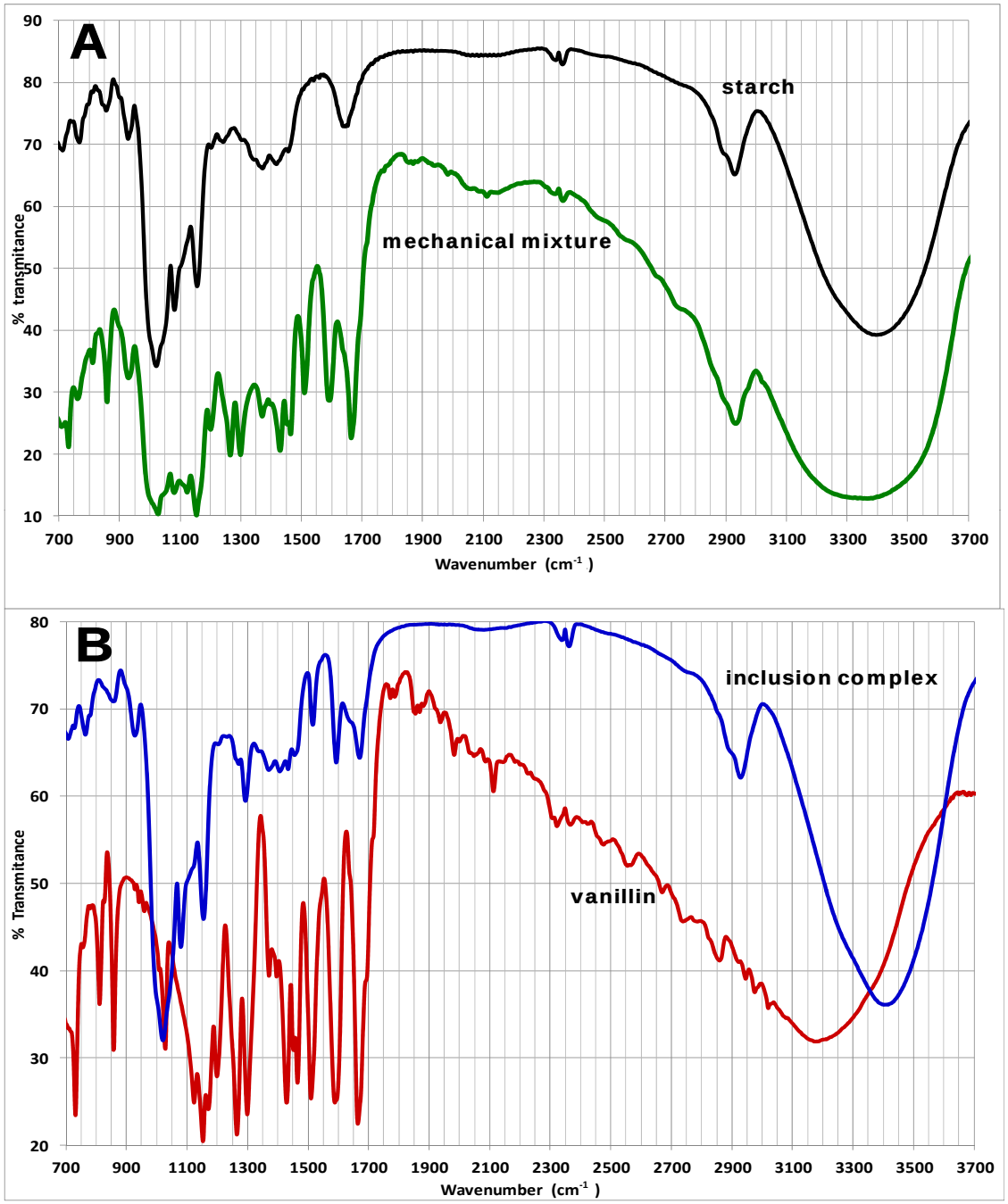
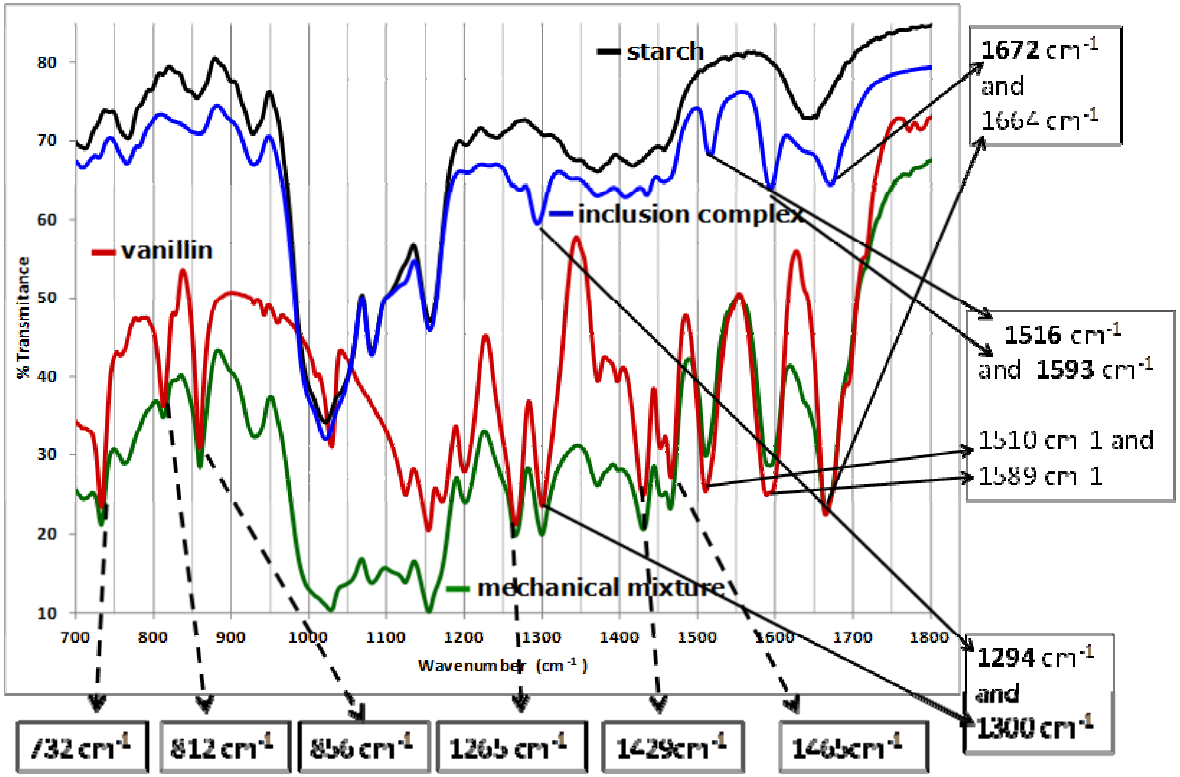
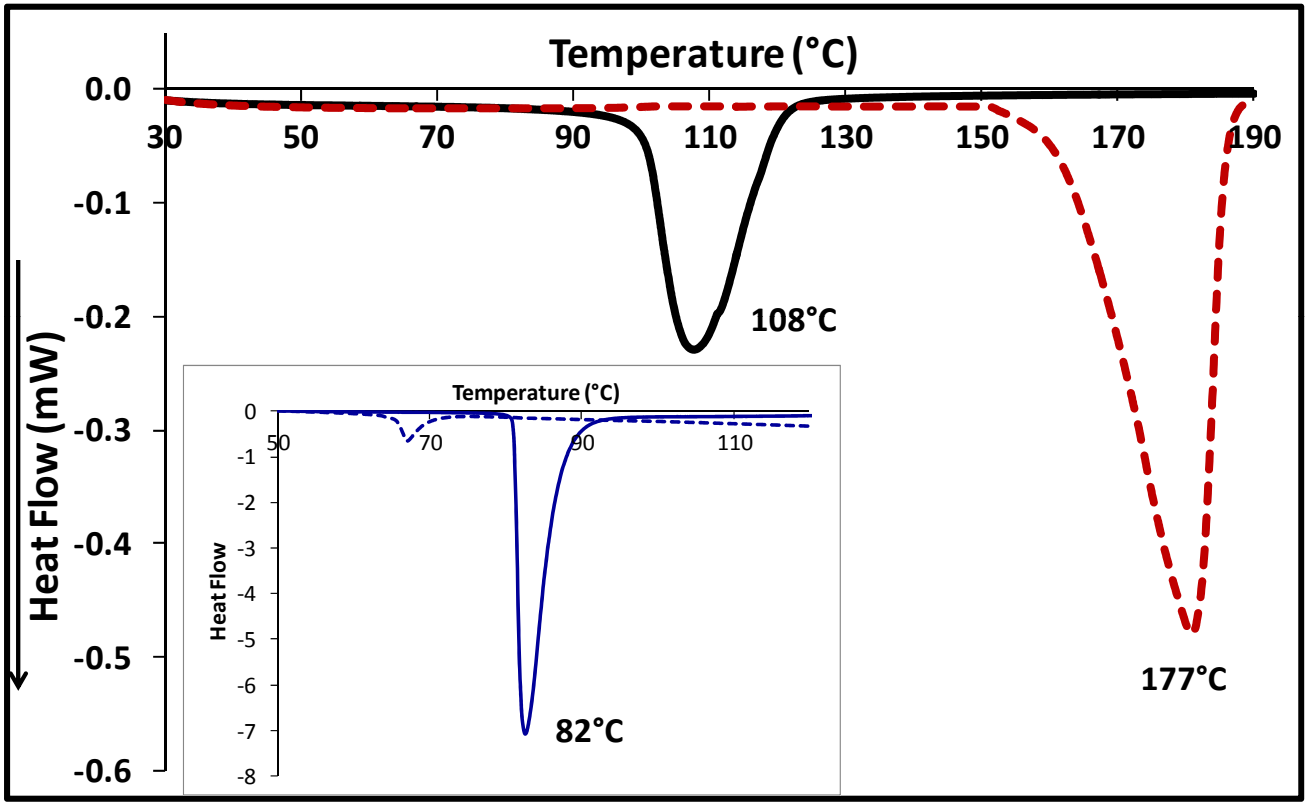
564
565

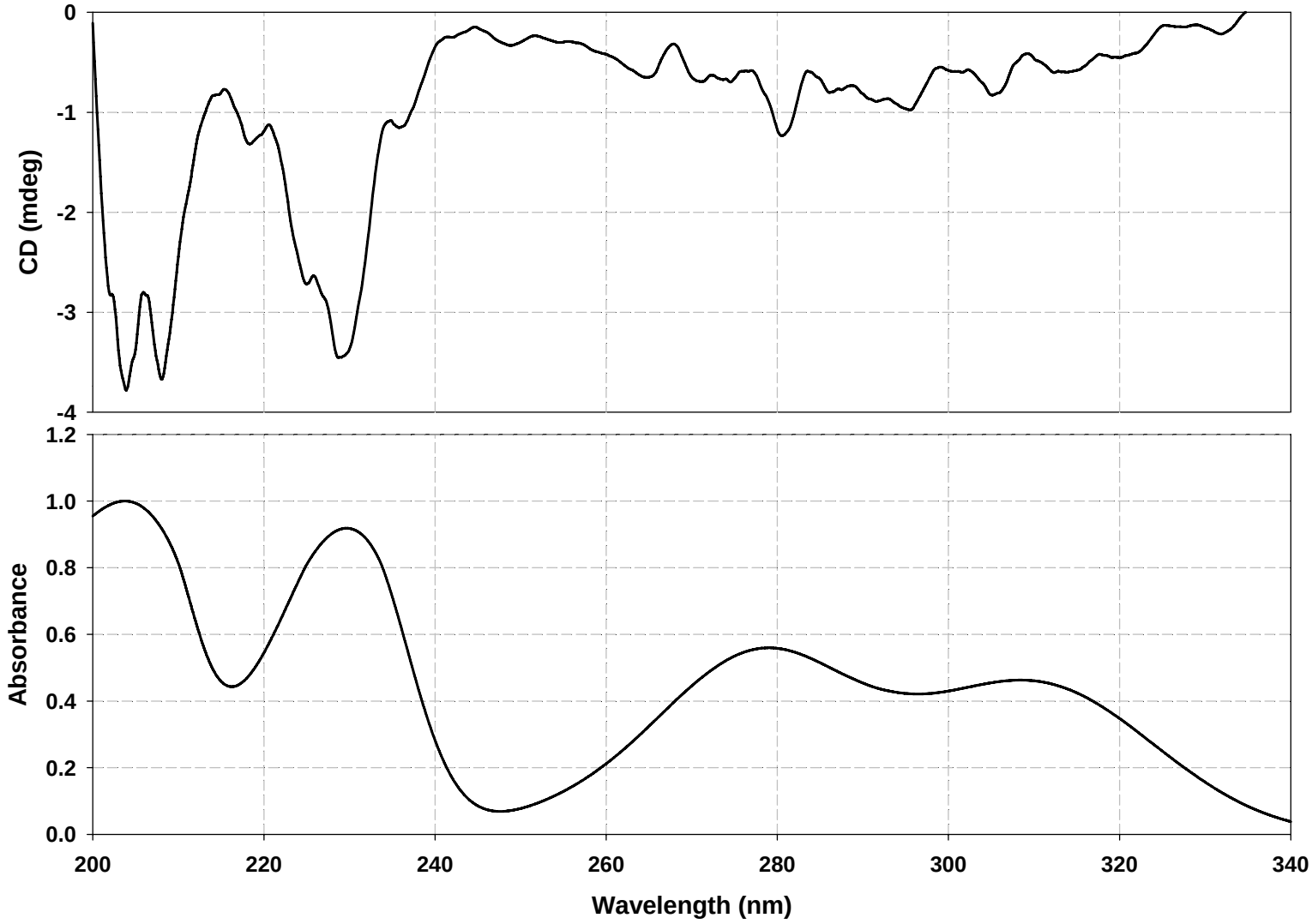
Table II: Blue values obtained for Hylon VII-iodine and APTII-iodine complexes at each starch concentration, according to Eq. 1.

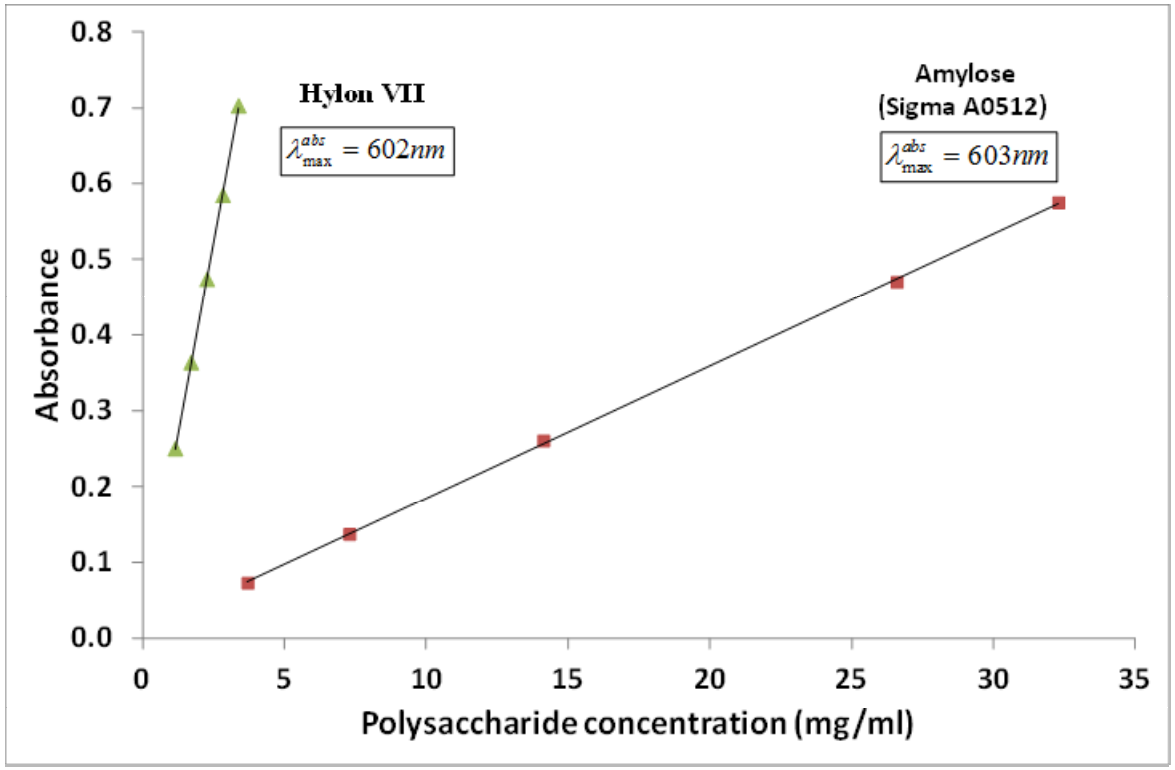
Hylon VII		iodine complex λ_{max}^{abs} = 603 nm	APTIII		iodine complex λ_{max}^{abs} = 602 nm
C (starch mg /ml)	Absorption at 680 nm	Blue value	C (starch mg/ml)	Absorption at 680 nm	Blue value
1.14	0.201	0.070	3.70	0.058	0.0063
1.70	0.292	0.069	7.29	0.111	0.0061
2.26	0.381	0.069	14.13	0.213	0.0060
2.82	0.470	0.067	26.59	0.384	0.0058
3.37	0.566	0.067	32.29	0.470	0.0058
		$BV_{Hylon\ VII}$ ≈0.07			BV_{APTIII} ≈0.006


A
starch
mechanical mixture
% transmitance
Wavenumber (cm-1)
B
inclusion complex
vanillin
% Transmitance
Wavenumber (cm-1)

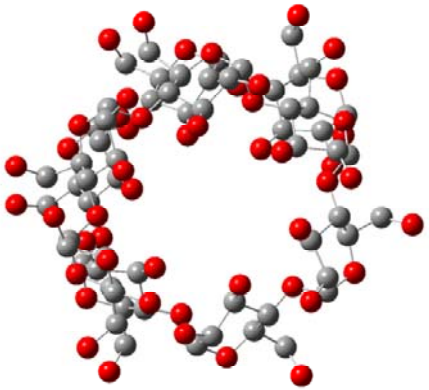

starch
inclusion complex
vanillin
mechanical mixture
% Transmitance
Wavenumber (cm-1)
1672 cm-1 and 1664 cm-1
1516 cm-1 and 1593 cm-1
1610 cm 1 and 1589 cm 1
1294 cm-1 and 1300 cm-1
732 cm-1
812 cm-1
856 cm-1
1265 cm-1
1429cm-1
1465cm-1


Temperature (°C)
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
Heat Flow (mW)
30
50
70
90
110
130
150
170
190
108°C
177°C
Temperature (°C)
0
-1
-2
-3
-4
-5
-6
-7
-8
Heat Flow
50
70
90
110
82°C

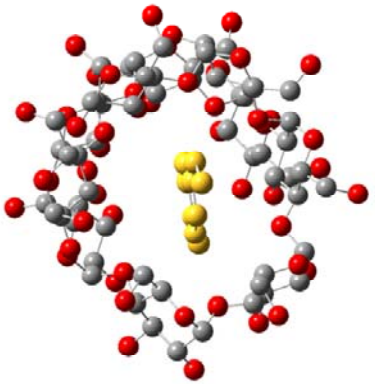

0
-1
-2
-3
-4
CD (mdeg)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Absorbance
200
220
240
260
280
300
320
340
Wavelength (nm)


Hylon VII
$\lambda_{max}^{abs} = 602nm$
Amylose
(Sigma A0512)
$\lambda_{max}^{abs} = 603nm$
Absorbance
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
5
10
15
20
25
30
35
Polysaccharide concentration (mg/ml)

A



B



C