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Structure–properties relationship in cross-linked high-amylose starch for use in controlled drug release☆

Pompilia Ispas-Szabo [a], François Ravenelle [a], Iman Hassan [a], Michel Preda [b], Mircea Alexandru Mateescu [a,*]

[a] *Department of Chemistry–Biochemistry, Université du Québec à Montréal CP 8888, Succ. A, Montreal, Quebec, Canada H3C 3P8*

[b] *Department of Earth Sciences, Université du Québec à Montréal CP 8888, Succ. A, Montreal, Quebec, Canada H3C 3P8*

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Abstract

Cross-linked high-amylose starch (CLHAS), obtained by high-amylose starch cross-linking, was recently introduced as an excipient (Contramid™) for monolithic dosage forms that are able to control drug release over 18–24 h. These control properties are related to tablet swelling and are strongly dependent on the degree of the cross-linking of CLHAS. The permeability of solutes through CLHAS hydrogels depends on the chemical structure of the polymer. The aim of this study was to obtain a better understanding of how modifications in CLHAS molecular structures at the level of long-range and short-range order during the cross-linking and processing conditions relate to the release properties of the CLHAS matrices. Structural parameters such as crystallinity contribute significantly to the physical and mechanical aspects of starch products. X-ray diffractometry, FTIR spectroscopy, dissolution tests in vitro, and mechanical hardness (of dry tablets) were found to be sensitive to the cross-linking degree (cld) variation. Best release properties and highest mechanical hardness were obtained from CLHAS matrices with low-to-moderate crystallinity, where the V- and the B-type structures coexist with amorphous regions. X-ray and FTIR profiles of dry CLHAS powders were found to be predictive for release properties of CLHAS tablets.

Keywords: Amylose; Cross-linking; Crystallinity; X-ray diffraction; FTIR; Drug release

1. Introduction

There is a continuously growing interest for oral drugs in controlled-release pharmaceutical forms that allow once or twice-a-day dosage administration [1]. Cross-linked high-amylose starch (CLHAS) was introduced a few years ago as an excipient (Contramid™) for controlled drug release [1–5]. It swells in water to form an elastic gel and the ability to regulate the swelling and thus regulate drug release in aqueous media as a function of cross-linking density makes this hydrogel particularly suit-

Abbreviations: CLHAS, cross-linked high-amylose starch; cld, cross-linking degree, defined as the amount of epichlorohydrin (g) used to cross-link 100 g of polymer under specific conditions, i.e., CLHAS-6 is obtained with an initial ratio of 6/100 cross-linking agent/high-amylose starch.

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* Corresponding author. Fax: +1-514-987-4054.

E-mail address: mateescu.m-alexandru@uqam.ca (M.A. Mateescu)

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able as a pharmaceutical excipient. The permeability of solutes across the hydrogel barrier (a dynamic state throughout the polymer device) depends on the texture of the hydrogel. Thus, polymer hydrophilicity and crystallinity play an important role in drug release [6]. Appropriate resistance of swollen tablets and good control of the drug release (over 15–20 h) were only obtained for CLHAS with moderate cross-linking degrees (clds): CLHAS-6 and CLHAS-8 (Fig. 1). Higher clds (CLHAS-20 and more) generate a sharp decrease in the release time (1–3 h), and under certain conditions can even afford disintegrant properties for CLHAS [9,10]. The non-monotonic variation of the drug release time with cld (Fig. 1) is a particular characteristic of the CLHAS matrix that differs from those of other classical polymeric matrices for which increasing clds lead to longer release times [7,8]. This behavior of CLHAS was ascribed to the particular structure of the matrix where, in the case of low cld, covalent linkages, interchain hydrogen bonds, and water-promoted hydrogen associations stabilize the network [3,8,11,12], thus controlling the access of water into the matrix. Aspects of the water uptake as a function of cld [9] and its role in the release behavior of CLHAS tablets [6,11,12] were reported.

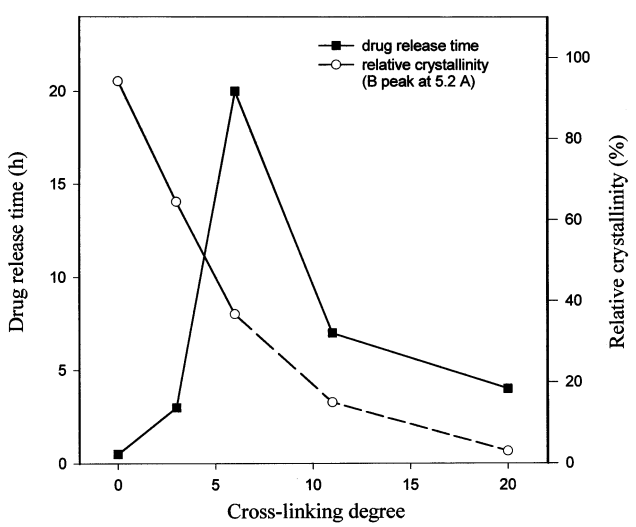

Fig. 1. Relationship between drug-release time, relative crystallinity and cross-linking degree (cld) of CLHAS. The dissolution kinetics were followed with acetaminophen as tracer released in 1 L of phosphate buffer at pH 7 and 37 °C from tablets of CLHAS (500 mg with 20% drug), compressed at 2.3 T/cm^2. The relative crystallinity was evaluated from X-ray analysis of CLHAS powders of various clds.

The native starch granule is heterogeneous both chemically (e.g., amylose and amylopectin) and physically (e.g., crystalline and noncrystalline regions). The presence or absence of crystalline order is often a basic factor underlying starch properties [13]. Depending on their origins, various types of native starches present specific morphologies giving distinctive X-ray powder patterns termed type A, B or C polymorphs [13–15]. The sharpness of the X-ray diffraction pattern of starch granules depends on their water content, the B type being more sensitive to hydration than the A-type starch [16–18].

When the cld changes, the morphology of powder, tablet or film forms also changes [19]. In a previous report, the variation of X-ray diffractograms as a function of cld for powders and tablets was described [12]. In this present study, for a better understanding of the role of crystallinity in release control, a series of powders, tablets and films was analyzed for high-amylose starches with different clds. A hypothesis that the compression of powders leads to molecular rearrangements and possibly to more extended hydrogen association has also been advanced [5]. When powders are compressed, it is hypothesized that the aggregates of the polysaccharide chains come closer together, and their ability to resist an external force could be related to this new structure. The crushing strength of CLHAS tablets has been shown to depend on the cld [9].

The presence of water and its influence on solid carbohydrate structure is often reflected in their spectra [20–22]. In a previous FTIR study [12], a variation of the 1646 cm^{-1} band as a function of cld was found and correlated with the hydration state of CLHAS powders. FTIR studies of conformational changes due to the retrogradation of starch–water systems during storage [23,24] pointed out that the 1300–800 cm^{-1} region is sensitive to the conformation of the polysaccharides. Since CLHAS films represent, in fact, another type of dry structure, it was interesting to observe the evolution of the 1300–800 cm^{-1} region in powder and film forms versus the cld and to correlate this with the morphological transitions from B- to V-type helix observed by

X-ray diffraction. The X-ray and FTIR analysis correlated with dissolution kinetics and mechanical hardness of the dry tablets can generate interesting information on the structure–properties relationship in CLHAS matrices.

2. Experimental

Materials.—High-amylose starch (Hylon VII) was obtained from National Starch and Chemical Corp., USA. Epichlorohydrin, (Sigma Chemical Co.), acetone, and acetic acid (BDH), as well as the other chemicals, were reagent grade and used without further purification.

Preparation of cross-linked high-amylose starch.—High-amylose starch was covalently cross-linked with epichlorohydrin (a bifunctional agent), obtaining different clds as a function of the ratio of epichlorohydrin to polymer. The cld was conventionally expressed as the initial amount (g) of epichlorohydrin added to 100 g of polymer. For each CLHAS synthesis, high-amylose starch was thermally and chemically treated in the same manner for gelatinization (in a Hobart® planetary mixer tank N-50), only the amount of cross-linking agent varying between 0 and 20 g/100 g polymer. Thus, 300 g of Hylon VII was first gelatinized in 1.7 L of 0.85 M NaOH at 50 °C for 20 min. Various quantities of epichlorohydrin (depending on the chosen cld, i.e., 9 g for CLHAS-3, 18 g for CLHAS-6, etc.) were slowly added to each batch under continuous stirring, and the reaction was continued for 1 h at 50–55 °C; then the suspension was neutralized (pH 6.9) by the addition of acetic acid solution. The soluble byproducts of the reaction were eliminated by centrifugation, and in all cases, salt elimination was controlled by conductivity measurements (values under 100 μS were considered acceptable). For washing and drying, the suspensions of CLHAS were washed with acetone–water solutions of increasing concentration of acetone by successive filtrations [25]. In the first step a solution of 17:3 acetone–water was used for thoroughly washing the product on a Buchner funnel, followed by a wash (three times) with 2:3 acetone–water. For final drying only pure acetone was used. The powders were dried at room temperature (rt) for 48 h. The desired powders (particle size 300–75 μm) were obtained by sieving, and the products were stored in closed bottles at rt. Before X-ray and FTIR measurements, the powders and films were heated for 24 h at 105 °C and then kept at rt, over P_2O_5 powder to prevent hydration.

Tablet preparation.—Several types of tablets were prepared from CLHAS with different degrees of cross-linking: (a) for X-ray diffraction analysis, tablets of 400 mg were obtained by compression of powders at 2.3 T/cm^2 in a Carver hydraulic press using conventional tableting equipment with a flat face punch die assembly. (b) For dissolution tests in vitro, 400 mg of polymeric powders were mixed with 100 mg acetaminophen as tracer and compressed at 2.3 T/cm^2 to obtain tablets with a diameter of 13 mm and a thickness of 2.6–2.8 mm. (c) For FTIR analysis, tablets of 100 mg KBr with 6% (w/w) native or CLHAS were prepared at 7.7 T/cm^2 under vacuum. (d) For hardness of dry tablets, 400 mg of powders with various clds were compressed at different compression forces ranging from 0.4 to 2.3 T/cm^2.

Film preparation.—An aqueous suspension of 1% (w/v) CLHAS for each cross-linked amylose was heated at 90–100 °C, then cast at rt and dried until a film was obtained. By the same procedure, films of CLHAS-0 and CLHAS-6 were prepared from solutions in Me_2SO.

X-ray diffraction patterns.—X-ray diffraction patterns were obtained using a Siemens D-5000 diffractometer equipped with a silicon detector and operating in the reflectance mode at a Co K_α wavelength of 1.79018 Å over the angular range 2θ from 5 to 50°. The recorded spectra were analyzed using the Diffract-AT software and tentatively, a quantitative estimation of long-range order as a function of cld was carried out. As the crystallites in starch were very small (100–150 Å), an estimation of the degree of crystallinity could not be rigorously or unambiguously considered. In semicrystalline biopolymers such as starch, the crystallinity is usually defined as the ratio between the intensity (area) of the maximum

crystalline diffraction and the total intensity (area) of the diffractogram [26]. In both cases only a relative crystallinity could be estimated. Since for CLHAS materials, peak intensities change as a function of the morphological structure induced by cross-linking [12], this study presents the estimation of the relative crystallinity related to the peak at 5.2 Å as a measure of B-type organization. The relative crystallinity was evaluated for native high-amylose starch, and CLHAS-0, CLHAS-3, CLHAS-6 powders, following the calculations of van Soest et al. [23].

The intensities of 5.2 and 4.5 Å peaks were used in the relation

$$\%X_{\mathrm{RDH}} = [R(X_{\mathrm{H}}) - 0.095]/0.0055 \quad (1)$$

where $\%X_{\mathrm{RDH}}$ represents the relative crystallinity of the chosen peak and $R(X_{\mathrm{H}})$ is defined as the ratio between crystalline diffraction height (H_{c}) and total diffraction height (H_{t}) as measured from baseline [23]. For native high-amylose starch, the value of $\%X_{\mathrm{RDH}}$ was considered as 100% and all the other CLHASs were reported to this maximal value.

FTIR spectroscopy.—Absorbance spectra of CLHAS powders (6% in KBr pellets) and films were recorded on a Bomem MB-100 spectrometer with a deuterated triglycine sulfate (DTGS) detector. First, for all CLHAS films, FTIR spectra were carried out just after casting and drying at rt, without any special treatment. Then, the same films were heated for 1 and 24 h at 90 °C, and the spectra were recorded again after equilibration at rt. The spectra were obtained at a 1 cm^{-1} resolution as an average of 50 scans and with air as background. For the 1200–800 cm^{-1} region, the spectra were baseline corrected, and the deconvolution was conducted using Voigt-type equations for all found peaks. Intensities of bands at approx. 1047, 1022 and 1000 cm^{-1} were analyzed as a function of cld, and the data were interpreted in terms of short-range changes induced by cross-linking. Calculations for peaks found by deconvolution were done as a function of intensity or area of each band compared with the total intensity or area of the 1300–800 cm^{-1} spectral region. The results were practically identical, and thus only the surface results are hereby presented. The same method of FTIR deconvolution and analysis was applied to the 1500–1200 cm^{-1} region.

Tablet hardness.—For evaluation of changes induced by compression for each CLHAS material as a function of cld, tablets of 400 mg pressed at different compression forces (0.4, 0.8 and 2.3 T/cm^2) were prepared as outlined above. Hardness (resistance that tablets may offer towards a stress and a strain) was measured with a Dr Schleudinger tablet hardness tester (model 6D), and the values are expressed in Kp/cm^2 (mean of four measurements).

Dissolution tests in vitro.—Dissolution tests were carried out in a USP dissolution type II apparatus (Distek system with paddle) with 1000 mL of 0.05 M phosphate buffer (pH 7) at 37 °C and 100 rpm. Acetaminophen was the tracer for all tests. Samples were taken automatically every 30 min during 24 h and assayed with a Hewlett–Packard diode array spectrophotometer at 245 nm.

3. Results and discussion

X-ray diffraction.—The diffraction spectra of CLHASs in powder, tablet and film forms showed differences with varying clds (Figs. 2–4). It was also found that the drug release kinetics are related to the X-ray patterns of the CLHAS matrix (Table 1). For native high-amylose starch Hylon VII powder, a predominant B-type with elements of V-type diffraction pattern was found (Fig. 2(a,b)). Fig. 2(a) presents diffraction patterns as recorded in original form without any mathematical treatment. By using the Diffract-AT software (which eliminates the background noise and part of the amorphous signal), the diffraction spectra obtained (Fig. 2(b)) are very similar to the originals, and, at the same time, allowed better observation of diffraction maxima. The same treatment was applied to diffractograms for tablets and films for which only the resulting spectra are shown (Figs. 3 and 4).

The diffraction maxima (Fig. 2(a,b)) at 5.7, 5.2, 3.9 and 3.7 Å are typical for the B-type diffraction pattern [27], whereas the shoulder

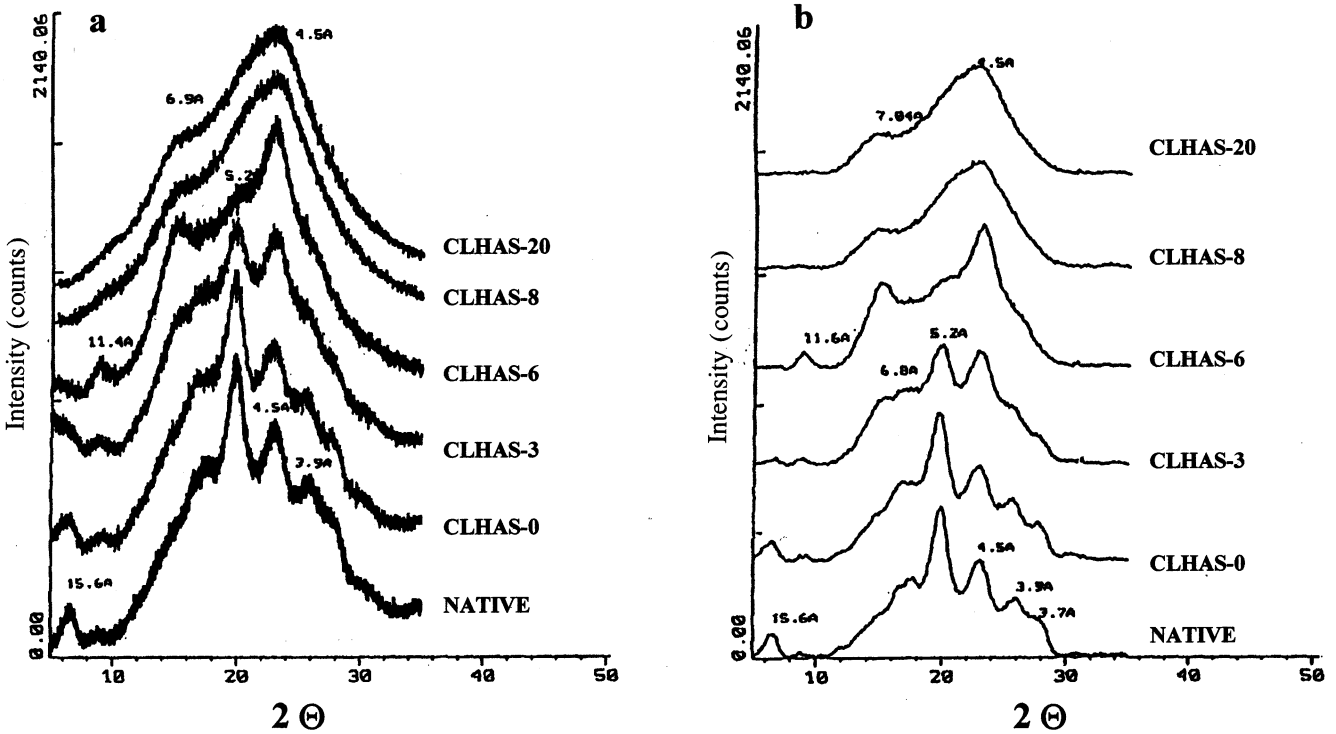

Fig. 2. X-ray diffraction patterns of native and CLHAS powders at various clds (a) without mathematical treatment and (b) treated with Diffract-AT software. Diffractograms were obtained with a Siemens D-5000 apparatus operating in reflectance mode at $\lambda = 1.79018$ Å.

at 6.8 Å and the peak at 4.5 Å are related to the V-type structure [17]. For CLHAS polymers with increasing cld, the intensity of 5.7, 5.2, 3.9 and 3.7 Å peaks diminishes, whereas the 4.5 Å peak becomes more important. At the same time, the shoulder at 6.8 Å becomes more and more separated, and for the CLHAS-6 powder becomes a quite well-defined peak. The small peak appearing at 11.8 Å is also characteristic of V-type single-helix structure. The general feature for CLHAS powder diffractograms is the loss in crystallinity with increasing cld. CLHAS-20 shows no discrete diffraction between 18 and 30°, and the broader profile suggests a more amorphous structure.

Modification of X-ray diffraction patterns for powders could be correlated with physical and chemical transformations that occurred during gelatinization, cross-linking and drying, all having a contribution to the structural changes. For CLHAS powders with low and moderate cld (CLHAS-3, CLHAS-6), both B- and V-type patterns are still present, but the proportion between them changed in comparison with native high-amylose starch and gelatinized (but not cross-linked) CLHAS-0, for which B-type is predominant. For higher cld (CLHAS-20) the broader diffractogram can be ascribed to a low-ordered structure that may contain some single V-helices. Our cross-linking procedure involves a gelatinization step (heating in an alkaline aqueous media) that leads to partial or complete disruption of the predominant B-type order existing in native high-amylose starch. As a general feature, gelatinization induces changes in double-helix conformation, even an unraveling or unwinding of the double helices [28]. It was reported that amylose also exists as an interrupted helix in aqueous solution [29]. With increase of pH, the helix–coil transformation occurs, and the molecule may be regarded as a flexible coil [28]. By cross-linking, neutralization and drying, a new type of order and a new structure become possible. In solution, amylose is supposed to change first in single helices (pseudo V-type diffraction pattern). However, under particular conditions, insoluble amylose can keep the initial B-type pattern [28]. For CLHAS powders synthesized under our conditions, the transition from a predominant

double-helix B-type diffraction pattern in native Hylon VII and CLHAS-0 to a predominant pseudo-V form (single-helix conformation) of CLHAS can be followed by X-ray diffraction analysis (Figs. 2–4). It is worth mentioning that the CLHAS-0 diffractogram is similar to that of native high-amylose starch. Since only gelatinization and no cross-linking was done (CLHAS-0), the native arrangements can be almost restored. Only when the chemical structure was modified by cross-linking, the X-ray profiles gradually changed. Therefore, cross-linking appears to be the main treatment that induces structural modifications.

The transformation from semicrystalline to a more disordered structure can be quantitatively estimated by the values of relative crystallinity at the 5.2 Å peak (Fig. 1) associated with the double helix (B-type). Only the treated materials CLHAS-0, CLHAS-3 and CLHAS-6 were chosen for the graphic presentation (Fig. 1) because their preparation was done in similar conditions (differing from the native high-amylose starch, which is not gelatinized). For CLHAS-8 and CLHAS-20 powders, the spectra showed peak overlaps and did not allow crystallinity estimation, but all three maxima observed at 11.8, 6.9 and 4.5 Å are characteristic of the V-type structure. The intensity ratio $I_{5.2\ \text{Å}}/I_{4.5\ \text{Å}}$ of double helix:pseudo V-type single helix (Table 1) decreased with an increase in cld.

The same aspects were observed in the diffraction pattern of the tablets (Fig. 3). As a result of compression, the peak at 22–24° (corresponding to 4.5 Å) becomes predominant in CLHAS-3 and CLHAS-6. Tablets of native high-amylose starch and pre-gelatinized CLHAS-0 keep almost the same diffraction pattern as the corresponding powders, differing only by a diminution of intensities. For CLHAS-20 tablets a broad diffraction is present between 16 and 26°.

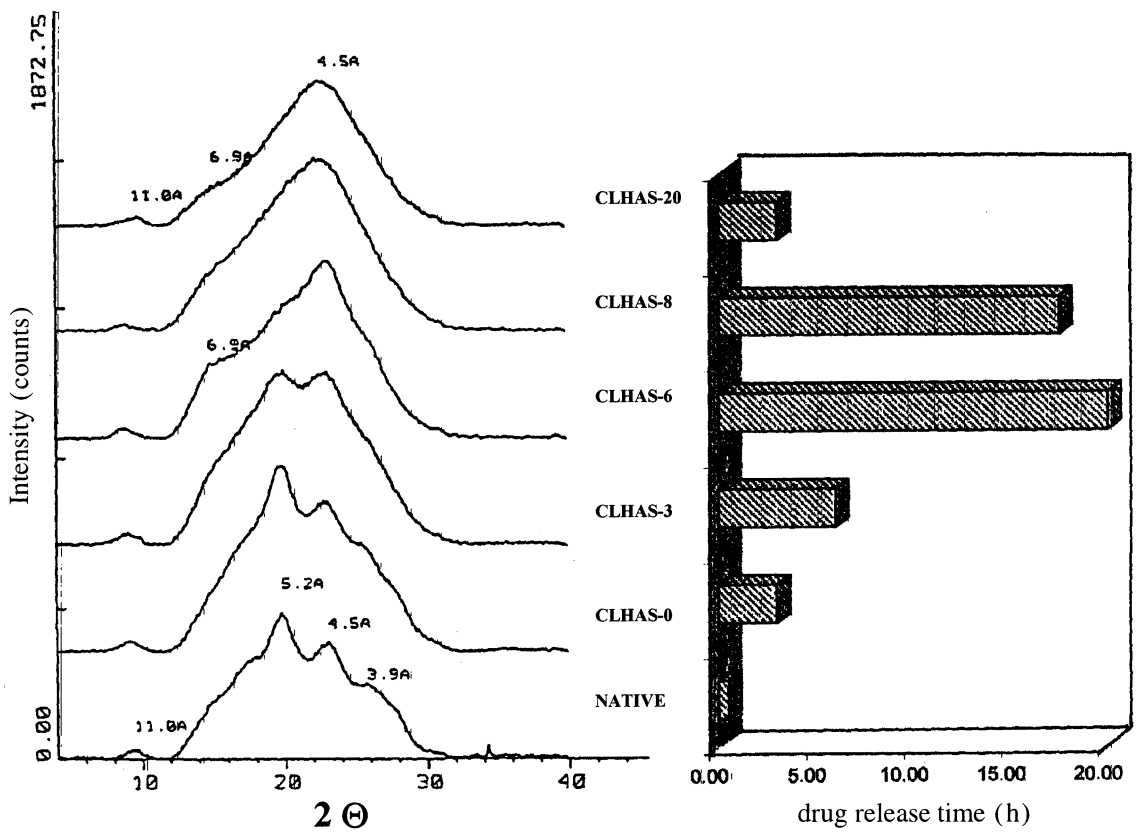

Fig. 3. X-ray diffraction patterns of native and CLHAS tablets at various clds and the corresponding drug-release times. Diffractograms were obtained with Siemens D-5000 apparatus operating in reflectance mode at $\lambda = 1.79018$ Å. The dissolution kinetics were followed with acetaminophen as tracer released in 1 L of phosphate buffer at pH 7 and 37 °C from tablets of CLHAS (500 mg with 20% drug), compressed at 2.3 T/cm^2.

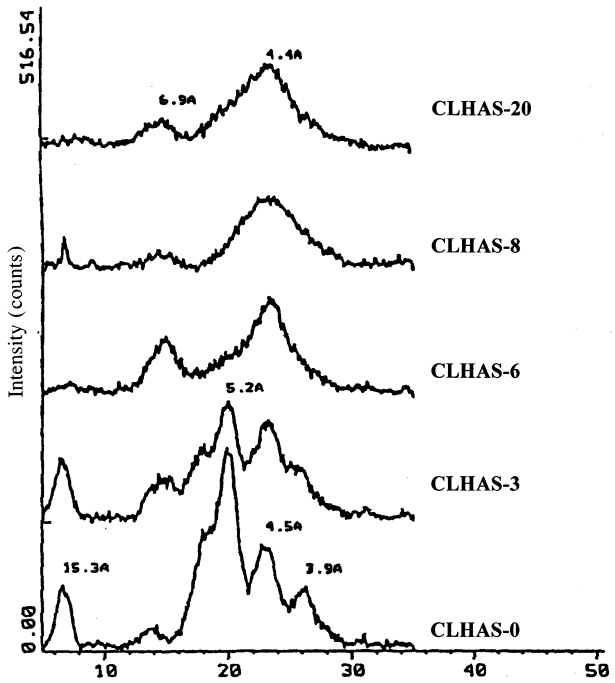

Fig. 4. X-ray diffraction patterns of pregelatinized and CLHAS films at various clds. Diffractograms were obtained with a Siemens D-5000 apparatus operating in reflectance mode at $\lambda = 1.79018$ Å.

The correlation of X-ray diffraction data for both powders and tablets with in vitro dissolution tests (drug release time and mechanical behavior for swollen tablets containing 20% of drug) suggests that an optimal order/disorder (crystalline/amorphous) ratio can be responsible for tablet integrity during swelling and thus is a factor for good control of drug release. These order/disorder features of CLHAS starches are directly involved in the network formation. If a stable network is formed, the slow water penetration (and the drug release) can be controlled. Slow swelling contributes toward preventing tablet disintegration. This is the case for CLHAS-6 tablets, which present good release properties and diffraction patterns with moderate crystallinity (B and V forms co-exist with the amorphous structure).

The relatively high level of double-helical order of native high-amylose starch powder and of CLHAS-0 is consistent with poor mechanical resistance of the tablet in the dissolution test. The access of water into this crystalline structure is difficult, as the tablet is not able to swell, and capping (cracking of tablets during swelling) was observed. For higher cross-linked starches (CLHAS-6–CLHAS-8), the amorphous part becomes more extended, and the less-ordered chains have more flexibility. When compressed there are probably rearrangements, which can generate a structure favorable to inducing a stable network formation at swelling. When the cld is too high (CLHAS-20), the high density of transversal cross-linking between polysaccharide chains can hinder achieving a favorable conformation during swelling, and the structure remains almost unordered. Hydroxyl groups of chains are not involved in network stabilization by interchain hydrogen bonding; they are only available for fast hydration [12], leading to tablet disintegration.

It is well known that starches have good film-forming properties [30–33]. Interpretation of X-ray diffraction patterns of films cast

Table 1
Correlation of X-ray data for CLHAS powders and drug-release properties

Polymer	X-ray diffraction data		Dissolution test in vitro [a]	
cld	Powders		Drug-release time (h)	Mechanical resistance and tablet shape during release
	$I_{5.2\ Å}$ (AU) [b]	$I_{4.5\ Å}$ (AU)		
Native	315	200	0.50 ± 0.04	disintegration
CLHAS-0	310	200	2.0 ± 0.5	capping [c]
CLHAS-3	240	230	7.0 ± 0.4	capping
CLHAS-6	180	290	20.0 ± 0.5	good shape and stability
CLHAS-20			3.0 ± 0.6	disintegration

[a] Tablets (500 mg) were obtained by dry compression at 2.3 T/cm^2 of CLHAS powders with 20% acetaminophen as tracer.
[b] AU = arbitary units.
[c] Capping means the cracking of the tablets during swelling.

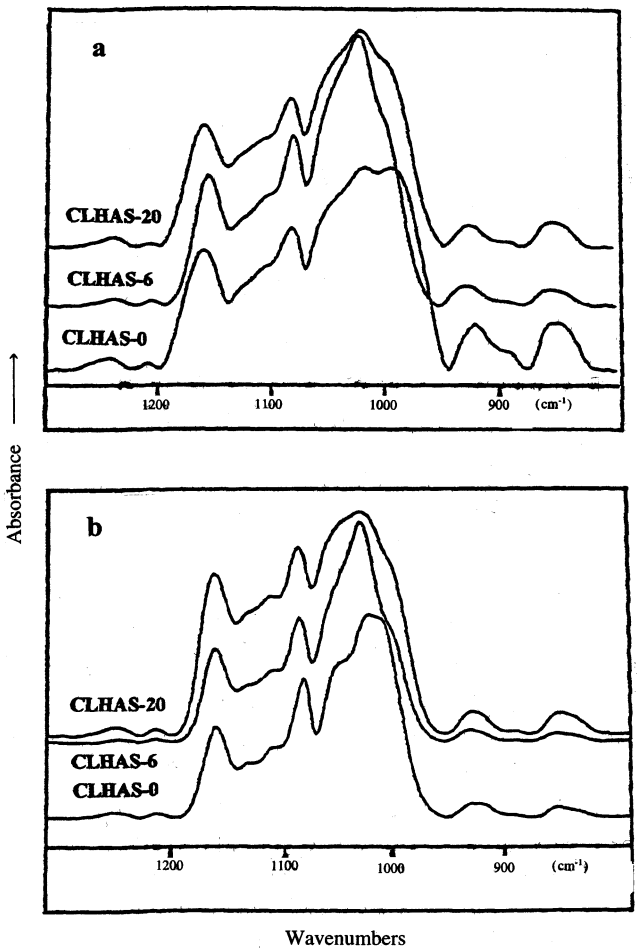

Fig. 5. FTIR spectra of CLHAS at different clds in powders (a) and films (b). Spectra were carried out with a Bomem MB-100 spectrophotometer at 1 cm^{-1} resolution.

from the different CLHASs, indicates a morphological change, suggesting a different structural order (Fig. 4). When CLHAS powders are suspended in water and boiled, the amorphous part can swell, and on cooling can adopt new conformations. By slow water evaporation, the resulting structures, spontaneously achieved, become stable. To a certain extent, the film could be considered a 'print' of these structures. In the X-ray patterns (Fig. 4) obtained from films, the double-helical order is present in CLHAS-0 and CLHAS-3 films (maximum at 20°) and becomes less evident in CLHAS-6. For the CLHAS-20, only the peak at 4.5 Å was observed. The general features of film diffractograms are very similar to those obtained for CLHAS powders.

FTIR data.—Fig. 5(a,b) shows FTIR spectra in the 1300–800 cm^{-1} region for three representative clds (CLHAS-0, 6 and 20) in dry powders and films. The changes in spectral profiles as a function of cld were found to be similar for both dried forms, and they consisted in different intensities of the shoulders on each side of the band around 1022 cm^{-1}. By deconvolution of the 1300–800 cm^{-1} region, 13 bands were found. The elimination of the less-intense bands below 950 cm^{-1} and above 1200 cm^{-1} gave spectra with only seven deconvoluted bands, and no change in quantitative estimation was observed. The same procedure was applied for all analyzed spectra, and an example of the CLHAS-6 deconvoluted spectra (powder and film) is shown in Fig. 6(a,b). For the various CLHASs, it was observed that the three bands at about 1047, 1022 and 1000 cm^{-1} were sensitive to cross-linking. For quantification, the intensities (or areas) of the chosen peaks were normalized to the total intensity (or surface) of the massive band between 1200 and 900 cm^{-1}. The spectra of powders show wider bands than those of corresponding films, suggesting a lower scattering. For different clds, the normalized surfaces (and intensity) of selected bands at 1047, 1022 and 1000 cm^{-1} were calculated. Since they were practically identical, only the normalized surfaces are shown (Fig. 7). With the increase of cross-linking density, the bands at 1022 and 1047 cm^{-1} increased, whereas a decrease in the 1000 cm^{-1} band was observed.

On the basis of the results of the correlation of X-ray data on crystallinity and morphological changes with the variation of bands in the 1200–900 cm^{-1} region, it is possible to give an FTIR band assignment for the analyzed polymers. As shown above by X-ray data, increasing the degree of cross-linking induced a decrease of the B-type double-helix morphology, whereas the presence of the pseudo V-type and amorphous structures was increased. This strongly suggests that the decreasing band at 1000 cm^{-1} could be associated with the crystalline order (B-type morphology), becoming less important in CLHASs with higher clds. The increase of the 1022 and 1047 cm^{-1} bands (Fig. 7) could be related to the amorphous phase and to a pseudo V-type structure. Both bands have almost the same evolution, and because the

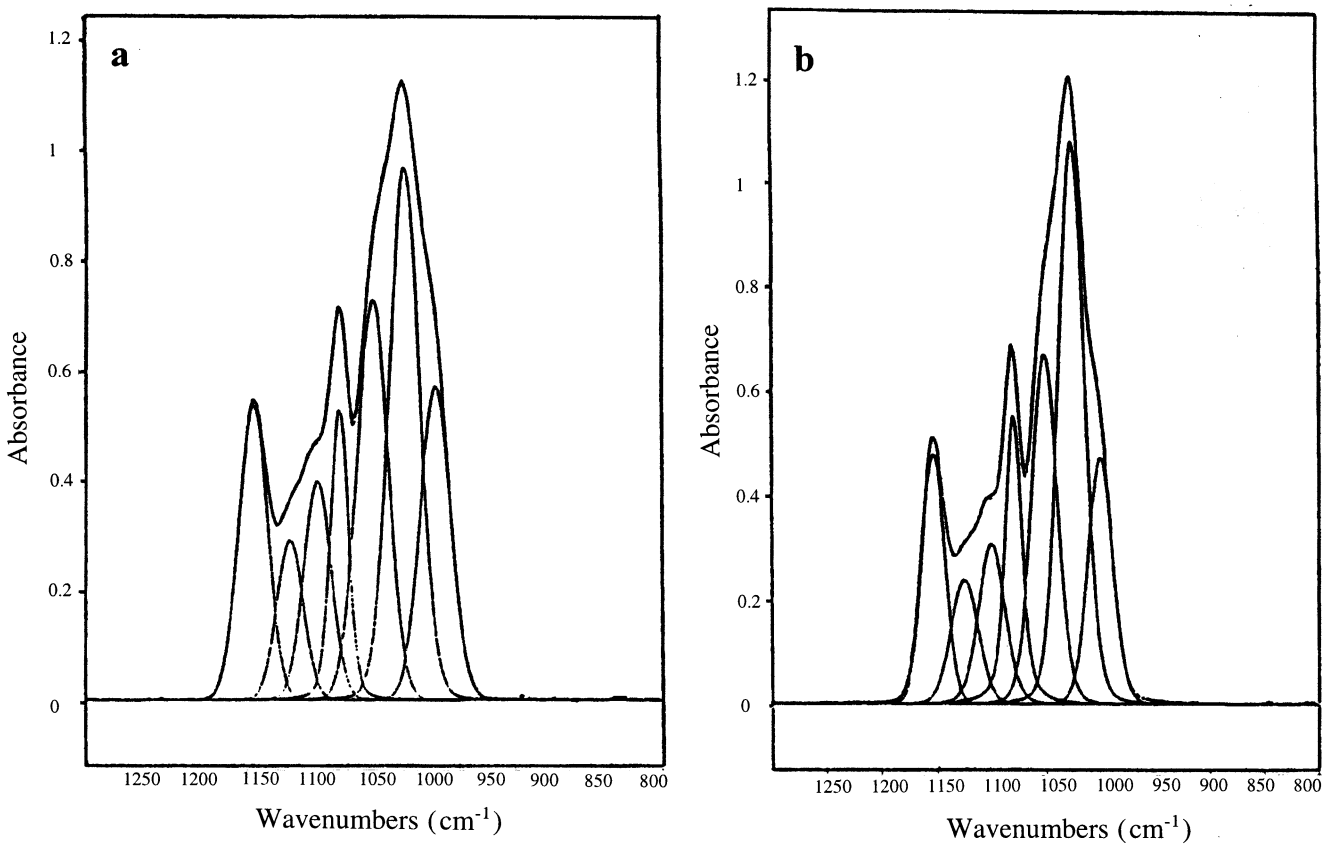

Fig. 6. Deconvoluted FTIR spectra for CLHAS-6 in (a) powder and (b) film forms. The CLHAS powders were analyzed in KBr pellets (100 mg) containing 6% polymeric powder. The initial spectra of powders and films were baseline corrected and a Voigt-type equation was used for all deconvoluted bands.

helix conformation of polysaccharidic chains in noncrystalline and V-type structures (observed by ^{13}C CP/MAS NMR) is the same in solid starch [34], it is difficult to make an unambiguous band assignment. Gidley and Bociek [35] suggested, via ^{13}C NMR spectroscopy of solids, that double and single helical chains can be associated with ordered and non-ordered conformational states, respectively. A ^{13}C CP/MAS NMR study (Marchessault et al., in preparation) of the same series of CLHAS powders is now underway with the aim to confirm our X-ray and FTIR data on double/single helix evaluation.

The diffraction maxima broadening in CLHAS-20 (Figs. 2–4) could have the same explanation and seems useless for a good distinction between the bands discussed. For other starch systems, it has been previously shown [23,24] that the band at 1022 cm^{-1} can be associated with the amorphous phase, and that the band around 1000 cm^{-1} is water sensitive. For elimination of water interference all our powders and films were kept at 105 °C for 24 h. Only for gelatinized, not cross-linked HAS-0 films, a decrease of the absorbance values in the 3200–2900 cm^{-1} and 1200–900 cm^{-1} regions was observed, whereas the films of various CLHASs exhibited no spectral change. Under these conditions, the evolution

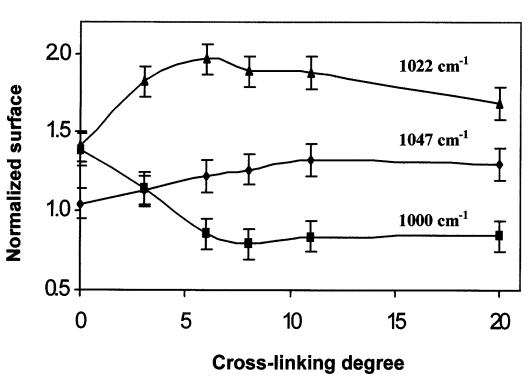

Fig. 7. Dependency of 1000, 1022 and 1047 cm^{-1} FTIR bands as a function of cld of high-amylose starches. Spectra were carried out in films of CLHAS-0, CLHAS-3, CLHAS-6, CLHAS-8, CLHAS-11 and CLHAS-20 with a Bomem MB-100 spectrometer at 1 cm^{-1} resolution.

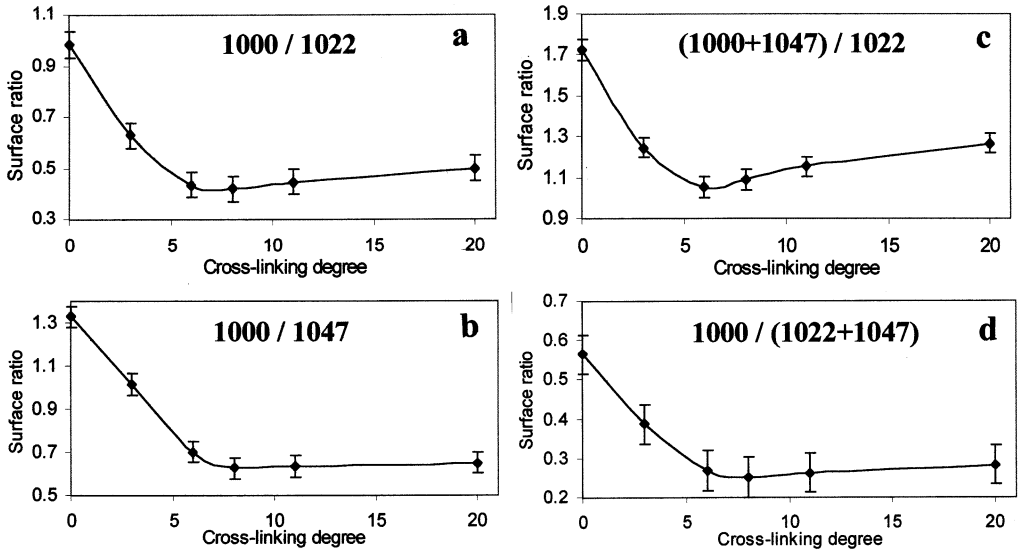

Fig. 8. Crystalline/amorphous ratio calculated as normalized surface ratio of bands to 1000/1022 cm^{-1} (a), 1000/1047 cm^{-1} (b), (1000 + 1047)/1022 cm^{-1} (c), 1000/(1022 + 1047) cm^{-1} (d), as a function of the cld of CLHAS. Spectra were recorded on films of CLHAS-0, CLHAS-3, CLHAS-6, CLHAS-8, CLHAS-11 and CLHAS–20 with a Bomem MB-100 spectrometer at 1 cm^{-1} resolution and a Voigt-type equation was used for all deconvoluted peaks.

of the 1000 cm^{-1} band could be related only to the modification induced by polymer cross-linking. It therefore seems possible that the intensity of the 1000 cm^{-1} band depends on the amount of associated chains. In our cross-linked system, increasing the cld generates fewer associations and more amorphous structures. From the correlation of our data with those of van Soest et al. [23], it is possible to assume that the band at 1000 cm^{-1} is related to poorly stabilized hydroxyl groups, which are sensitive to hydration in the case of starch or to conformational modification and loss of crystallinity in the case of CLHAS.

The amount of short-range order in the CLHAS samples (powders and films) can be expressed on the basis of the surface of the most characteristic bands of crystalline and amorphous structures. The calculated ratios 1000/1022 and 1000/1047 versus cld (Fig. 8(a,b)) showed a drastic decrease when cross-linking increases until CLHAS-6–CLHAS-8 and then remained almost constant. If the 1022 and 1047 cm^{-1} bands are related to the non-ordered structures in the CLHAS materials, the crystalline/amorphous ratio could be estimated from 1000/(1022 + 1047) values for various clds (Fig. 8(d)). Considering B-type and V-type structures together to be responsible for crystalline order in CLHASs, the crystalline/amorphous ratio was calculated as (1000 + 1047)/1022 surface ratio (Fig. 8(c)). The 1000/1022 ratio (Fig. 8(a)) could also be interpreted as a crystalline B-type/amorphous feature of the analyzed system and 1000/1047 ratio as the proportion between the two polymorphs B/V (Fig. 8(b)). Irrespective of the fact that the 1047 cm^{-1} band (related to V-type structure) was associated with the crystalline or amorphous part containing dispersed V helices, the crystalline/amorphous ratio versus cld exhibited the same type of variation. The FTIR analysis shows that the major tendency is the loss in crystallinity until CLHAS-6–CLHAS-8, with no important changes at higher clds. It clearly appears that for moderate clds, a stable structure with a moderate crystallinity is responsible for the best swelling and release properties.

At the same time, another region at 1500–1350 cm^{-1} of the FTIR spectra was deconvoluted for powders and films with different clds (spectra not shown). The same method was used, and from the nine bands obtained by deconvolution, the band around 1254 cm^{-1} exhibited a shift with increase of cld. Similar data were found for powders and films. It was previously shown [36] that the band at 1265–1254 cm^{-1}, assigned to a $-CH_2OH$ related mode, shifts for various polymorphic forms of

amylose, from 1263 cm^{-1} in V_a amylose to 1254 cm^{-1} in B-amylose. Fig. 9 shows the wavenumber shift as a function of cld. It is evident that when the cld increases, the tendency is to a conformational change from a B-type helix (characteristic of low cld CLHAS-0, CLHAS-3) to a V-type helix conformation (CLHAS-11). If the best-organized and compact structures (B-type) have smaller wavenumbers and those less-organized (Vh-type) higher wavenumbers, the shift to higher values (1267 cm^{-1} for CLHAS-20) could be interpreted as a B- to V-type transition. It is worth mentioning that in all FTIR analyses as a function of cld (Fig. 8), the major modifications were found for CLHAS-6, which gives the best control of drug release. With the increase of density of cross-linking bridges between the polysaccharide chains, the conformational arrangements in B- or V-type structures become more difficult, and a helix to random coil transition seems to occur following the previously proposed model [12] in which only CLHAS at low cld can be associated by hydrogen bonding.

More support in assigning the bands at 1000 and 1047 cm^{-1} was obtained by FTIR analysis of CLHAS-0 and CLHAS-6 films cast from Me_2SO (dimethyl sulfoxide) suspensions with 1% (w/v) solid. The deconvolution of the 1200–800 cm^{-1} region for CLHAS-0 and CLHAS-6 films cast from water and Me_2SO showed that the normalized surface of the 1000 cm^{-1} band is slightly lower in Me_2SO films than in aqueous films (Fig. 10(a)). The difference is more evident for CLHAS-0 than for CLHAS-6. In the case of the 1047 cm^{-1} band, the change from water to Me_2SO solvent for film casting is reflected in an increase of band surface for both CLHAS-0 and CLHAS-6 (Fig. 10(b)). It is known that Me_2SO exerts its solvent action as a powerful hydrogen-bond acceptor, thereby breaking associative hydrogen bonds in both polysaccharide and water [37,38]. Under special conditions of heating, Me_2SO is also capable of complexing with amylose to form V_{DMSO} [39,40]. The increase of the 1047 cm^{-1} band in $CLHAS_{(Me_2SO)}$ films could be an argument for the presence of the complex V_{Me_2SO}, which is present in a higher extension in CLHAS-6 than in CLHAS-0 (Fig. 10(b)). The breaking of morphological organization of double-helix B-type (existing in CLHAS-0) by Me_2SO could be reflected in the decrease of the 1000

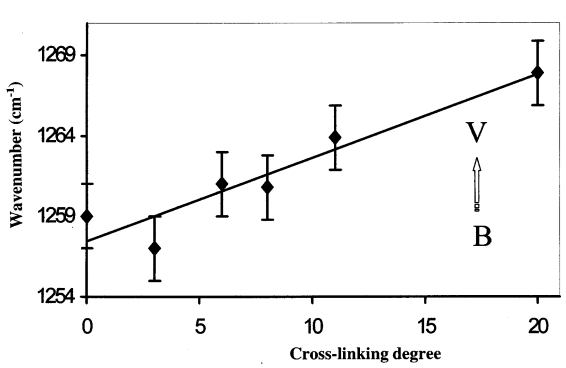

Fig. 9. Variation of the band corresponding to related modes –CH_2OH as a function of cld of CLHAS. The spectra of CLHAS films were deconvoluted in the 1500–1200 cm^{-1} region and a Voigt-type equation was used for all bands.

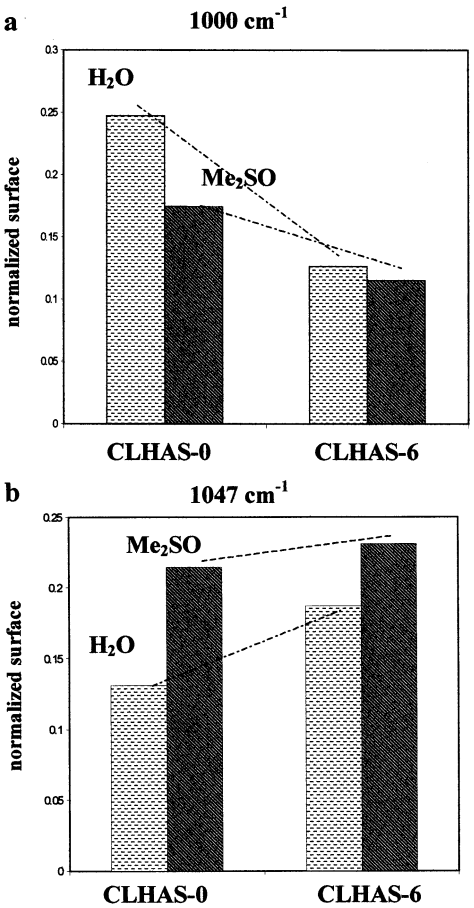

Fig. 10. Influence of solvent on the (a) 1000 cm^{-1} and (b) 1047 cm^{-1} band intensities for CLHAS films prepared by casting from aqueous and Me_2SO suspensions. Spectra were carried out on films of CLHAS-0 and CLHAS-6 with a Bomem MB-100 spectrometer at 1 cm^{-1} resolution, and a Voigt-type equation was used for all deconvoluted bands.

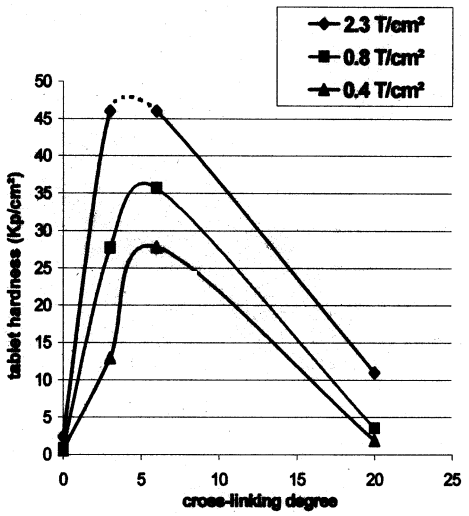

Fig. 11. Dependency of CLHAS tablet hardness on the cld obtained at different compression forces. Tablets of 400 mg were crushed for each CLHAS in a Dr Schleudinger (model 6D) apparatus.

cm^{-1} band surface (Fig. 10(a)). For CLHAS-6 this morphology is present to a lesser extent in the structure, and thus the surface decrease is less pronounced. Further studies will be carried out for all clds in Me_2SO to confirm these preliminary data.

Tablet hardness.—The effect of covalent cross-linking on the mechanical properties of dry tablets showed that for all compression forces (0.4, 0.8 and 2.3 T/cm^2) the maximal hardness was reached by CLHAS-6 tablets (Fig. 11). An hypothesis that compression leads to polymeric chain rearrangements and to formation of an extended interchain association, enhanced by a hydrogen-bonding network, has been advanced [3,5]. The dependency of tablet hardness on cld suggests that compression brings the polymeric chains into closer contact. Since the conformation of chains seems to be a function of cld, and following our hypothesis that this hydrogen association also depends on the cld, this might be reflected in tablet hardness. The maximal values obtained for CLHAS-6 tablets could be interpreted in terms of maximal stabilization of the system by both covalent and physical forces (hydrogen bonding). It is also worth mentioning that tablet hardness, although indirect, can be a proof in favor of the hypothesis of interchain physical associations, which depend on cld. It is therefore possible to assume that physical associations following (a) compression and (b) hydration (when swollen) control the drug release.

4. Conclusions

Maximal stabilization seen in CLHAS-6 dry powders and films (by X-ray and FTIR analysis), as well as in tablets (by X-ray and tablet hardness) is clearly reflected in the best drug-release properties found for this matrix. Increased cross-linking induced morphological changes from predominant B-type to the pseudo V-type and amorphous structures. The conformational modification can be observed in short-range order (by FTIR) or in long-range order (by X-ray diffractometry) arrangements. Both series of results indicate that an optimal order/disorder (crystalline/amorphous) ratio is required for optimal release properties of CLHAS matrices. Adequately cross-linked CLHAS (i.e., CLHAS-6) forms a network (highly stabilized by covalent and hydrogen bonding) that is able to control water penetration and drug release. These tablets of CLHAS-6 also exhibit highest hardness, longest drug release times, and best mechanical resistance during and after swelling. Higher cross-linking prevents hydrogen bonding [3,5,12]; thus hydration and swelling are rapid and the release is fast. These structural data help to understand better the transition from helix to random coil, which occurs by the increase of cross-linking. Therefore, it appears that X-ray and/or FTIR analyses can give important information on the behavior of this polymeric excipient for controlled drug release.

The similarity of FTIR profiles corresponding to CLHASs, powders and films could be an argument for the analysis of films, which are less water sensitive and easier to prepare than KBr pellets.

The study of the structure–properties relationship of CLHAS matrices contributes to the understanding of the drug-release mechanism and can open new strategies for controlled drug delivery.

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