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Modeling a system of phosphated cross-linked high amylose for controlled drug release. Part 2: Physical parameters, cross-linking degrees and drug delivery relationships

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ABSTRACT

High amylose cross-linked to different degrees with sodium trimetaphosphate by varying base strength (2% or 4%) and contact time (0.5–4 h) was evaluated as non-compacted systems for sodium diclophenac controlled release. The physical properties and the performance of these products for sodium diclophenac controlled release from non-compacted systems were related to the structures generated at each cross-linking degree. For samples at 2% until 2 h the swelling ability, G' and η^* values increased with the cross-linking degree, because the longer polymer chains became progressively more entangled and linked. This increases water uptake and holding, favoring the swelling and resulting in systems with higher viscosities. Additionally, the increase of cross-linking degree should contribute for a more elastic structure. The shorter chains with more inter-linkages formed at higher cross-linking degrees (2%4 h and 4%) make water caption and holding difficult, decreasing the swelling, viscosity and elasticity. For 2% samples, the longer drug release time exhibited for 2%4 h sample indicates that the increase of swelling and viscosity contribute for a more sustained drug release, but the mesh size of the polymeric network seems to be determinant for the attachment of drug molecules. For the 4% samples, smaller meshes size should determine less sustained release of drug.

1. Introduction

There has been a continuous interest of the pharmaceutical industries in the research and development of sustained or controlled drug release. Polymers are almost indispensable to prepare oral drug delivery systems and among them, polysaccharides play an important role due to their biocompatibility and biodegradability. In 1991, high amylose cross-linked with epichloridrin was introduced as excipient, assuring a theophylline sustained release of about 20 h (Lenaerts et al., 1991). In fact, different cross-linking degrees could be obtained by varying the epichloridrine to amylose ratios, but it was found that the maximal drug release time occurred at lower cross-linking degrees (Dumoulin et al., 1998). This non-monotonous relation between release time and cross-linking degree is a particular property that differs this material from other polymers for which, generally, the increase in cross-linking degree results in slower drug release (Vandelli et al., 2001; Dini et al., 2003; Kurkuri and Aminabhavi, 2004).

It was proposed that the high amylose could be properly cross-linked with the non-toxic sodium trimetaphosphate (STMP) (Le Bail et al., 1999). Since it was impossible to carry out the pre-gelatinization under the temperature conditions proposed by these authors, because the samples carbonized, the pre-gelatinization step was eliminated from the cross-linking process of high amylose (Cury et al., 2008). In this way, different cross-linking degrees were reached by fixing the original amounts of polymer and STMP and the temperature, but varying the contact time with sodium hydroxide (NaOH) and the base strength (Cury et al., 2008).

Following that routine, eight samples at 0.5 h, 1 h, 2 h and 4 h at base strengths of 2% and 4% were prepared. C and H elemental analysis, IR, SEM and solid state NMR of the samples indicated that the incorporation of phosphate groups into the polymers followed the same basic trend irrespectively of the strength of the base, and this fact was interpreted as if the cross-linking reaction would follow at the beginning (up to 2 h contact times) a kinetically controlled pathway and ending in a possibly thermodynamic preferred cross-

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Table 1
Mean diameters of cross-linked samples.

Samples	Mean diameter (μm)
2%0.5 h	351.25
2%1 h	332.33
2%2 h	329.87
2%4 h	305.87
4%0.5 h	481.98
4%1 h	412.32
4%2 h	410.31
4%4 h	373.00

linked polymer after the rearrangements which occurred in several conditions (Cury et al., 2008).

In the present work, these cross-linked polymers were used as excipients for the preparation of non-compacted solid systems (physical mixtures) for drug delivery purposes. This study was performed evaluating the influence of the cross-linking degrees on the physical characteristics (particle size distribution, swelling degree and rheological properties) of these systems as well as on the *in vitro* release behavior of sodium diclophenac.

2. Materials and methods

2.1. Raw materials

Acetone and hydrochloric acid (Synth, Diadema, Brazil), purified water (Milli-Q Plus System Millipore), high amylose (HYLON VII, National Starch and Chemical, 70% amylose, 30% amylopectin, New Jersey, EUA), sodium diclofenac (Henrifarma, São Paulo, Brazil), sodium hydroxide (Grupo Química, Rio de Janeiro, Brazil), trisodium trimetaphosphate (Sigma–Aldrich Co., St. Louis, USA) were used as purchased.

2.2. Preparation of cross-linked high amylose

High amylose was cross-linked as described earlier (Cury et al., 2008). Briefly, eight samples with different cross-linking degrees were obtained by reacting high amylose in aqueous medium alkalinized by NaOH (2% or 4%) at four different times (0.5 h, 1 h, 2 h, and 4 h). The amount of the cross-linker STMP was kept constant by 30% and the polymer pre-gelatinization step was skipped. The samples were labeled as 2% (2%0.5 h, 2%1 h, 2%2 h, 2%4 h) and 4% (4%0.5 h, 4%1 h, 4%2 h and 4%4 h).

2.3. Particle size distribution

The analyses of the size distribution of the samples at 16-fold magnification were performed with a *Leica Qwin* image analyzer coupled to a Leica MZAPO stereoscope. The Feret's diameters at 0° of 300 particles were measured.

2.4. Water uptake

The swelling dynamics was determined with an Enslin's device (Voigt, 2000). For the assay, 0.05 g of powdered samples were placed on the sintering filter and the volume of water absorbed

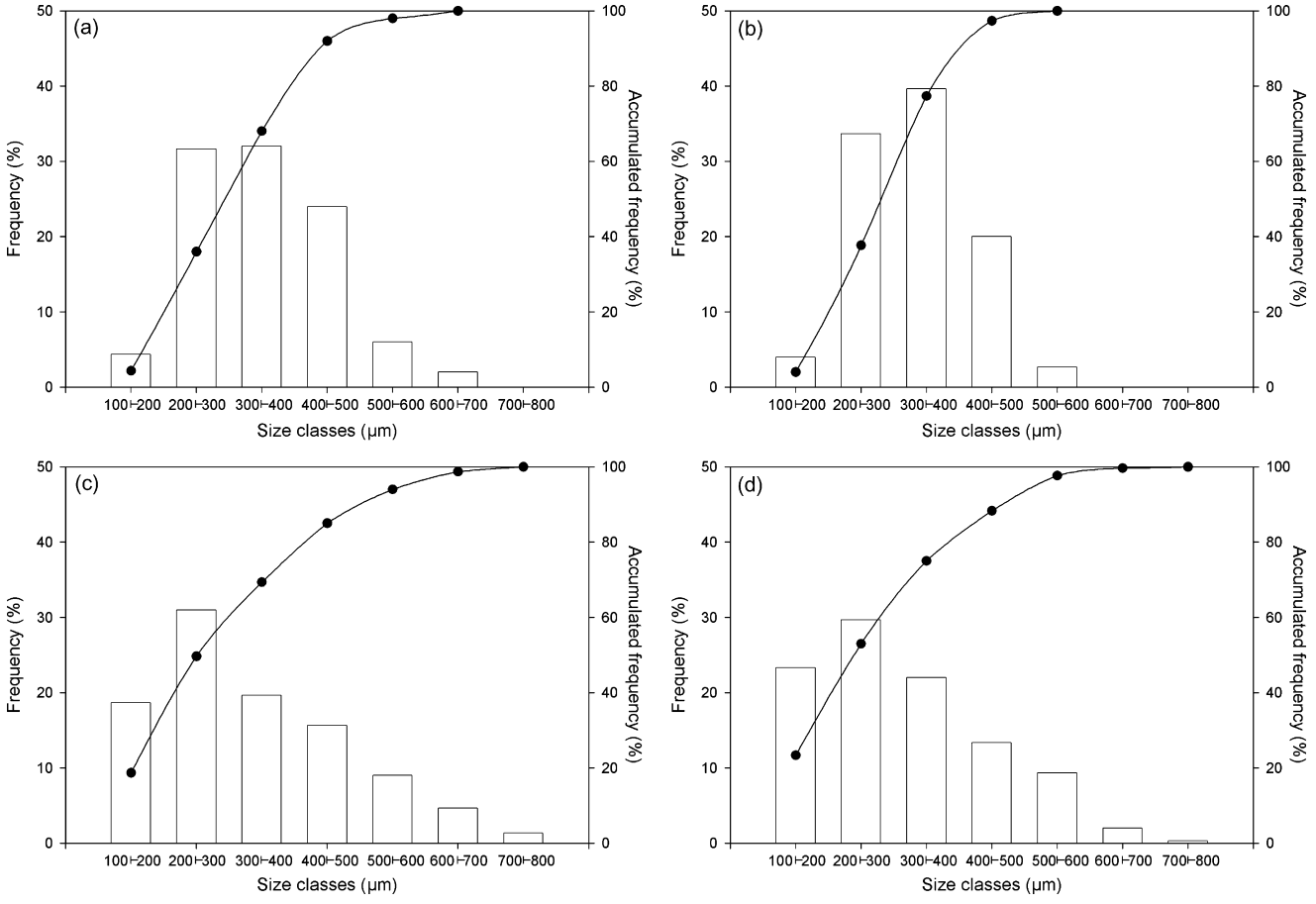

Fig. 1. Particle size distribution. (a) 2%0.5 h, (b) 2%1 h, (c) 2%2 h, and (d) 2%4 h

after 5, 15, 30, 60, 90 and 120 min was measured with the graduated pipet. The assays were carried out in triplicate and the results expressed as % of water uptake in relation to the initial mass of the samples. Statistical analysis of the results was performed by ANOVA/Tukey with a significance level α of 0.05.

2.5. Analysis of the viscoelastic behavior of the polymers

The viscoelastic properties of the 1% aqueous dispersions of the samples were assessed through dynamic oscillatory measures on a stress controlled cone-plate Carrie Med SLM 100 rheometer (diameter 4 cm, angle 2° and gap 61 μm). The storage modulus (G'), loss modulus (G'') and complex viscosity η^* were assessed at the frequency of 1 Hz and under a stress of 30 Pa, at 37 °C. The mechanical spectra at angular velocities from 0.6 to 188 rad/s were measured at the same conditions.

2.6. In vitro drug release

For this analysis, samples were prepared by manually mixing 50 mg of sodium diclophenac with 230 mg of each type of high amylose (cross-linked or not). These physical mixtures were poured into n° 0 hard gelatin capsules, which were placed in a Hanson Dissolution Test Station SR8-Plus (Chastworth, USA). The tests were performed in triplicate in 900 mL distilled water at 37 °C and stirring set at 50 rpm using apparatus 1 (USP 26, 2003). Drug release was followed measuring the absorbance of the samples at 276 nm. Statistical analysis of the results was performed by ANOVA/Tukey with a significance level α of 0.05.

3. Results and discussion

3.1. Particle size distribution

The size distribution of the samples prepared with 2% NaOH is depicted in Fig. 1. It can be observed that the 0.5 and 1 h samples presented the lowest particles frequency within the 100–200 μm range. According to the cross-linking mechanism proposed earlier (Cury et al., 2008), this interesting behavior can be attributed to the initial, random addition of long phosphate branches to the polymers, which, however, resulted in only few effective cross-linking points. Therefore, these structures would be more dilated and heterogeneous than those samples taken at 2 and 4 h at the same base strength. In fact, the formation of large clusters with a vast number of fragility points makes the comminution process easier hence reducing the amount of small particles, because of the low stress required for the samples preparation.

The same principle may explain the size distribution of the 2 and 4 h samples at base strength of 2% (Fig. 1a–d), since structures with higher mechanical resistance would have been expected from a polymer with a higher cross-linking degree and a lesser amount of long, dangling phosphate chains. Therefore, higher stresses must be applied for the comminution of the samples, resulting in a greater frequency of small particles.

The samples prepared at 4% base strength (Fig. 2a–d) showed the same wide size distribution patterns as the later samples at 2% base strength (2 and 4 h), however with a lower frequency of finer particles. This finding may be evidence that the chemical processes occurring at the longer contact times at 2% base are somewhat sim-

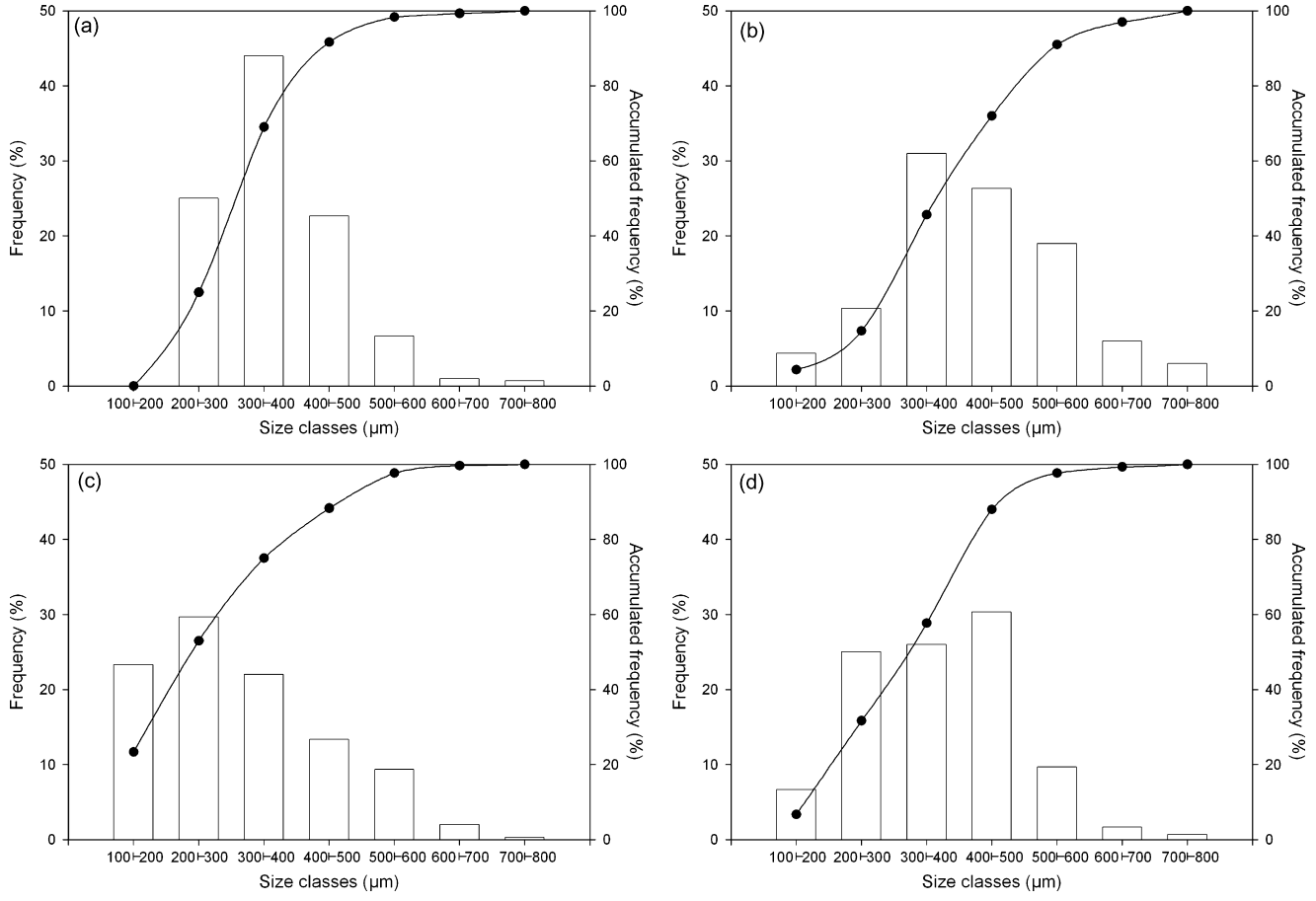

Fig. 2. Particle size distribution. (a) 4%0.5 h, (b) 4%1 h, (c) 4%2 h, and (d) 4%4 h

ilar to those at the early stages of the reactions performed at 4% base strength. Since the mechanical strength directly influences the behavior of the materials during the comminution process (Hickey and Ganderton, 2001), the similarities expected for the size distribution of the samples at 4% base and that from 2%4 h could be explained by the analysis of the size distribution profiles. Therefore, a quantitative evaluation of the particle sizes was performed, and the particles mean diameters were calculated from the cumulative frequency data.

Table 1 shows the systematic reduction of the mean diameters as a function of the reaction time for both series of base strengths used. This trend agrees well with the proposed mechanism in which the time-dependent increase of cross-linking would create polymeric networks with meshes of reduced sizes. Moreover, the samples at 4% base presented mean values higher than those at 2%. This also suggests the formation of more extended tri-dimensional polymer networks as the result of some reorganization of the already bound phosphate groups (Cury et al., 2008).

3.2. Rheological behavior

The characterization of the rheological behavior of the cross-linked polymers was performed by the evaluation of the storage (G') and loss (G'') modules, since it is expected an increase of both the mechanical resistance and elasticity of the samples as the cross-linking degree of the polymer chains also increases (Romani et al., 2002; Schulze et al., 2003). The variation of G', G'' and η^* values for the cross-linked polymers as function of frequency are shown in Figs. 3 and 4. Interestingly, G'' seems to be dominant at low frequencies, but the increase of this parameter causes the progressive increase of G', which then becomes more important at higher frequencies. This predominantly elastic behavior at high frequency is typical for materials presenting networks, which are entangled and interconnected (Nystrom and Walderhaug, 1996).

Table 2
Values of G', G'' and η^* obtained from oscillatory test at 1 Hz frequency.

Samples	G' (Pa)	G'' (Pa)	η^* (Pa s)
2%0.5 h	0.021198	0.120125	0.019403
2%1 h	0.075373	0.919175	0.146693
2%2 h	0.128304	2.441055	0.388818
2%4 h	0.013443	0.078878	0.01485
4%0.5 h	0.010546	0.078018	0.012523
4%1 h	0.011533	0.011811	0.002626
4%2 h	0.032035	0.187095	0.030193
4%4 h	0.010090	0.107600	0.017189

The values of G', G'' and η^* obtained in oscillatory tests at 1 Hz frequency are exhibited in Table 2. It can be observed that the samples cross-linked with 2% NaOH exhibited a constant increase in G', G'' and η^*, with a maximum at 2 h contact time. This evidences the progressive increase in the cross-linking degree and, therefore, of the samples viscosity. The steep fall of the values for the samples obtained at 4 h can surely be attributed to the breakdown and reorganization of shorter cross-linked chains resulting in structures with lower entanglement level (Grassi et al., 2006; Cury et al., 2008). The same partial breakdown of long polymeric chains may explain the lower values for the rheological parameters obtained for the samples prepared with 4% NaOH.

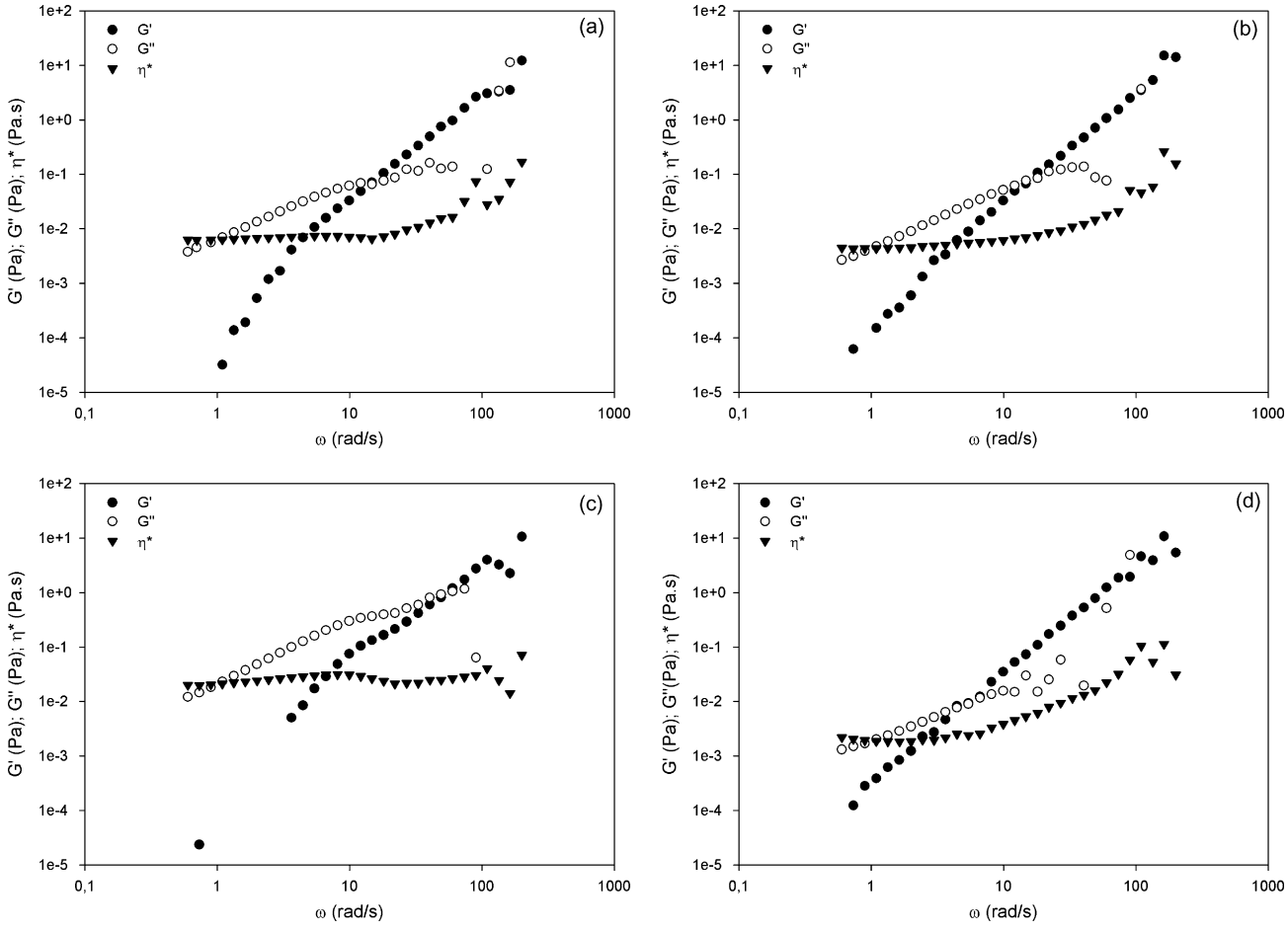

Fig. 3. Viscoelastic behavior of 2% samples. (a) 2%0.5 h, (b) 2%1 h, (c) 2%2 h, and (d) 2%4 h

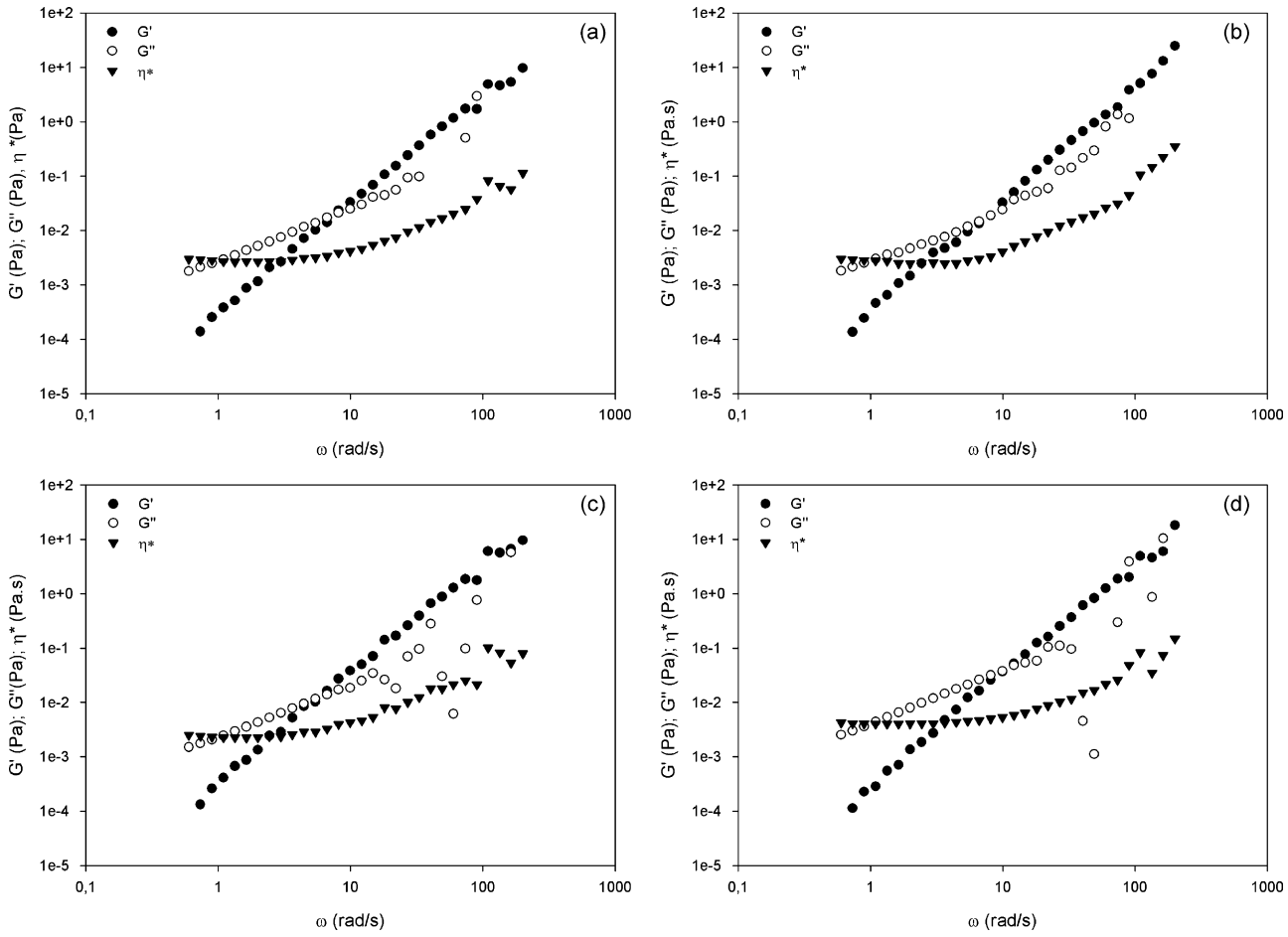

Fig. 4. Viscoelastic behavior of 4% samples. (a) 4%0.5 h, (b) 4%1 h, (c) 4%2 h, and (d) 4%4 h

3.3. Swelling behavior

All cross-linked samples showed an increase in the water uptake in relation to native high amylose ($\alpha < 0.05$) (Figs. 5 and 6). This sharp increase in the swelling capacity may be attributed to the introduction of terminal phosphate groups, which would build dilated and highly branched polymer chains (Cury et al., 2008). This new chains would surely be more prone to a different type of chain entanglements, which, together with the new cross-linking structural points, would facilitate the penetration and imprisonment of the water molecules. The comparatively low water absorption of pure high amylose may be due to the fact that such polymer adopts the conformation of helicoidal ribbons (Imberty et al., 1988), which allows the chains to be closer from each other, but with a high interchain mobility, which restricts their water uptake ability.

At the initial stages of cross-linking, the introduction of terminal phosphate groups may serve to increase the hydrophilic character of the cross-linked polymers, since the ionization of those dangling

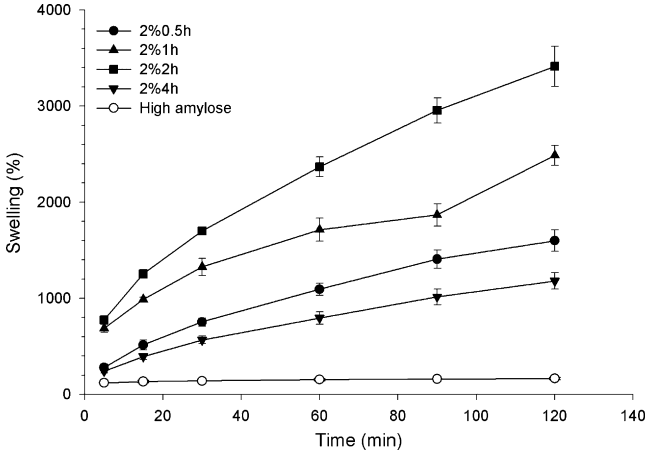

Fig. 5. Swelling profiles of 2% samples.

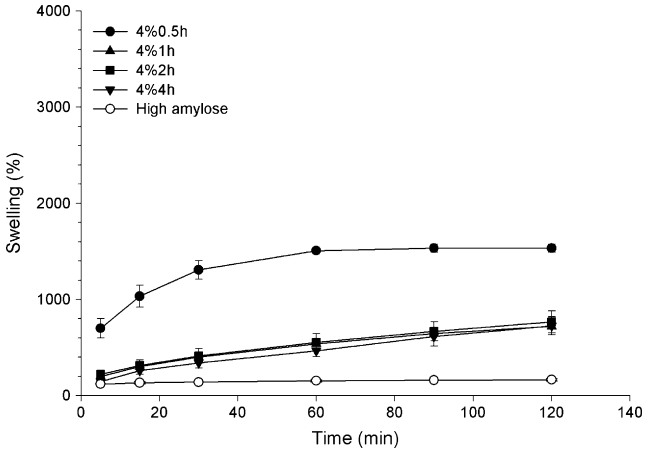

Fig. 6. Swelling profiles of 4% samples.

groups shall increase their water uptake (Peppas and Khare, 1993). As the cross-linking process evolves, the interchain mobility shall decrease and the new pockets formed may be increase even more the water uptake up to the limit where the size of the meshes of the new network will prevent the swelling of the polymer (Gehrke and Lee, 1990). It is also possible that at high cross-linking degrees there may be a reduction of available hydroxyl groups, which are important to the swelling processes (Ruiz et al., 2001). It is also interesting to speculate that a severe degree of cross-linking may rearrange the amylose chains, exposing the originally internal ether groups, which would diminish even more the hydrophilicity of the cross-linked polymers.

The combined factors, which seem to accompany the phosphate cross-linking processes of high amylose, point to a maximum swelling capacity for the samples obtained at 2 h contact time of the reactants to the 2% base solutions. An increase in the reaction time, or in the base strength from 2 to 4%, apparently contributes to lessen this capacity. The similar swelling behavior of the series of samples obtained at 4% base strength, with those of the 2%4 h polymers ($\alpha < 0.05$), also supports the idea of a thermodynamically driven conformational rearrangements for those structures, as pointed out earlier (Cury et al., 2008).

3.4. Sodium diclophenac release patterns

Figs. 7 and 8 clearly show that both sets of samples obtained at different base strengths can release the drug more slowly and gradually than native high amylose ($\alpha < 0.05$). Considering that the interchain links introduced by the cross-linking process should result in greater rigidity of the polymer, there should be expected an elastic matrix that would be denser than the original polymer after the swelling process, thus inducing the slower release of the drug (Moussa and Cartilier, 1997).

The release profile of the samples cross-linked at 2% base strength (Fig. 7) showed that there was no difference in the total release time of the drug as from the samples 2%0.5 h and 2%1 h. However, for the first 5 h of the release process, the samples 2%0.5 h allowed a slower release ($\alpha < 0.05$). This can be directly attributed to the lesser swelling capacity of these samples in relation to the 2%1 h samples, establishing therefore fewer interactions among the hydrophilic matrix, water and drug (Colombo et al., 2000). After the period of 1 h, the 2%0.5 matrices must have absorbed enough water to hydrate the polymers, resulting in a gel of good physical stability, which resulted in the practically constant release profile shown in Fig. 7a.

It is also interesting to note that the release profiles for the 2% samples shows a increase of the time of drug release with the increase of the cross-linking reaction time. Although samples produced at shorter reaction times released circa 80% of the drug between 7 and 9 h, the $t_{80\%}$ for 2%4 h sample was reached after 18 h. It seems, therefore, that the effects of swelling and gel formation are important factors for the drug release from samples synthesized up to 2%2 h. For the other samples synthesized under stronger conditions, the size of the polymer meshes produced during the synthesis is perhaps the most important factor influencing a slower and controlled drug release. In this context, it is important to remember that the samples 2%4 h were those that showed the lowest complex viscosity, a factor that had been pointed out earlier to force

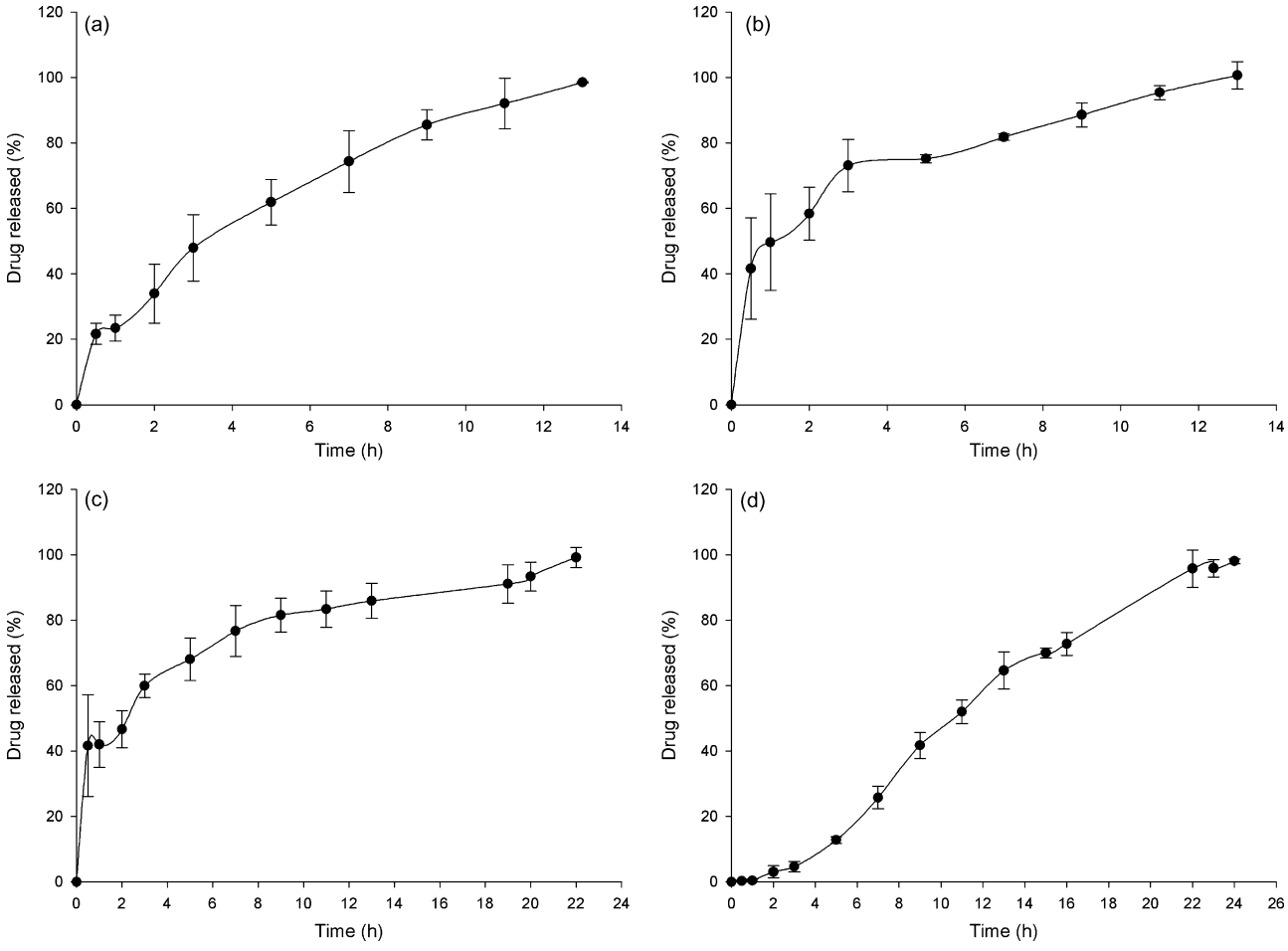

Fig. 7. *In vitro* drug release profiles from 2% samples.

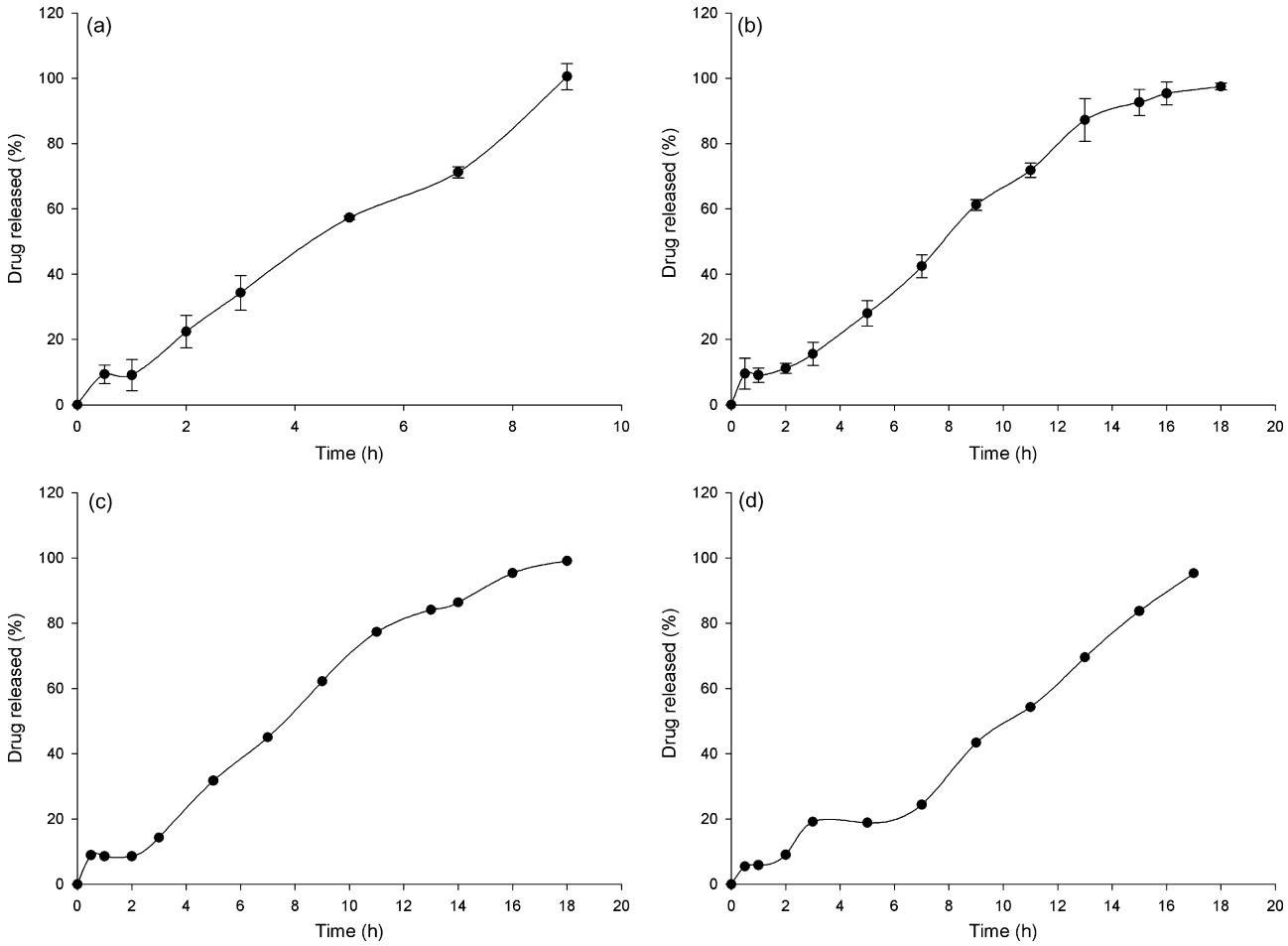

Fig. 8. *In vitro* drug release profiles from 4% samples.

a shortening, not a lengthening, of drug release times (Ford et al., 1985; Sung et al., 1994; Vandelli et al., 1998; Velasco et al., 1999; Espinoza et al., 2000; Roy and Rohera, 2002). The formation of small sized meshes during the preparation of the 4% samples should be the main factor involved in the lesser sustained sodium diclophenac release showed for these samples (Fig. 8).

4. Conclusions

The particle size distribution correlated well with the cross-linking degree. The rheological behavior presented, in which G'' predominates at low frequencies whereas G' prevails in high frequencies, is typical for cross-linked and entangled networks and it evidences the occurrence of the cross-linking reaction. The lower values of G' and η^* presented by samples with higher cross-linking degree indicates the breakdown of chains, resulting in more rigid structures with lower level of entanglement. The swelling capacity increases with cross-linking degree until a maximum for the 2%2 h. After that, the more rigid structure, the smaller polymer meshes and the decrease of polymer hydrophilicity make water uptake more difficult. Despite of some cases of burst effect, the release profiles from samples with cross-linked polymers evidenced the slow and gradual sodium diclophenac release. Although gel formation is very important for release control, the viscosity is not an imperative factor in such process, since the maximal release prolongation was exhibited for the 2%4 h sample, which was not the most viscous sample. Consequently, the mesh sizes of polymer network that can promote an adequate attachment of drug molecules must be the most important factor in controlling drug release. The fact of different cross-linking degrees leading to various release profiles also evidences the possibility to adjust the release patterns for diverse therapeutic necessities.

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