

BEHAVIOUR OF AMPHIPHILIC POLYSACCHARIDES IN AQUEOUS MEDIUM

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ABSTRACT

This paper describes the rheological and thermal behaviour of alkylated chitosan and hyaluronan derivatives and methylcellulose. Their behaviour is characterized by the formation of hydrophobic domains which may be considered as crosslinked points. Then, depending on the thermodynamic conditions (temperature, salt concentration), an aqueous solution transforms to a 3D-physical network formed on the basis of hydrophobic cooperative interactions.

Key Words: Alkylated chitosan, alkylated hyaluronan, amphiphilic polysaccharides, methylcellulose, rheological behaviour.

RESUMEN

Este trabajo describe el comportamiento reológico y térmico de quitosano y hialuronano alquilados y de metilcelulosa. Dicho comportamiento está caracterizado por la formación de dominios hidrofóbicos que pueden considerarse como puntos de entrecruzamiento. Por tanto, en dependencia de las condiciones termodinámicas (temperatura, salinidad), las disoluciones acuosas pueden transformarse en una red física tridimensional basada en interacciones cooperativas de carácter hidrofóbico.

Palabras Clave: Quitosano alquilado, hialuronano alquilado, polisacáridos anfífilicos, metilcelulosa, comportamiento reológico.

INTRODUCTION

In this paper, different amphiphilic polysaccharides based on chitin, cellulose and hyaluronan are described and few of their properties are given. First, chitin is a poly(β (1-4)-N-acetyl-D-glucosamine); it is a natural polysaccharide of major importance, first identified in 1884 (Figure 1a). This biopolymer is synthesized by an enormous number of living organisms (shrimps, crabs...).¹ As many polysaccharides, chitin has some hydrophobicity and in aqueous NaOH it can form a lower critical solution temperature (LCST) solution with a critical temperature around 30°C.² This characteristic temperature is very important to characterize the influence of temperature on the behaviour of a polymer in solution: at a temperature lower than the LCST, the polymer is soluble and one gets one phase; over the LCST, two phases

separate corresponding to a concentrated and a diluted solutions at equilibrium. After deacetylation in alkaline conditions, one gets chitosan which becomes soluble in aqueous acid solution due to the protonation of the $-\text{NH}_2$ group at C-2 position of the D-glucosamine. The different chitosans are characterized by their average number of acetyl groups per monomeric unit (degree of acetylation, DA) (Figure 1a). Using the reductive amination, a series of amphiphilic chitosan derivatives were produced from aldehydes with different chain lengths (Cn from 3 to 14) at controlled DS (usually lower than 10% to maintain water solubility in acidic conditions)^{3,4}. Alkylated chitosans with good solubility in acidic conditions (pH < 6) have a number of very interesting properties.

Hyaluronic acid (HA), a linear polysaccharide composed of repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid (Figure 1b), is the only non sulfated glycosaminoglycan found in the extracellular matrix. HA is an

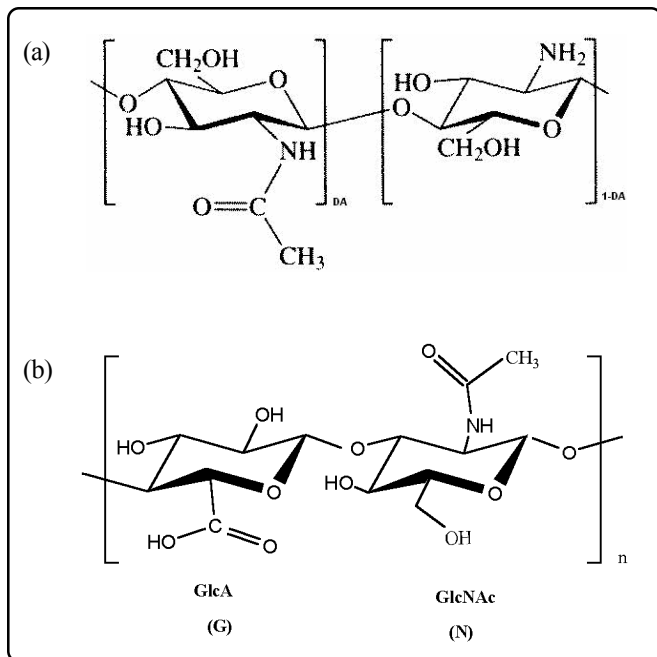


Figure 1. Chemical structure of the repeat unit of chitin (when $DA > 0.9$) and chitosan (when $DA < 0.5$) (a) and hyaluronan (b).

attractive polymer for the development of new biocompatible materials with many applications in viscosupplementation, tissue engineering, and drug delivery^{5,6}. This biopolymer has been the subject of various chemical modifications leading for example to chemically cross-linked materials with successful application in the treatment of osteoarthritis and HA-drug conjugates having controlled-release and cell-targeted properties^{7, 8}. We have recently prepared new water-soluble alkylated derivatives of hyaluronan (HA-C10)^{9,10}

The third polymer examined is the methylcellulose; this derivative of cellulose is a LCST polymer (the LCST is located at $29 \pm 2^\circ\text{C}$) which gives a strong physical gel on heating over 50°C ¹¹. It is often used in food applications.

In this paper, it is intended to summarize the behaviour of these three systems we have previously studied and which are based on polysaccharide derivatives. We intend to show the original properties that such amphiphilic copolymer-like systems which can be considered as smart materials being thermosensitive, salt and /or pH-sensitive.

EXPERIMENTAL

Methylcellulose used is a commercial sample (Methocel A4M Premium from Dow Chemical; the average degree of substitution was 1.8) (Figure 2). All the solutions tested were prepared by direct dissolution at ambient temperature in 0.1M NaCl solution and fixed at 6g/L.

Alkyl-chitosans were prepared by Rinaudo et al.⁴; they are

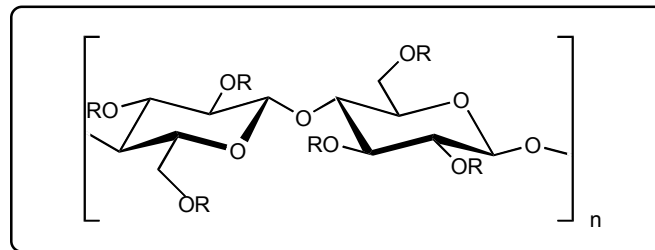


Figure 2. Chemical structure of cellulose backbone. $R=H$ cellulose; $R=H$ or CH_3 in methylcellulose.

dissolved in acidic conditions (acetic acid 0.3M) in the presence or the absence of an external salt (sodium acetate).

Alkyl-hyaluronan under its sodium salt form is dissolved in water; NaCl is added when necessary to get a 0.15 M NaCl salt concentration, reflecting the biological conditions. HA is produced from a bacterial strain by ARD Cy (Pomacle, France). The dynamic rheological measurements were performed on a AR 1000 from TA Instruments using a plate-cone geometry; the cone has 4 cm as diameter and $3^\circ 59'$ angle. The temperature was controlled by the Pelletier plane in the range of 20 or 37°C up to 65 or 70°C with a controlled rate of temperature variation ($3^\circ/\text{min}$ for methylcellulose). The strain percent was selected to be in the linear domain. A film of silicone oil was carefully disposed on the examined solution such as to prevent its evaporation.

DSC experiments were performed on methylcellulose with a microcalorimeter DSCIII (SETARAM, France) in the same range of temperature as adopted for rheological measurements and with the same solutions but with a rate of temperature change of $0.5^\circ/\text{min}$ for heating and cooling cycles.

RESULTS AND DISCUSSION

A- Alkylated chitosans

Alkyl-chitosans were prepared by reductive amination on the free $-\text{NH}_2$ in C-2 position of the D-glucosamine units with a degree of substitution around 0.05 to preserve the water solubility in acidic conditions. They were described previously in our laboratory^{3,4}. There are two important effects of the grafting of alkyl-chains on chitosan as soon as the alkyl-chain length is large enough (carbon chain $n = 10$ or better 12):

- first, these derivatives exhibit surface activity and they were compared with corresponding low molecular weight surfactants^{12,13}; for the same amount of alkyl chains with the same length, they have a relatively low effect on the decrease of the surface tension but they improve much the stability of the interfacial film; it was clearly demonstrated that a simple surfactant and modified chitosan have completely different behaviour¹²⁻¹⁴. It is interesting to mention that alkyl-chitosans are compatible with neutral and cationic surfactants; it was demonstrated that cationic surfactant adsorbed on the alkyl chain grafted on chitosan, promotes its solubilisation.

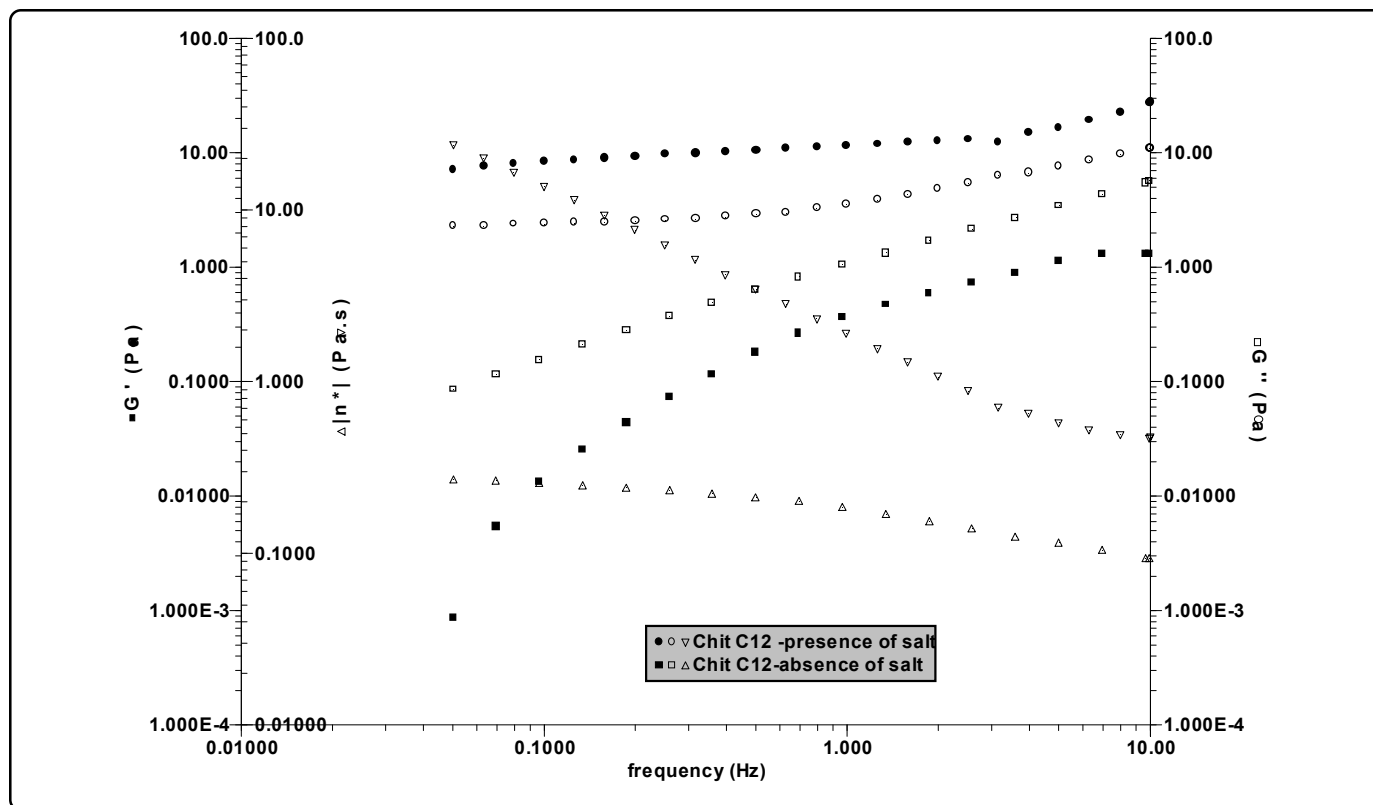


Figure 3. Rheological behaviour of alkylated chitosan (chit-C12) at 7g/L in 0.3M acetic acid (G' ■, G'' □, $|\eta^*|$ △) and in 0.3M acetic acid/0.2M sodium acetate (G' ●, G'' ○, $|\eta^*|$ ▽).

- secondly, they increase considerably the viscosity of aqueous solution due to hydrophobic inter-chain interactions; especially for C-12 chain length and a DS~0.05, a physical gel is obtained in dependence on the pH¹⁵ and on salt concentration⁴. Figure 3 indicates that in absence of external salt, the behaviour is that of a solution with the elastic modulus G' lower than the viscous modulus G'' in all the range of frequencies covered; in the presence of salt able to screen the electrostatic repulsions between chains, hydrophobic interactions dominate and a gel-like behaviour is observed with $G' > G''$.

These gels are characterized by rheology and correspond to a storage modulus G' larger than the loss modulus G'' ; the physical gel formation results from a balance between electrostatic repulsions between the positively charged chitosan chains and hydrophobic attraction due to alkyl chains mainly in relation with their length^{3,4}. The hydrophobic domains formed in the systems are important to adsorb hydrophobic molecules such as pyrene (a fluorescent probe used to evidence these domains in dilute solution); it has been previously shown⁴ that these associations can be destroyed by addition of cyclodextrins which are known to complex the alkyl chains of surfactants in their hydrophobic cavity¹⁶.

Alkylated hyaluronan

The chemical structure of hyaluronan (also named hyaluronic

acid or HA) is prepared under the sodium salt form. It is a very soluble polymer in water⁸. Based on our previous knowledge on amphiphilic polymers, it was decided to modify HA and to graft alkyl chains of different lengths and different amounts on the HA backbone. These derivatives were synthesized by Creuzet et al.⁹ and Rinaudo et al.¹⁰ under mild aqueous conditions. The first step was to prepare a HA derivative selectively modified with adipic dihydrazide (HA-ADH) with a substitution degree of 0.08; then, alkyl chains with different chain lengths were introduced with a substitution degree around 0.05^{9,10}. These polymers being still water-soluble exhibit associating properties in the presence of external salt. Formation of hydrophobic domains is demonstrated from fluorescence measurements in the presence of pyrene as a probe. Then, it is shown that the viscosity of polymer solutions in 0.15 M NaCl becomes non-Newtonian for HA grafted with C-10 and C-12 chains and increases rapidly when the polymer concentration increases over 1g/L. When the polymer concentration increases, a gel-like behaviour induced by external salt addition was observed from rheological measurements (Figure 4). In absence of salt, the alkylated hyaluronan behaves as a viscoelastic solution but when external salt is added, a gel formation occurs due to the screening of electrostatic repulsions between polyanionic chains.

B- Gelation of methylcellulose

Commercial water soluble methylcelluloses (with a degree of

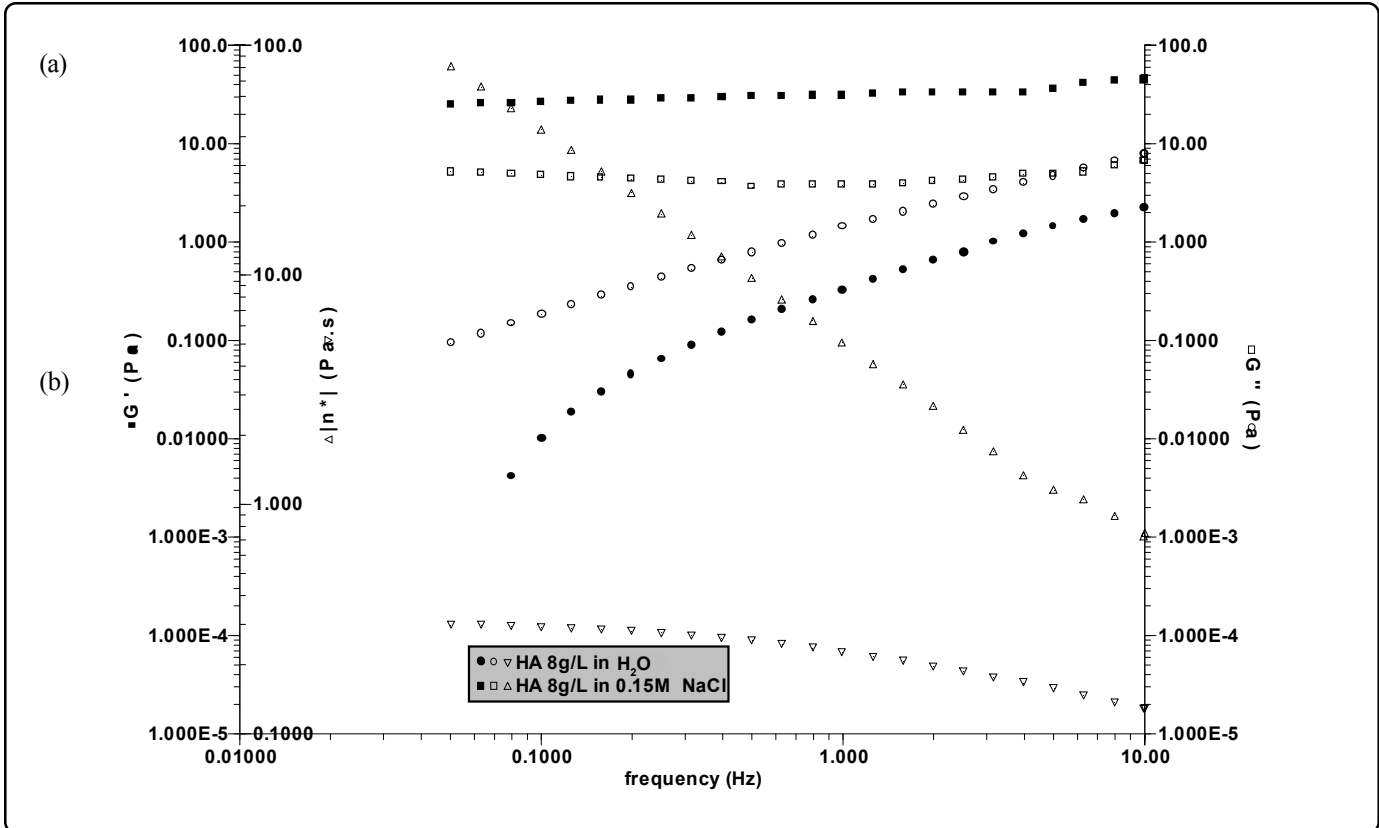


Figure 4. Rheological behaviour of alkylated hyaluronan (HA-C10) at 8g/L in water (G' ●, G'' ○, $|\eta^*|$ ▽) and in 0.15M NaCl (G' ■, G'' □, $|\eta^*|$ △).

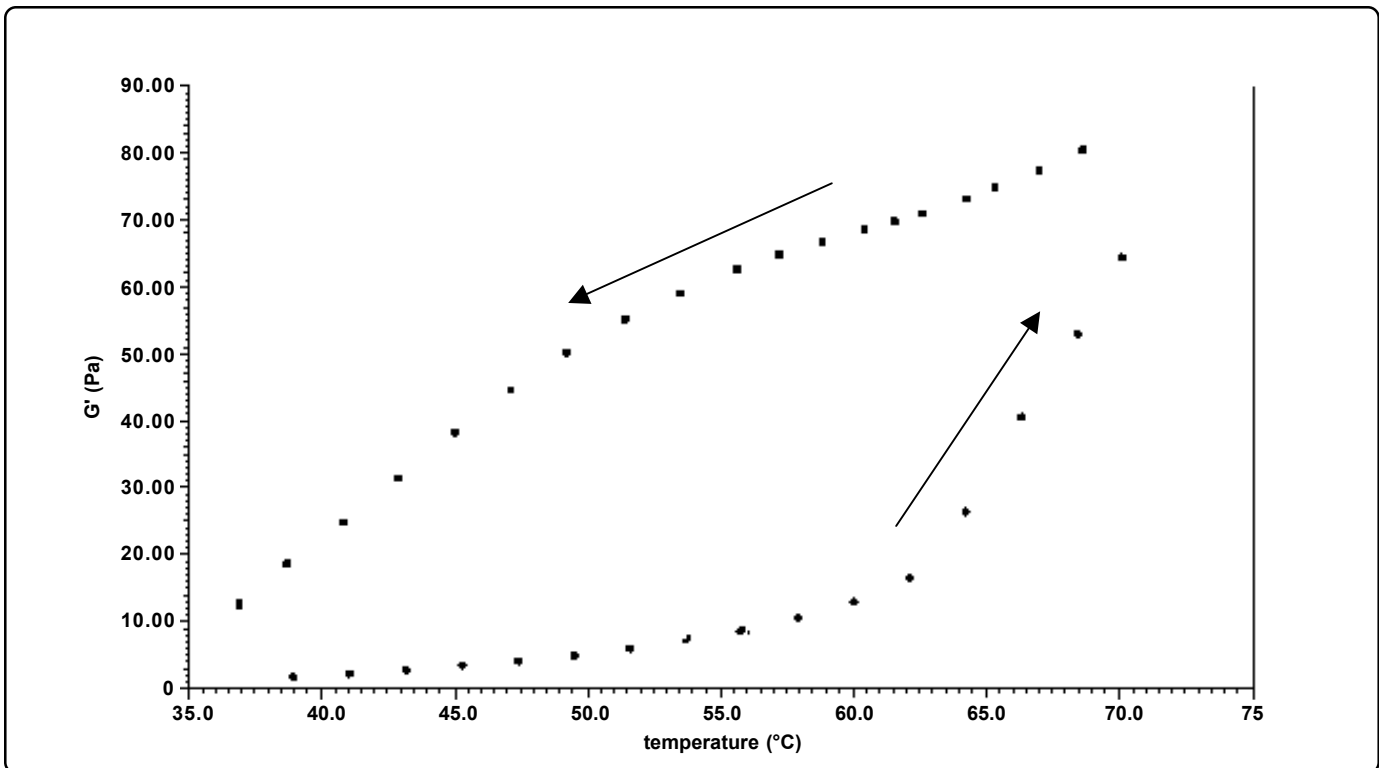


Figure 5. Influence of temperature on the rheological behaviour of a 6g/L solution of methylcellulose in 0.1M NaCl.

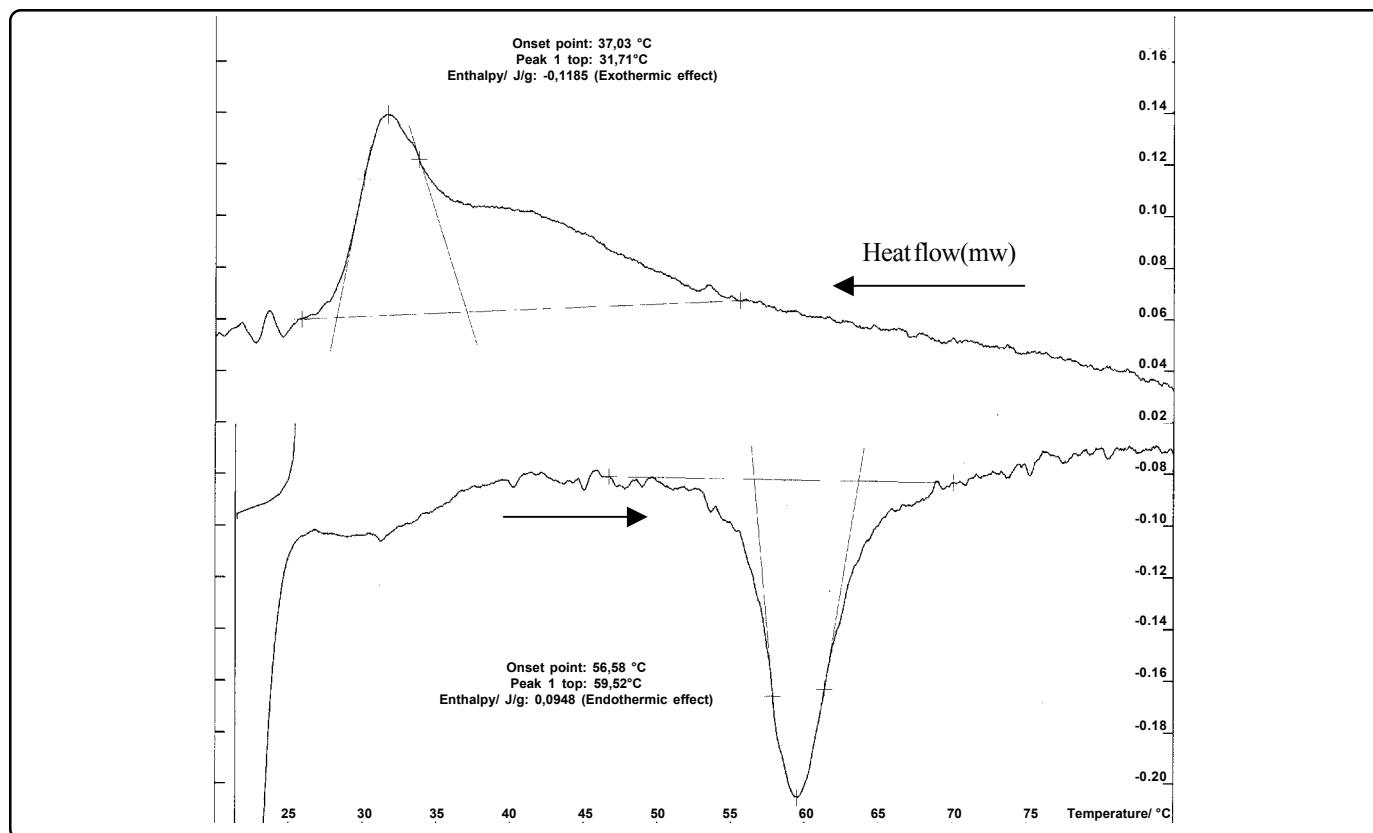


Figure 6. DSC curves for methylcellulose at 6g/L in 0.1M NaCl on heating and cooling at 0.5°C/min.

substitution in the range of 1.7) are amphiphilic polymers and they associate when temperature increases due to hydrophobic interactions. The characteristic temperatures for phase transition depend on the rate of temperature change; in addition, a large hysteresis is observed between gel formation on heating and melting of the gel on cooling curves as obtained in rheometry or DSC. An example is given in Figures 5 and 6.

The mechanism was previously analyzed and a two steps gelation was clearly demonstrated^{11,17,18,19}: a clear gel forms over 30 °C in relation with the presence of highly substituted zones on the cellulosic backbone and a phase separation with formation of a turbid stronger gel is obtained over 50 °C. In fact, this behaviour is directly related with the chemical modification process. Commercial methylcelluloses are produced from cellulose in a heterogeneous process in which amorphous zones are more substituted than crystalline ones; the resulting microstructure is a blockwise distribution of the methyl substituents along the cellulose backbone. This was proved when methylcelluloses were prepared under homogeneous conditions and have a completely different phase diagram¹⁹. It is concluded that the interchain interactions are based on hydrophobic interactions depending on the degree of substitution of cellulose but also on the distribution of methyl substituents along the cellulosic backbone.

Figure 5 demonstrates the strong increase of the storage modulus G' when temperature increases; a strong gel is formed with methylcellulose at 70 °C (with G' independent on the frequency in the range covered in this experiment and $G' > G''$)¹⁹. After cooling down to 37°C, the rheological parameters remain higher than after direct dissolution at low temperature in relation with the temperature range for clear gel stability. The change in the behaviour of methylcellulose is also clearly shown by DSC as presented in figure 6; on heating, the phase transition has a peak at 59.5°C (formation of the turbid gel corresponding to a large increase in G') and on cooling the peak occurs at 31.7°C (gel-sol transition). The temperature of 37°C is still in the range of the clear gel.

CONCLUSION

This paper summarizes the behaviour of three derivatives of polysaccharides which have the chemical structure of amphiphilic block and randomly grafted copolymers. These amphiphilic polymers have surface active properties (and behave as protective colloids) but especially they increase the viscosity of aqueous solution in dependence of the temperature, pH and salt concentration.

In given thermodynamic conditions, a reversible 3D-network is formed. Figure 7 presents a schematic description of these

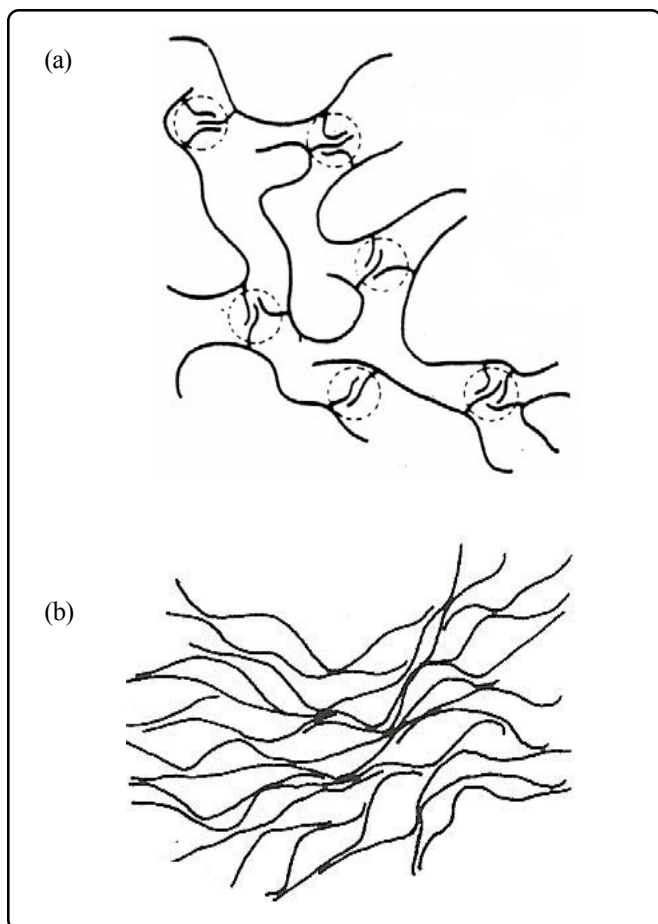


Figure 7. Schematic representation of physical gel formed by hydrophobic interactions on alkylated chain grafted copolymers (a) and on block wise copolymers (b).

physical gel: (a) represents the alkyl chain grafted chitosan or hyaluronan; (b) corresponds to gelation of methylcellulose.

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