New pH sensitive network : combination of an amphiphilic degradable polyester with a β-cyclodextrin copolymer

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SUMMARY : A novel hydrophobic monomer, ethyladamantyl malolactonate, has been synthesized and copolymerized with benzyl malolactonate by anionic ring-opening polymerization. The ratio of adamantyl monomer varied from 0 to 100 mol%. Deprotection of benzyl groups leads to a water soluble copolyester with carboxylic acid lateral functions which give a polyelectrolyte character to the corresponding polymers. The mixture of a copolyester containing 10% of adamantyl groups and a β-cyclodextrin/epichlorohydrin copolymer in aqueous solution leads to a new pH-dependant associating system. The solution behavior of this system was studied by viscosimetry as a function of pH, concentration and ratio of both copolymers. At the initial solution pH (pH=2), the copolyester adopts a coiled structure as a result of hydrophobic interactions between the pendant adamantyl groups. Consequently , no network formation is observed as shown by a very low viscosity. As the pH increase, the viscosity of the medium increases and reaches a maximum at pH=5. At this pH, the copolyester expands because of electrostatic repulsions between the carboxylate pendant functions. Consequently, the adamantyl groups are accessible and can be encapsulated into the β-cyclodextrin cavities resulting in a significant increase of the viscosity.

Introduction

Hydrophobic modified polyelectrolytes consist in water soluble charged polymers containing a small fraction of hydrophobic groups. When dissolved in water above the overlap concentration (C*), they tend to self-associate by intermolecular hydrophobic interactions, generating a reversible network[1]. The properties of aggregates formed by associating polymers in aqueous medium have attracted widespread interest in recent years.

We have studied a new class of associating systems consisting in the mixture of two different copolymers : a degradable copolyester bearing adamantyl pendant groups as hydrophobic groups and a water soluble β-cyclodextrin (β-CD) / epichlorohydrin polymer. The inclusion of the hydrophobic groups inside the β-CD cavities can lead to the associating network above critical values of the polymers concentrations. The adamantyl group[2] is a highly symmetric tricyclic hydrocarbon which consits in three fused cyclohexane rings as shown in figure 1. It has been chosen because of its high hydrophobicity and because its size corresponds to the β-CD cavity which is a necessary condition for a good inclusion phenomenum[3-6].

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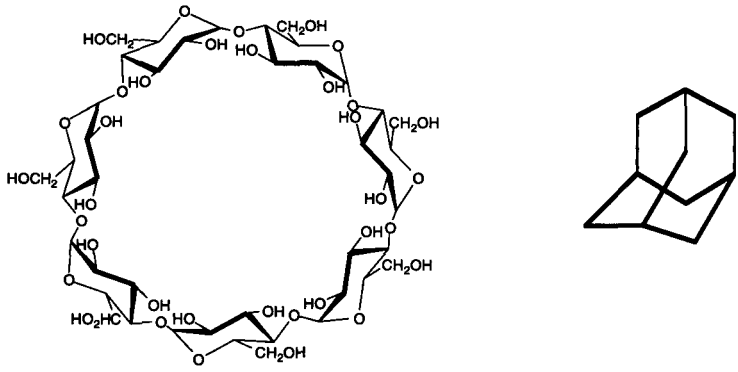

Fig. 1 : Schematic representations of β-cyclodextrin and adamantane

Amphiphilic degradable copolyester, poly(β-malic acid-co-ethyladamantyl β-malate), was synthesized by copolymerization of benzyl malolactonate which is conducive, after deprotection, to the hydrophilic part of the polymer and the ethyladamantyl malolactonate which corresponds to the hydrophobic part. These polymers are degradable by simple hydrolysis of main chain ester bonds[7-8]. The hydrophobic pendant groups arranged in different proportions along the main chain allow the modification of the hydrophilic / hydrophobic balance. Presence of charged groups in the amphiphilic polymer increases its solubility and provides systems sensitive to ionic strength and pH changes.

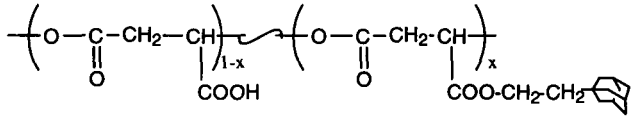

Fig. 2 : Poly(β-malic acid-co-ethyladamantyl β-malate)

In the first part of this paper, we wish to report on the synthesis and characterization of copolymers of malic acid and adamantyl malate. The associating properties of the copolyester and β-CD copolymer in aqueous solutions, studied by viscosity measurements, will be presented in the second part of the paper. Network formation will be discussed as a function of pH and concentrations.

β-CD copolymer

The β-CD polymers[9] are prepared by polycondensation of β-CD with epichlorohydrin (EP) under strongly alkaline conditions. The molecular weights of the water soluble polymers

obtained depend on several parameters : initial ratio EP/ β-CD, NaOH concentration and reaction time. It is possible to obtain very high molecular weight (Mw = 12 10^6 g/mol) polymers by stopping the reaction just before gelation. After ultrafiltration through a membrane, only high molecular weight samples are isolated from the initial mixture. The β-CD content, titrated by ^{1}H NMR spectroscopy, was 50 weight%. All the following study has been done on this purified sample containing only the high molecular weight fraction.

Amphiphilic degradable polyester

Preparation of the hydrophobic monomer, ethyladamantyl malolactonate, was based on the chemical synthesis route established for benzyl and alkyl malolactonates starting from malic acid[10]. Despite the sterical hindrance of adamantyl group, the three steps synthesis led to the corresponding β-substituted β-lactone with a quite good yield and high purity.

The second monomer, benzyl malolactonate, was prepared starting from either aspartic acid or malic acid as already described elsewhere[11-12].

These two monomers were copolymerized in different ratios by anionic ring-opening copolymerization with tetramethylammonium benzoate as initiator, leading to hydrolyzable copolyesters containing from 0 to 100% of adamantyl groups. Table 1 shows characteristics of these different copolymers by Steric Exclusion Chromatography and Differential Scanning Calorimetry.

Tab.1. Characterization of the protected copolyesters

%Adamantyl	Tg (°C)[a]	Mn(g/mol)[b]	Mw(g/mol)[b]	Ip
0	32	22 100	34 100	1.5
4	20	26 200	45 500	1.7
8	22	20 600	35 000	1.7
11	40	40 600	66 300	1.6
100	50	19 500	37 700	1.9

[a]Tg were measured by DSC using a Setaram 92-DSC apparatus under normal atmosphere at a heating rate of 10°C/min

[b]Mn and Mw were determined by SEC using a Spectra Physics P100 equipped with three columns of PL-gel and a Shodex RI-71 refractive index detector in tetrahydrofuran with a flow rate of 1ml/min and PS standards

Corresponding poly(β-malic acid-co-ethyladamantyl β-malate) were obtained after deprotection of benzyl pendant groups by catalytic hydrogenolysis. This reaction does not affect main chain ester bonds and is specific of the benzyl lateral group. Deprotected copolyesters displayed a high water solubility. These copolymers show a polyelectrolyte behavior, comparable to the one

observed for poly(β-malic acid). pKa, determined by acid-base titration, is equal to 3.8. This value is very close to the one of the homopolymer (pKa=4.4) [13].

Rheological behavior

The viscosity measurements were performed on a couette viscometer "Low Shear Contraves 30" at 20°C. We have used the copolyester containing 7.5% of adamantyl pendant groups for all this rheological study.

It is worth noting that poly(β-malic acid) and β-CD copolymer are incompatible. Indeed, we have observed the precipitation of the polyester in presence of 2% β-CD copolymer solution. For poly(β-malic acid-co-ethyladamantyl β-malate) copolymers, complexation of adamantyl groups inside β-CD cavities allows the interconnection between both structures, leading to the compatibilization of the copolyesters and β-CD copolymer.

1 - Influence of pH

Solutions of each copolymer were prepared by dissolving 80mg of polymer in 2ml of ultrapure water ($C_{copolyester}=C_{\beta\text{-CD copolymer}}=40g/l$). Then, both solutions were mixed ($C_T=40g/l$). Adjustements of pH were accomplished by adding small aliquots of a concentrated NaOH solution.

Figure 3 shows the effects of increasing pH on the viscosity of the copolyester alone at C=20g/l and on the mixture of copolyester/β-CD copolymer at the same copolyester concentration.

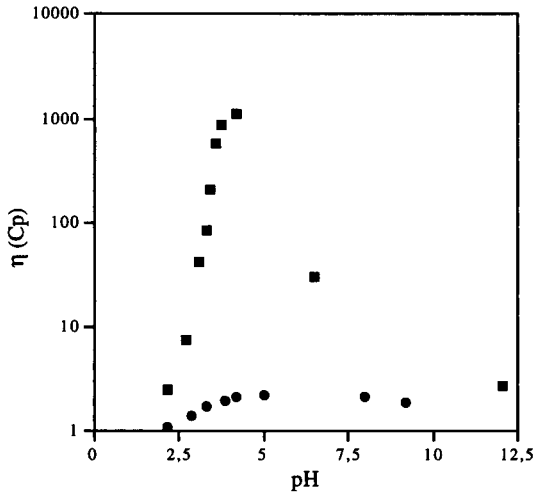

Fig. 3 : Viscosity of copolyester alone (•) and copolymers mixture (■) as a function of pH

Solution of the sole copolyester does not display significant viscosity variation as a function of pH.

On the other hand, observation of the copolymers mixture displays drastic viscosity variations as a function of pH. At the initial solution pH (pH=2.5), the copolyester is almost neutral and adopts a coiled structure as a result of hydrophobic interactions between the pendant adamantyl groups. Therefore, these groups cannot interact with β-CD as shown by a very low viscosity. As the pH increases, viscosity of the medium increases and reaches a maximum at about pH5. Carboxylic groups are progressively dissociated, and electrostatic interactions between these negative charges cause a stretching of the polymer coil. Thus, adamantyl groups are accessible and can form inclusion complexes with β-CD cavities. At higher pH, the viscosity decreases. The high charge density of the copolyester and presence of cations in the medium are unfavorable for the complexation[14].

2 - Influence of concentration

We have studied variation of viscosity as a function of pH at different total copolymers concentrations. We have observed that at optimun pH(pH=5), the viscosity increases with total concentration as shown in table 2.

Tab.2. Viscosity of copolymers mixture as a function of the total concentration

C_T (g/l)	η (Cp)
2	1.8
10	28
40	1000

At low concentration, the viscosity of the medium is the same as the viscosity of the copolyester alone. In fact, at low polymer concentrations, the proportion of adamantyl groups included in β-CD cavities is lower than the one expected from the stoechiometry of the mixture because of the finite value of the complexation constant. Moreover, probabilities to form interchains complexes are too low. When total concentration is increased, we observed a drastic increase of the viscosity. Indeed, above a critical concentration, chains of both polymers can overlap and interchains complexes are favored. This leads to an associating network.

3 - Influence of medium composition

Stock solutions of each copolymer were prepared separately at 10g/l and pH=4. Before measurements, solutions were mixed at the correct ratio, the total concentration being 10g/l.
Figure 4 illustrates the viscosity behavior of the mixture containing varying ratios of both copolymers at pH4. The maximum value of viscosity is observed at about 40/60 copolyester/β-CD copolymer mixture.

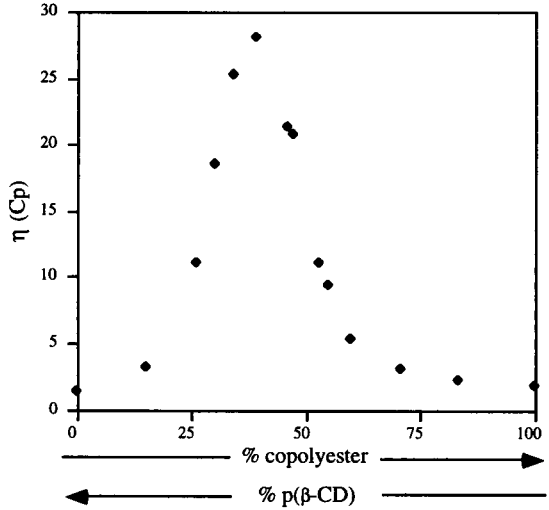

Fig. 4 : Viscosity of the mixture as a function of the copolymers ratio

In theory, position of the maximum corresponds to the stoechiometry between adamantyl groups and β-CD cavities, i.e. one adamantane for one β-CD cavity. Under this assumption, we should find the maximum for a ratio of 43/57 copolyester/β-CD copolymer. Predicted and experimental values are in agreement within experimental range of error. However, the picture is less simple than described above because further experiments[15] have shown different results for others percentages of adamantane that can be illustrated by the two following arguments :
- Firstly, all β-CD cavities or/and adamantyl groups are not accessible for the complexation. The conformation adopted by chains in the mixture decreases the mobility of the adamantyl groups and can avoid accessibility to β-CD cavities ;
- Secondly, the viscosity response to the associations is not linear when the chains are overlapping, as it should be the case at the total polymer concentration of this experiment (see table 2). As both copolymers do not have the same molecular dimensions, the overlapping conditions are varied when the composition of the mixture is varied.

Conclusion

The preparation of a copolyester based on ring-opening polymerization of an hydrophilic malolactonate with an hydrophobic malolactonate provides an effective route for the control of the hydrophilic / hydrophobic balance.

Association of this copolyester with a water soluble β-CD copolymer leads to a new associating network. The study of the mixture viscosity as a function of the pH has shown that the formation of inclusion complexes between adamantyl moieties and cavities of the β-CD copolymer depends largely on the conformation of the copolyester in solution.

This new pH-sensitive partially degradable associating network is a promising system for site-specific drug delivery.

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