Covalent Immobilization of Biological Molecules to Maleic Anhydride and Methyl Vinyl Ether Copolymers—A Physico-Chemical Approach

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ABSTRACT: The covalent grafting of biological molecules to copolymers of maleic anhydride and methyl vinyl ether (MAMVE) has been used in various applications in diagnostics. To tentatively elucidate the phenomena involved in the control of the immobilization of oligodeoxynucleotides and proteins, the physico-chemical properties of MAMVE copolymers were investigated. Because the grafting mixture contains water, to allow dissolution of the biomolecules without loss of biological properties, the anhydride-based copolymer evolves from a neutral to a negatively charged macromolecule due to hydrolysis of the anhydride moities. The properties of both hydrolyzed and nonhydrolyzed polymers were investigated. As demonstrated by light-scattering measurements in batch, the copolymers showed some level of aggregation in DMF, DMSO, and aqueous DMSO. The presence of aggregates was confirmed by size-exclusion chromatography in DMF. However, partial deaggregation occurred for the lowest molecular weight sample, on adding 1% w/v of LiBr. The nonhydrolyzed copolymers exhibited a rigid conformation in a 5% water/DMSO mixture, as well as their hydrolyzed counterpart at a low ionization degree. The rate of the hydrolysis reaction was shown to be dependent on the pH of the reaction medium and on temperature. The activation energy of the hydrolysis reaction was 14 kJ/mol, and the rate constant in the order of 10^{-4} s^{-1}. On the basis of these data, the effect on the grafting reaction of biomolecules of different parameters such as ionic strength and the nature of the solvent, along with some other results were interpreted in terms of interactions between the synthetic and bioactive macromolecules. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 71: 927–936, 1999

Key words: maleic anhydride copolymers; light scattering; size-exclusion chromatography; hydrolysis; kinetics

INTRODUCTION

In previous works, we demonstrated that molecules of biological interests could be immobilized covalently onto linear reactive polymers, in particular, for diagnostics applications.[1–3] The most easily available functional group on biological molecules is the amino moiety; so, to achieve the covalent grafting, we selected a polymer-bearing functional group reactive toward an aminated counterpart. We used the polymer with an aldehyde group,[4,5] with activated esters,[3] and with anhydride.[5–7]

Maleic anhydride copolymers proved, so far, the most capable at binding molecules as different

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as nucleic acids[6] and proteins.[7,8] Biological molecules are only soluble in water and, even if oligonucleotides can withstand high content of organic solvent, proteins lose their biological specificities in medium containing too much organic solvent. So, for our purposes, water was a prerequisite. But, water being not an inert solvent, the hydrolysis of anhydride groups takes place during the immobilization process. This reaction transforms an originally neutral polymer into a polyelectrolyte whose physico-chemical properties are quite different from the originating polymer. Therefore, during the grafting process, the chemical properties of the polymer change, which can have an effect on the immobilization reaction itself. Creating a covalent bond between two species requires that the two counterparts get close enough for the chemical bond to form, and the physico-chemical conditions under which two macromolecules interact may vary if the chemical nature of one of the counterpart evolves.

This article aims at studying the physico-chemical properties of maleic anhydride and methyl vinyl ether copolymers, widely used in the lab, under the nonhydrolyzed and hydrolyzed forms. Some kinetics parameters of the hydrolysis reaction will be determined as well. Finally, these data will be used to tentatively rationalize the results obtained over the years, on the immobilization mechanism of biological molecules, onto maleic anhydride–methyl vinyl ether copolymers.

EXPERIMENTAL

Polymer Samples

Copolymer samples of maleic anhydride and methyl vinyl ether (PMAMVE) were supplied by Polysciences, Inc. [samples (PMAMVE) 1 and 1A] and by Scientific Polymer Product, Inc. [for samples (PMAMVE) 2, 3, and 4]. The supplier indicated that the polymers were obtained by free radical polymerization in aromatic solvent, and that the molecular weight determination was carried out by membrane osmometry in methyl ethyl ketone.

Other chemicals were from Aldrich and used as received, unless stated otherwise.

Thermogravimetric Analyses (TGA)

A TGA 2950 thermobalance (Dupont Instrument) and the Thermal Analyst 2000 software were used to perform the TGA analyses of polymer samples. Experiments were run in a helium atmosphere using a ramp of temperature of $10°C \cdot min^{-1}$.

Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectra were recorded on a P.E.1760 spectrometer (Perkin-Elmer) (resolution of 2 cm^{-1}, 10 scans). KBr–polymer pellets were observed by transmission.

^{13}C-NMR

High resolution ^{13}C-NMR spectroscopy was carried out at 323K using a Bruker AC200 apparatus working at 50.3 MHz. In organic solvent (d6-DMSO), tetramethylsilane was used as the internal standard ($\delta = 0$ ppm), and in aqueous solvent (deuterated water), it was substituted by trimethyl silyl-3 propionic acid ($\delta = -2.35$ ppm).

Viscosity Studies

Viscometry measurements were performed with an automatic SEMATech apparatus, using a capillary viscometer fitted with a 0.58-mm diameter capillary tube thermostatted at $37 \pm 0.1°C$. The polymer samples were dissolved in a DMSO–water (95/5 volume ratio) mixture, then filtered through a 0.45-μm Millipore membrane before measurements.

Light Scattering Analyses

Experiments in batch were performed using a Multiangle laser light-scattering photometer (M.A.L.L.S.) DAWN F (WYATT Technology), operating at 632 nm. The refractive index increments (dn/dc) were measured in DMSO–water (95/5 volume ratio) polymer solutions by differential refractometry (Brice-Phoenix) equipped with a laser source at 632 nm. Light-scattering data were used to determine the average molecular weights, the radius of gyration, and the second virial coefficients.

Size Exclusion Chromatography

The experiments were carried out in an organic medium, DMF/LiCl (0.4% w/v), with a Styragel HT column (Waters) at 60°C, and DMF/LiBr (1% w/v) with a TSK gel mixed B column at room temperature, a Waters-Millipore differential refractometer and a LC isochrom pump (Spectra-Physics), at a flow rate of 0.5 $mL \cdot min^{-1}$. The

Table I Comparison of Different Characteristics of the Polymer Samples (PMAMVE), Either Measured or Provided by the Suppliers

Polymer Sample	Data from Suppliers	Water Content (wt %)	$[\eta]$ ($cm^3 \cdot g^{-1}$)	$\overline{M_{w_{app}}}$ LS (g · mol^{-1})	$\overline{M_{n_{app}}}$ SEC[c] (g · mol^{-1})	$\overline{M_{w_{app}}}$ SEC[c] (g · mol^{-1})	PD
(PMAMVE) 1	M_n[a] = 67,000	0.78	210 ± 2	407,000	190,000	325,000	1.71
(PMAMVE) 1A	M_n[a] = 20,000	0.94	31.5 ± 0.5	167,000	100,000	150,500	1.50
(PMAMVE) 2	$[\eta]$[b] = 150–200	8.26	228 ± 3	527,000	290,000	800,000	2.76
(PMAMVE) 3	$[\eta]$[b] = 260–350	9.56	314 ± 9	584,000	—	—	—
(PMAMVE) 4	$[\eta]$[b] = 50	6.59	82 ± 1	187,000	340,000	960,000	2.82

[a] units: g · mol^{-1}.
[b] units: $cm^3 \cdot g^{-1}$.
[c] The calibration was achieved with Polystyrene standards.
$[\eta]$: intrinsic viscosity.
PD: polydispersity index.

calibration were achieved using polystyrene standards (Polymer Laboratories). The solutions were filtered through a 0.45-μm Millipore membrane before measurements.

Hydrolysis Experiments

Various amounts of P(MAMVE) 1 sample were mixed in 100 mL of hydrolyzing medium and stirred at the required temperature.

For experiments run at room temperature, pH was directly monitored from the reaction solution with a Minisis 800 pH-meter (Taccussel) fitted with a Xerolyt electrode (Ingold).

For experiments run at higher temperatures, 6-mL aliquots were pipetted and cooled to room temperature before measurement of pH with the same equipment as above. The hydrolysis rate has been determined by extrapolation to time equal to zero with the assumption that, when the pH remained constant, every maleic anhydride group was hydrolyzed.

Polymer Titration

Polymer (340 g) was dissolved in 50 mL of milliQ grade water (Millipore) at 67°C, and let to stand on this temperature for 5 h. After cooling to room temperature, the polymer solution was titrated with a 1*M* sodium hydroxide solution (Merck) using the above described pH-meter.

RESULTS

Chemical Analysis of the Polymers

The overall composition of the copolymers was determined by elemental analysis, and proved to be a 1/1 molar composition of maleic anhydride and methyl vinyl ether. The presence of the anhydride moities in the polymers was checked by IR spectroscopy, TGA and ^{13}C-NMR. A loss of water, observed at 160°C by TGA, is indicative of cyclization of two carboxyl groups into an anhydride moiety, proving that hydrolysis had previously occurred. When the chemical shift of the carbon atom of the carbonyl group was 172 ppm, it corresponded to the anhydride group. When the chemical shift was in the 176–183 ppm range, the carbon atom corresponded to a carboxylic acid moiety, depending on the ionization degree. In samples with a high water content, as determined by TGA (Table I), a band due to the free COOH group was observed at 1720 cm^{-1} in IR spectroscopy.

Physico-chemical Studies

Molar Mass Determination

Because copolymers are heterogenous systems in terms of molecular weight, composition, and structure, the data obtained are apparent (Table I). Nevertheless, maleic anhydride copolymers are alternating copolymers,[9–11] with no significant composition distribution, so, light scattering measurements can be interpreted as for homopolymers.

Molar mass determination using light scattering and 5% water/DMSO polymer solutions yielded results in complete disagreement with the data provided by the suppliers. Most probably, in our solvent system, aggregates were formed as higher molar masses were observed. Changing the solvent to pure DMSO or pure DMF containing 0.4 to 1% w/v of lithium salt to disrupt aggre-

gates[12] and using SEC, with polystyrene standards for calibration, was not effective at suppressing the aggregation phenomenon (Table I).

Hydrolyzed polymers were analyzed in 5% water/DMSO solutions containing 0.1*M* LiCl to minimize the electroviscous phenomena observed with polyelectrolytes.[13] By comparison with their nonhydrolyzed counterparts, hydrolyzed polymers were in a better solvent in this mixture, as shown by higher intrinsic viscosities and second virial coefficient A_2 (Table II).

As we observed a concomitant increase of molecular weight and intrinsic viscosity, we used the Mark-Houwink-Sakurada relationship: $[\eta] = K.M^a$ to estimate the "a" and *K* parameters, considering aggregates as individual macromolecules. The parameter "a" = 1.10 reveals that the nonhydrolyzed polymer adopts a rigid conformation in this solvent. The "a" parameter and the constant *K* for nonhydrolyzed and hydrolyzed copolymers (*K* nonhydrolyzed = $1.32.10^{-4}$ mol · g^{-2} · cm^3 and *K* hydrolyzed with ionisation degree of 0.2 = $1.64.10^{-4}$ mol · g^{-2} · cm^3) were identical. Most probably, at 20%, the degree of ionisation, α', was not high enough to induce any conformational perturbation in the polymer. Taking into account the accuracy of measurement of the radii of gyration (R_g), there is a limited variation of R_g associated to the hydrolysis of the anhydride moieties.

As a conclusion, despite the presence of aggregates, we can ascertain the preservation of the rigidity of the system when hydrolysis of the anhydride occurs. The relatively high value of the "a" parameter should be in favor of oriented aggregates in which the chains would be parallely arranged via intermolecular links by DMSO molecules and/or by hydrogen bonding.

Investigation of the Hydrolysis Reaction

Due to the use of biological molecules, water was present in the immobilization media. The hydrolysis reaction scheme is shown in Figure 1. The kinetics of hydrolysis of the anhydride moieties was investigated under various conditions. The monitoring of the variation of pH, as the polymer hydrolyzed allowed to determine the time required for complete hydrolysis. Completion of hydrolysis was attained when the pH remained constant as all the anhydride moities were consumed, as checked by ^{13}C-NMR. The polymer hydrolysis rate increased with the pH of the reaction mixture (Fig. 2) and with temperature (Fig. 3). The hydrolysis rate (w) was determined

Table II Data Obtained from Static Light-Scattering Measurements on the Various Samples in 95% DMSO/5% Water Mixture

Polymer Sample	$\overline{M_{w_{app}}}$ (g · mol^{-1})		$[\eta]$ (cm^3 · g^{-1})		$\overline{R_{g_{app}}}$ (nm)		A_2 ($\times 10^4$ cm^3 · mol · g^{-2})	
	Non-hydrolysed	Hydrolysed $a' = 0.2$	Non-hydrolysed	Hydrolysed $a' = 0.2$	Non-hydrolysed	Hydrolysed $a' = 0.2$	Non-hydrolysed	Hydrolysed $a' = 0.2$
(PMAMVE) 1	407,000	626,000	210	310	32	24	8.18	9.40
(PMAMVE) 1A	167,000	155,000	31.5	41	20	23	1.50	2.28
(PMAMVE) 2	527,000	572,000	228	340	32	10	8.33	9.30
(PMAMVE) 3	584,000	769,200	314	460	28	29	6.80	6.67
(PMAMVE) 4	187,000	152,000	82.5	75	28	16	6.62	12.0

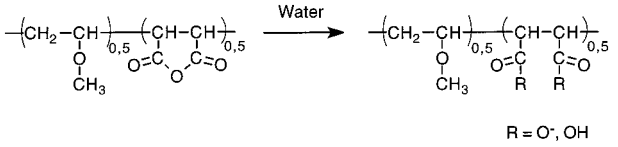

Figure 1 Hydrolysis reaction of copolymer.

for four polymer concentrations as a function of temperature. Due to the large excess of water in the reaction mixture, the reaction rate expressed in (1) can be simplified to (2). A Log plot of experimental values of "w" vs. the polymer concentrations yielded "δ" (the order of the reaction referring to the polymer), as the slope, $\delta = 1$, and k (hydrolysis rate constant) as the intercept.

$$w = k \cdot [\text{PMAMVE}]^{\delta} \cdot [\text{H}_2\text{O}]^{\gamma} \quad (1)$$

$$w = k' \cdot [\text{PMAMVE}]^{\delta} \quad (2)$$

$$\text{Log } w = \text{Log } k' + \delta \cdot \text{Log}[\text{PMAMVE}] \quad (3)$$

With the values of k' available for each temperature and using Arrhenius eq. (4), E_a could be determined as shown in Figure 4.

$$k' = A \cdot e(-E_a/RT) \quad (4)$$

For these measurements the (PMAMVE) 1 sample was used.

It turned out that $E_a = 14$ kJ/mol, proving that the hydrolysis reaction in a sodium borate buffer pH 9.3 was a favored reaction. Nevertheless, it appeared to be a slow process, because the rate constant was 10^{-4} s^{-1}.

Alkaline titration of hydrolyzed polymers (Fig. 5) featured two acidities as already reported.[14–18] The pKa's of polymeric carboxyl groups vary with the charge density in the macromolecule as titration proceeds. Therefore, the pKa can be expressed by eq. (5)[19,20]:

$$\text{pKa} = \text{pK}_o + 0.434\, \Delta G_{el}/RT \quad (5)$$

where ΔG_{el} is the electrical free energy ($J \cdot mol^{-1}$); R is the gas constant ($J \cdot mol^{-1} \cdot K^{-1}$); T is the absolute temperature (K).

The second term of eq. (5) is characteristic of electrostatic interactions between the ionizable groups along of the macromolecular chain.

Plotting pKa values vs. the dissociation degree "α'" for each acidity and extrapolation to $\alpha' = 0$,

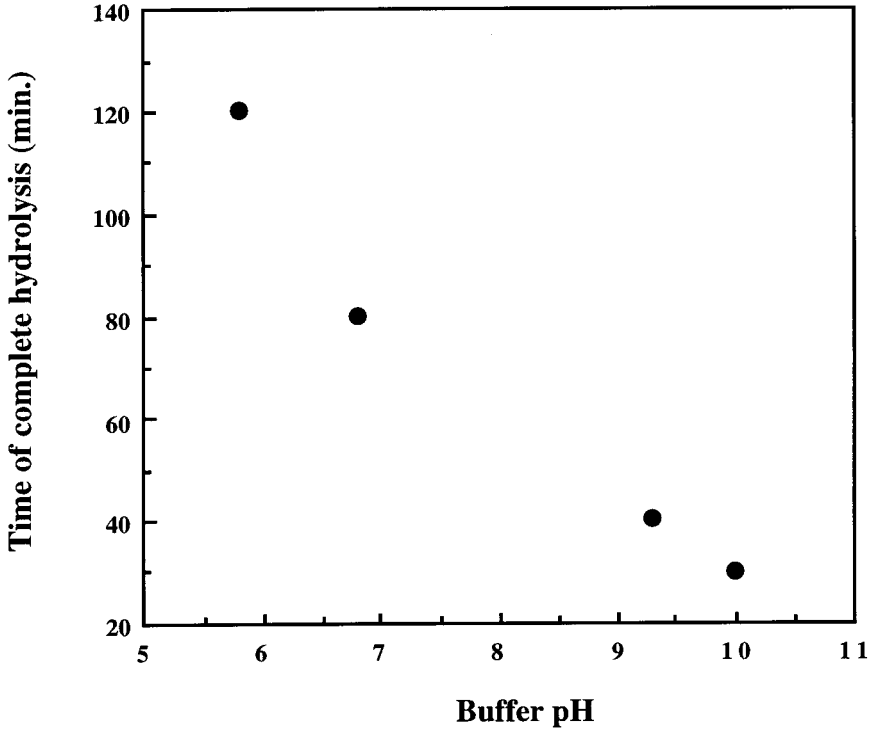

Figure 2 Effect of the buffer pH on the kinetics of the hydrolysis reaction at 67°C.

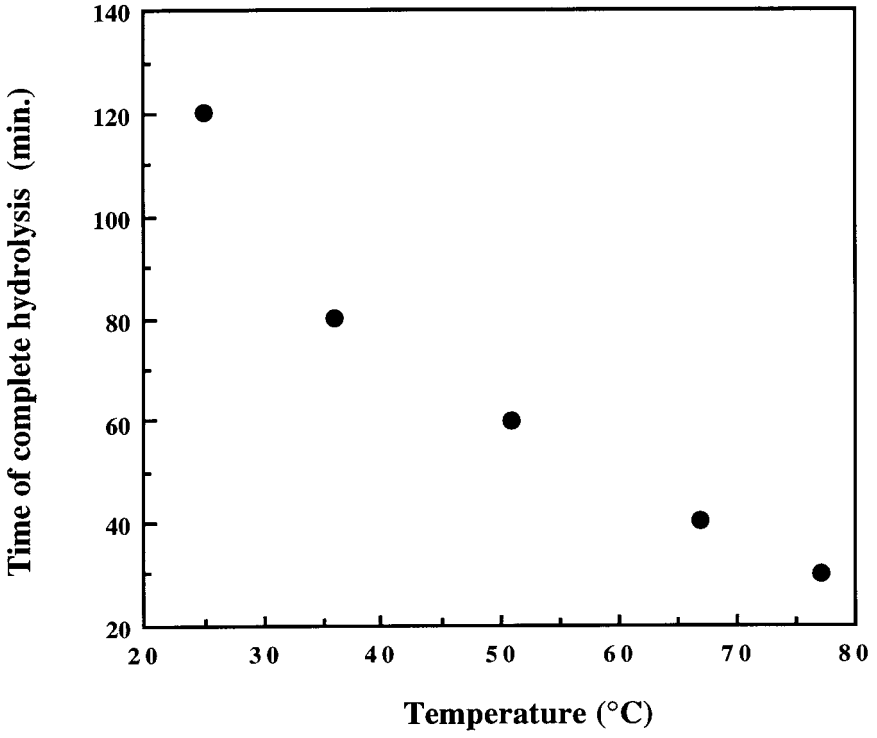

Figure 3 Effect of temperature on the kinetics of the hydrolysis reaction in the 0.5*M* sodium chloride, 0.1 sodium borate buffer pH 9.3.

afforded estimation of the two pKa values, such as $pK_{o1} = 3.5$; $pK_{o2} = 7.5$. pK_o, the intrinsic pKa, is indicative of the behavior of an isolated ionizable group, supposed to be undissociated.

DISCUSSION

Polymer Physico-chemistry

First, it is important to note that a change in the nature of the polymer from a neutral material to a polyelectrolyte occurs during the immobilization process of molecules of biological interest.

As a general feature, in a 5% water/DMSO mixture, both under the hydrolyzed or nonhydrolyzed forms, the copolymer formed aggregates as observed by light scattering (Tables I and II). These aggregates were observed as well in pure organic solvent, DMF, even in the presence of lithium chloride, which demonstrates the high tendency of this copolymer to self-associate. Wu et al.[21] analyzed PMAMVE samples by SEC using Waters Ultrahydrogel columns. In $LiNO_3$ containing DMF, they came across a problem of adsorption of the polymers onto the packing of the column leading to erroneous results (too low M_w and polydispersity index for a free radical process). With our sets of columns, the results we obtained were similar to those of Wu et al., when they ran SEC analyses of the copolymers under the diacid form, with a Tris buffer pH 9, 0.2*M* $LiNO_3$. Under those experimental conditions, they obtained high molecular weights and broad mass distributions, which suggest to us the presence of aggregates.

On a conformational standpoint, the presence of the anhydride ring confers stiffness to the macromolecular chains, which were found to adopt a rigid-rod conformation in aqueous DMSO. After hydrolysis, the stiffness was maintained for a dissociation degree of 20%, via hydrogen bonding between neighboring carboxylic acid groups. In the investigated solvent mixture (5% water in DMSO), the data reported in Table I concerning the intrinsic viscosity and A_2 values of the parent polymers were higher than those reported in the literature for poly(ethyl vinyl ether–maleic anhydride) in THF and acetone solutions.[9,22] Our coupling mixture corresponds to an efficient solubilizing medium in which segment–solvent interactions are favored.

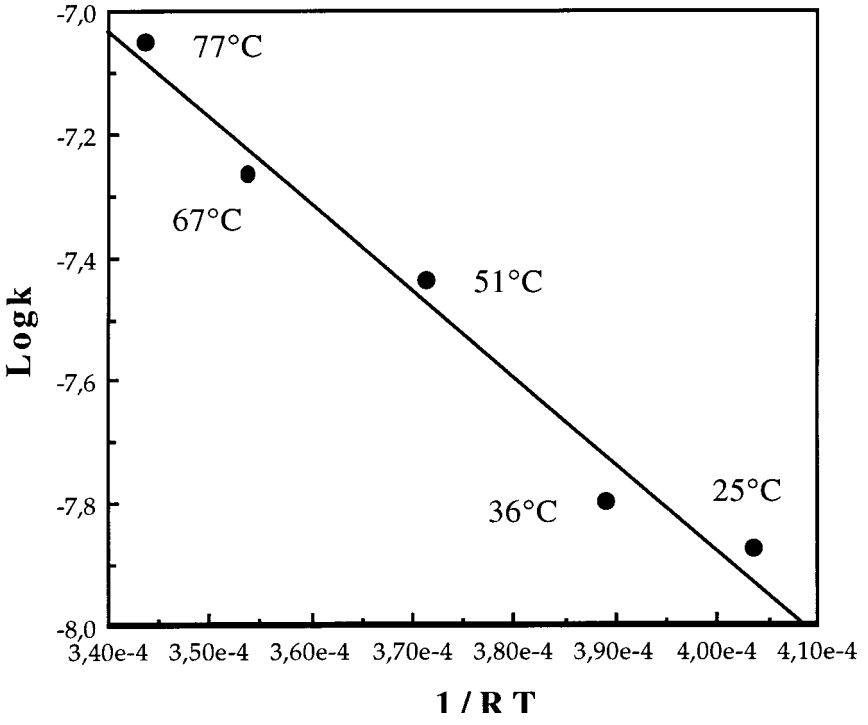

Figure 4 Determination of activation energy of hydrolysis reaction by Arrhenius's law in a 0.5*M* sodium chloride, 0.1*M* sodium borate buffer pH 9.3.

The alkaline titration of hydrolyzed polymers depicted two different acidities, as reported in the literature.[14–18] Dubin et al.[16–18] used the pKa curves deduced from titration curves to evidence transitions in the molecular structure of the copolymers, resulting from the variation of the ionization of the polymer chains. According to the authors, these transitions arose from hydrophobic

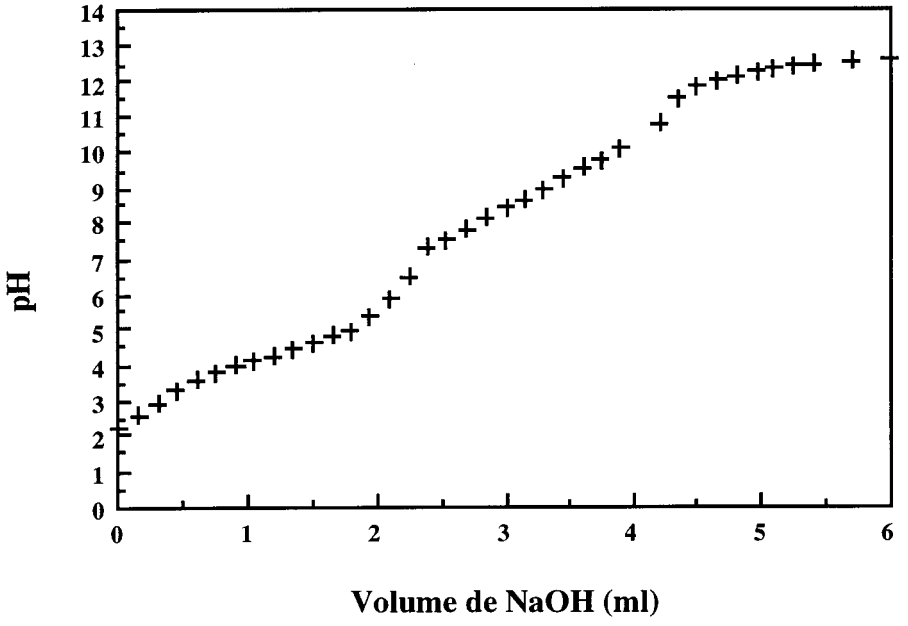

Figure 5 Alkaline titration of the hydrolysed polymer by 1*M* NaOH solution.

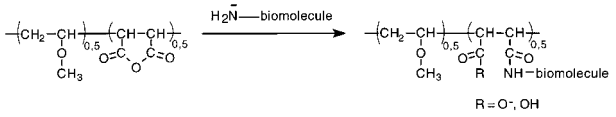

Figure 6 Coupling reaction between biomolecule and copolymer (PMAMVE).

interactions between the alkyl chains of the ether moieties. In our case, the methyl groups were not hydrophobic enough to observe any transition.

Effect of Physico-chemical Factors on the Immobilization Reaction of Molecules of Biological Interest

Two kinds of biomolecules were investigated—oligodeoxynucleotides (ODN) and proteins. The binding of biological molecule onto these polymers relies on the formation of an amide bond between the primary amine of the biological molecule and one carbonyl group of an anhydride moiety (Fig. 6). The conditions and results of coupling were reported elsewhere.[7,8]

ODN can sustain a fair amount of organic solvent and, actually, coupling mixtures consisted in 5% aqueous buffer in DMSO. For proteins, because their structure is modified by organic solvents, their immobilization was achieved in aqueous mixtures containing only 5% of DMSO. This latter solvent was chosen on account of the high intrinsic viscosity and A_2 values observed, compared with other solvents.[9,22]

Binding vs. Hydrolysis

The rate constant of the reaction of polymer hydrolysis was determined as 10^{-4} s^{-1}, which proves that it is a rather slow process, despite the fact that the energy of activation was low. At pH 9, complete hydrolysis of the polymer reactive groups was achieved within 4 min, although at a concentration four times higher than in the coupling media. For the optimized grafting conditions, the immobilization of ODN was achieved in 4 min,[6] and for proteins the required time for completion of the binding process varied from 10 to 40 min.[7,8] These data prove that the reaction of amine groups, under proper experimental conditions, can be favored over hydrolysis even in an aqueous medium. Therefore, the ratio of anhydride groups involved in the grafting reaction can be quite high, as shown, for instance, in the case of the immobilization of oligonucleotides. At maximum loading capacity, one out of four maleic anhydride group was involved in a chemical bond with oligonucleotides.[6] For BSA, a globular protein of molar mass 60,000 g/mol, maximal coupling efficiency involved 1 out of 21 anhydride groups in the grafting process.[7] Reporting the amount of polymer functional group involved in an amide bond, at maximum coupling capacity vs. the molar mass of the molecule of biological interest (Fig. 7), we can observe a regular decrease of the amount of reactive moieties involved in the binding as molar mass increases, a simple method of evidencing the effect of steric hindrance on the course of the reaction.

Effect of Conformational Factors

In a mixture containing 5% water in DMSO, the copolymer adopted a conformation with a high persistence length, as evidenced by «*a*» (from Mark-Houwink's law exponent) value of 1.10. In a rigid-rod conformation the functional groups of the polymer are readily available for the coupling reaction. In this respect, it is worth comparing the coupling efficiencies of MAMVE and *N*-vinyl pyrrolidone/*N*-acryloxy succinimide (NVP/NAS) copolymers, the latter, in the coupling medium (10% borate buffer in DMF), is under a compact coil.[3] This comparison was carried out over 10 different sequences,[23] the average coupling yield with the MAMVE copolymer was 64% ± 7 and 52% ± 16 for the NVP/NAS sample. This comparison proves that under the form of a rigid rod, the coupling reaction is more efficient and more reproducible, as seen by the lower variation range of the coupling yields obtained with the MAMVE copolymer.

The copolymer/ODN conjugates were under the form of high molar mass aggregates.[3–6] This aggregation can be due, at least in part, to the self-association characteristics of the copolymers observed by light scattering, even in the presence of lithium salt in the mobile phase (Tables I and II). These self-association can be due to the balance of segment/segment interactions and the segment/solvent interactions. The former interactions result from hydrogen bonding, which can arise from nonionizated COOH groups, and hydrophobic interactions from the structure of the hydrophobic segments.

Effect of Ionic Strength

Due to the hydrolysis of the anhydride groups, the copolymer acquires a polyelectrolyte character during coupling. From the modification of the

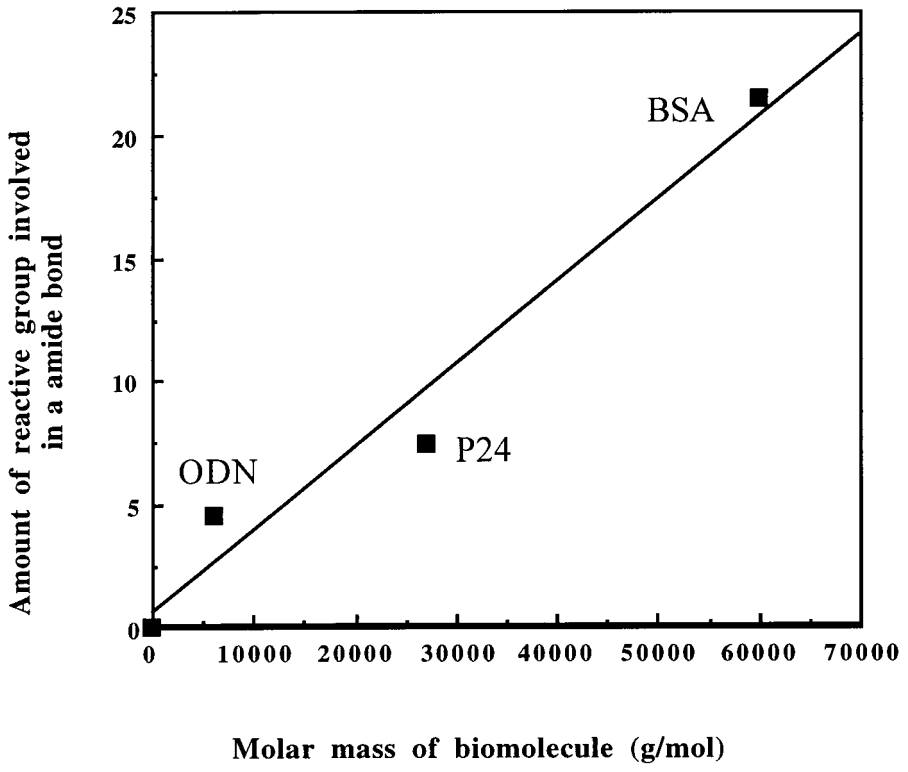

Figure 7 Amount of polymer functional group involved in a amide bond vs. molar mass of biomolecule. Results from publications 6, 7, and 8.

chemical nature of the copolymer, changes in physico-chemical properties can be expected. First, the hydrolysis of the polymers resulted in an increase in both the intrinsic viscosity and the second virial coefficient A_2, characterizing solvent–polymer interactions. This result demonstrated that hydrolyzed polymers were in a better solvent than their nonhydrolyzed counterparts. Electrostatic interactions play a dominant role in the control of the grafting reaction of molecules of biological interest. In a 0.1*M* borate buffer, the immobilization of nucleic acids better occurred in the presence of 1*M* sodium chloride (61% yield) than in the absence of salt (28%).[6] The role of the salt was to decrease electrostatic repulsive forces between the negatively charged oligonucleotides and the polymer. In contrast, immobilization with NVP/NAS in a DMF/borate buffer mixture, occurred in the absence of sodium chloride,[3] although this copolymer, like MAMVE, acquires a polyelectrolyte nature on hydrolysis of the *N*-hydroxysuccinimide moieties. This difference can be attributed to the fact that in the coupling medium, the NVP/NAS copolymer is under a compact random coil and relies on electrostatic repulsive forces to unfold the macromolecule. As a result of the unfolding, reactive groups get more available for further immobilization of nucleic acids.

The immobilization of proteins onto the MAMVE copolymer best took place at low ionic strength, even at pHs close to the isoelectric point. This proves that electrostatic factors, in particular attractive electrostatic forces, were a requirement for the binding reaction to proceed efficiently. These forces bring together the negatively charged, partially hydrolyzed polymer, close to positively charged domains of the proteins. Dubin et al.[24] have observed the same behavior in their studies of the formation of soluble noncovalent complexes between proteins and polymers bearing the same charge sign. That was interpreted as a result of a nonuniform charge distribution on the proteins. Hence, even at their isoelectric points, despite a null global net charge, proteins bear some sites that are positively and negatively charged.

CONCLUSION

The physico-chemical properties of maleic anhydride/methyl vinyl ether (MAMVE) copolymers were investigated in solution. It was found that

these polymers had a tendency to form aggregates in many different media, either under the hydrolyzed or nonhydrolyzed form. On a conformational standpoint, the macromolecules adopted a rigid conformation, as shown by related light-scattering data and intrinsic viscosity measurements using the Mark-Houwink law. The hydrolysis of the anhydride groups of the polymers was found to be a slow process, with a low energy of activation. The polymers resulting from the hydrolysis reaction were polyacids, more soluble in a water/DMSO mixture than the parent polymers, as demonstrated by viscosimetry.

These data were used for the interpretation of results obtained in the covalent grafting of biological molecules onto MAMVE copolymers. The rigid conformation of the synthetic macromolecules allowed its reactive groups to be readily available for reaction with amino moities of the biomolecules. Under optimized conditions, the binding reaction was quite fast, favoring the grafting process even in a 95% aqueous buffer. Due to the occurrence of the hydrolysis of some anhydride groups on the polymer during the immobilization step, the polymer has to be regarded as a polyelectrolyte, which explains why electrostatic interactions were the major factor controlling the tethering of molecules of biological interest onto the polymer.

On a mechanistic standpoint, our results showed that the chemical grafting of biomolecules onto reactive polymers is an issue that should not only be addressed with chemistry considerations. Physicochemical considerations must be taken into account such as polymer solubility, its conformation, and ability to interact with the biomolecules to get close enough for the chemical reaction to occur.

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