Surface Chemistry

DOI: 10.1002/ange.200500639

Electrografting of Poly(ethylene glycol) Acrylate: A One-Step Strategy for the Synthesis of Protein-Repellent Surfaces**

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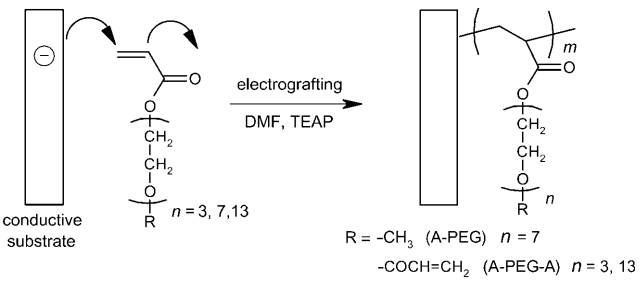
Nowadays, the availability of biocompatible surfaces is a key issue in the design and production of medical devices and implants to be used in contact with blood. Poly(ethylene glycol) (PEG) has the unique capacity to reduce protein adsorption and cell adhesion on a variety of hydrophobic substrates,[1] which explains the continuing efforts to develop new methods for the deposition of strongly adhering PEG coatings on gold[2] from thiols and disulfides, on silicon[3] and glass[4] from silanes or a poly(glycidyl methacrylate) primer,[5] and on metal oxides from poly(L-lysine)-g-poly(ethylene glycol)[6] or by plasma treatment.[7] In addition to these techniques, electrografting is often used for the chemisorption of poly(meth)acrylate chains onto electrically conductive substrates whatever their shape (plates, stents, fibers, powders,…).[8–11] Compared to the chemical methods, electrografting has the advantage of being applicable to a large variety of substrates, including metals and alloys, semiconductors, ITO-glass, and carbon. Although originally reported for acrylonitrile,[11] electrografting has been extended to a large variety of (meth)acrylates, thereby allowing the surface properties to be tuned by the appropriate choice of the ester substituent. For example, reactive, hydrophobic, and electroactive coatings have been deposited by the electrografting of various acrylates bearing *N*-succinimidyl,[12] norbornenyl,[13] and pyrrolyl[14] moieties, respectively. To the best of our knowledge, the one-step electrografting of highly hydrophilic coatings has not yet been reported.

Herein, we want to report the direct electrografting of acrylic end-capped PEG (macromonomers). In the following, A-PEG and A-PEG-A will be used as abbreviations for the number of polymerizable end-groups A (one or two, respectively; Scheme 1). The electropolymerization of PEG macromonomers was conducted by voltammetry in DMF containing a conducting salt (tetraethylammonium perchlorate, TEAP). As is usually observed for the cathodic polymerization of (meth)acrylates, two reduction phenomena can be distinguished[9] in solvents of a high donor number,[8] (Figure 1 A,



Scheme 1. Electrografting of PEG macromonomers by cathodic polarization of the conductive substrate.

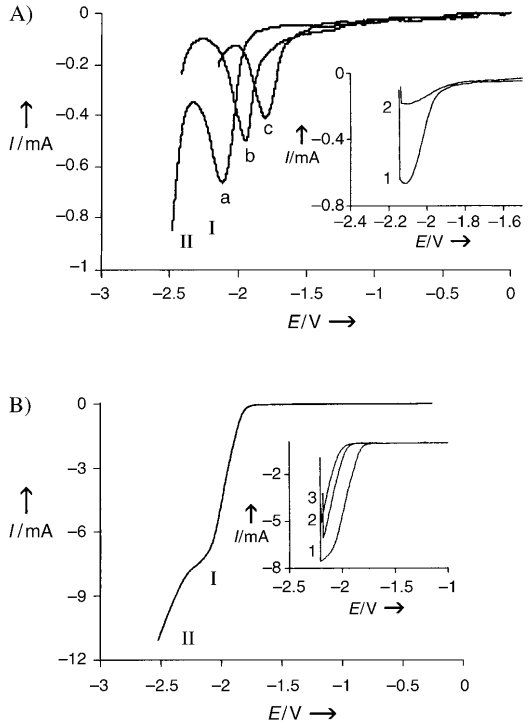


Figure 1. A) Voltammograms on glassy carbon for the reduction of A-PEG-A (M_n = 258): a) 0.1 M, b) 0.5 M, c) 1 M in DMF/5×10^{-2} M TEAP at 20 mVs^{-1}; I: grafting peak; II: polymerization in solution. Inset: Reduction of A-PEG-A (M_n = 258, 0.1 M): 1) first and 2) second scan. B) Voltammograms for the reduction of A-PEG (M_n = 454, 0.5 M) on glassy carbon in DMF/5×10^{-2} M TEAP at 20 mVs^{-1}; I: grafting peak; II: polymerization in solution. Inset: Reduction of A-PEG (M_n = 454, 0.5 M): 1) first, 2) second, and 3) third scan.

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[**] The authors are grateful to the Belgian Science Policy for financial support in the framework of the Interuniversity Attraction Poles Programme (PAI V/03). C.J. is a research associate of the "Fonds National de la Recherche Scientifique" (FNRS).

curve a). At the less-cathodic potential (peak I), a species is formed which is responsible for both the initiation of the macromonomer polymerization and the chemisorption of the growing chains,[11] whereas at a more-cathodic potential (Figure 1 A, signal II), the initiating species are also formed by electron transfer from the cathode to the monomer; however, the chains now propagate in solution and are no longer chemisorbed onto the cathode surface. Whenever the cathodic potential equals the maximum of peak I, the electrode is passivated within a few seconds as the result of the grafting of an insulating polyacrylate film. This polymer grafting was also confirmed by the extremely low current intensity observed during a second potential scan on the same electrode (curve 2 in the inset of Figure 1 A).

In a second part of the work, the macromonomer concentration was varied and the intensity of the passivation peak I (Ip1) was determined from the voltammograms, as exemplified in Figure 1 for A-PEG-A. As previously reported for ethyl acrylate electrografting,[8] the data summarized in Table 1 confirm that an increase in monomer concentration

Table 1: Current intensity at the potential of peak I in relation to the A-PEG-A concentration.[a]

[A-PEG-A]	0.1 M	0.5 M	1 M
Ip1 for A-PEG-A ($M_n = 700$) [mA]	−1.67	−1.28	−0.70
Ip1 for A-PEG-A ($M_n = 258$) [mA]	−0.66	−0.50	−0.40

[a] In a DMF solution of TEAP (0.05 M) at 20 $mV s^{-1}$.

results in a smaller Ip1 at a constant scan rate (20 $mV s^{-1}$). Indeed, only the polymerization initiation is an electrochemical process, in which only the adsorbed monomer participates. The kinetics of this step are thus weakly dependent on the monomer concentration. In contrast, chain propagation, and thus the increase of the degree of polymerization (DP) of the insulating brushes, is a chemical process which is fast when the monomer concentration is high. Therefore, passivation occurs rapidly when the chain propagation is fast and Ip1 decreases accordingly. In the case of the monoacrylate macromonomer, the voltammetric peaks I and II overlap and the passivation of the electrode is less efficient, as shown by the current intensity, which decreases more slowly when the potential scan is repeated (Figure 1 B, inset). These observations are consistent with a less-effective adsorption of the A-PEG macromonomer at the surface. The overlap of peaks I and II is indeed known to be extensive when the monomer concentration is low.[8] The lower efficiency of the electrografting of A-PEG also has a direct influence on the electrode passivation. As a rule, these electrochemical observations are observed irrespective of the substrate (glassy carbon, stainless steel, and gold).

Grafting of poly(ethylene glycol) was confirmed by ATR-FTIR analysis of the electrode surface after intensive washing with DMF, which is a good solvent for PEG; the three main absorptions at 2861, 1732, and 1106 cm^{-1} are characteristic of the CH_2, C=O, and C–O–C vibrations of poly(A-PEG), respectively (Figure 2). Moreover, a careful analysis of the spectra for all the electrografted films (PEG bands) shows

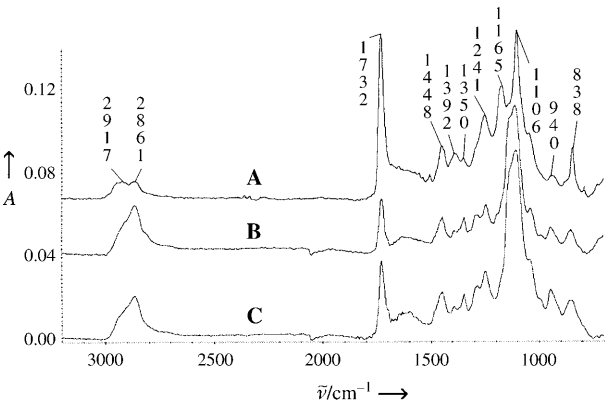

Figure 2. ATR-FTIR spectra for A-PEG-A ($M_n = 258$, 0.5 M) (A), A-PEG ($M_n = 454$, 1 M) (B), and A-PEG-A ($M_n = 700$, 0.1 M) (C) electrografted onto stainless steel.

that the CH_2 scissoring mode at 1448 cm^{-1} and the CH_2 twisting mode at 1241 cm^{-1} dominate the CH_2 wagging mode at 1350 cm^{-1}, which indicates that the PEG side chains are oriented parallel to the surface, as has been reported elsewhere.[6] Figure 3 illustrates this preferential

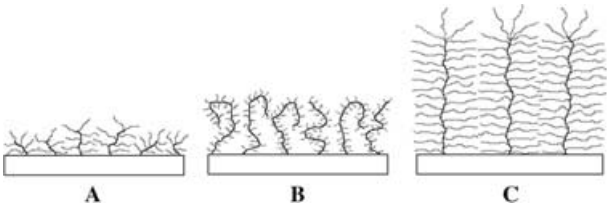

Figure 3. Expected architecture and conformation of poly(A-PEG) (A) and poly(A-PEG-A) (B and C) electrografted from A-PEG ($M_n = 454$, $DP_{EG} = 7$; A), A-PEG-A ($M_n = 258$, $DP_{EG} = 3$; B), and A-PEG-A ($M_n = 700$, $DP_{EG} = 13$; C). PEG side chains are in gray and the polyacrylic backbone in black.

orientation of the PEG teeth of the comb-like architectures. Interestingly, Raman analysis of the diacrylate PEG films showed that the electrografted coatings still contain unreacted acrylic functions. Indeed, although the intensity ratio of the C=C band at 1632 cm^{-1} to the C=O band at 1730 cm^{-1} has decreased, the C=C band does not disappear completely (Figure 4), which could offer a unique opportunity for further functionalization of the coating, for example by the Michael addition of nucleophiles.[15]

The film thickness was measured by ellipsometry at five distinct spots on the surface of films electrografted on stainless steel and glassy carbon (Table 2). The dispersion of the five data points for each film is lower than 10% error, which is a strong indication of the homogeneity of the films. Although the film thickness is systematically smaller when the substrate is stainless steel rather than glassy carbon (the difference being significant only for the A-PEG macromonomer), this characteristic feature depends more on the monomer concentration, as illustrated in Figure 5 for the glassy carbon substrate. The film thickness increases with the macromonomer concentration at a rate which is, however,

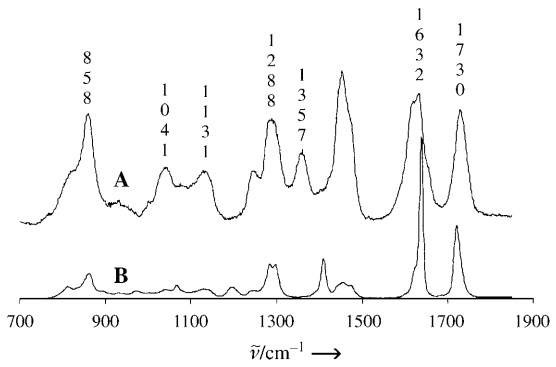

Figure 4. Raman spectra for A-PEG-A ($M_n = 258$, 1 M) electrografted (A) and solvent-cast (B) onto stainless steel.

Table 2: Thickness and contact angles of water (at 3 min) at the surface of PEG electrografted films.

		A-PEG-A ($M_n = 454$)			A-PEG-A ($M_n = 258$)		A-PEG-A ($M_n = 700$)	
		0.1 M	0.5 M	1 M	0.1 M	1 M	0.1 M	1 M
Carbon	Thickness [nm]	13 ± 1	24 ± 3	49 ± 5	14 ± 2	222 ± 20	112 ± 18	475 ± 37
	Contact angle [°]	43 ± 2	31 ± 3	25 ± 3	43 ± 3	48 ± 4	< 10	< 10
Stainless steel	Thickness [nm]	9 ± 1	18 ± 2	39 ± 4	11 ± 4	202 ± 31	98 ± 22	437 ± 39
	Contact angle [°]	45 ± 4	37 ± 4	29 ± 3	42 ± 4	50 ± 3	< 10	< 10

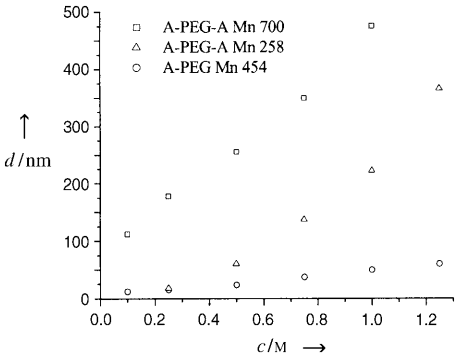

Figure 5. Dependence of the film thickness d on the macromonomer concentration c on polished glassy carbon.

much faster for the bifunctional than for the monofunctional macromonomer of comparable molecular weight. A greater thickness for the films formed by the bifunctional macromonomer was expected because the concentration of the polymerizable acrylic moieties is doubled in this case, thus making the propagation of the tethered chains much faster and, consequently, the DP and thickness greater, as illustrated in Figure 3 (A and B).

In the case of PEG monoacrylate films, a maximum thickness of 50 nm was observed for a coating prepared at high concentration (1M). This thickness is in agreement with the values previously measured for films synthesized starting from a monoacrylic monomer.[10,16] For example, for poly(ethyl acrylate)[10] or poly(acrylonitrile)[16] films, the thickness never exceeds 100 nm for a monomer concentration of 1M. In the case of PEG diacrylate, an increase in macromonomer concentration and molecular weight makes the coating thickness grow very rapidly such that a thickness of up to 500 nm can be reached when a macromonomer of $M_n = 700$ is used at a concentration of 1M.

This drastic evolution of the coating thickness can be explained by the peculiar architecture of the polymer chains that are formed by electrografting, namely a comblike polymer consisting of an acrylic backbone with PEG side chains grafted onto each acrylic unit. The conformation of such poly(acrylic) comblike polymers depends on the length of the macromonomer, i.e., on the size of the PEG side chain. As shown by Fredrickson,[17] the repulsive interaction between the side chains induces the rigidity of the comb polymer: the greater the length of the side chains, the higher the persistence length of the main chain. In other words, we can assume that when the M_n of the PEG side chains is increased, the stretching of the tethered chains is responsible for the rapid increase of the coating thickness (B and C in Figure 3). It should be emphasized that Figure 3 (B and C) does not consider the possible reaction of the second acrylic function of the macromonomer. However, this reaction most probably occurs and leads to additional branching of the brushes, cross-linking, loops, and/or network formation. All these additional reactions are also expected to increase the film thickness. These reactions have been neglected in Figure 3 for reasons of clarity as the extent of these reactions is limited, as already mentioned in the description of the Raman spectrum (Figure 4).

The water contact angles measured for the different PEG films are in good agreement with the thickness measurements. Table 2 shows that in the case of PEG diacrylate (thick films) the contact angles do not depend on the macromonomer concentration. These films fully cover the underlying substrate such that the contact angle only depends on the macromonomer composition and not on the macromonomer concentration, i.e., for A-PEG-A ($M_n = 258$), the DP of PEG is only 3, which makes the hydrophobic acrylic content quite high, and the contact angle levels at around 45°. When the M_n of the macromonomer is increased (from $M_n = 258$ to $M_n = 700$) very hydrophilic films are obtained (< 10°). As far as the monoacrylate PEG is concerned, the film thickness is low and concentration-dependent. As observed in Table 2, the contact angle decreases with increasing film thickness from 45 to 25°. When the films prepared with this hydrophilic macromonomer (A-PEG) are thin, the underlying hydrophobic substrate has an effect on the contact angle of water (Table 2), probably due to a weak tendency of these hydrophilic chains to spread out on the hydrophobic surface. For the thicker films, the contact angle reaches 25°, which is a value already lower than that usually reported for PEG monolayers 5–6 nm thick (30–35°).

The comblike architecture of the films obtained in this one-step process would be particularly interesting in cases where protein adsorption has to be avoided. Indeed, branched PEG chains, like PEG stars, have recently been shown to be more efficient at preventing unspecific protein adsorption

than linear chains[3a,18] because they combine both high grafting density and high PEG segment mobility.

Based on contact angle and ellipsometry data, films of monoacrylate PEG prepared at a 1M concentration combine high hydrophilicity and low thickness, which are two key criteria for reliable measurements of protein adsorption by surface plasmon resonance (SPR). The poly(A-PEG-A) films were disregarded because of the possible Michael addition of the proteins to unreacted acrylate groups. Liedberg and coworkers, the pioneers in the SPR field, have demonstrated the utility of this practical and commonly used technique for the characterization of bio-interfaces.[19] SPR substrates (glass slides coated on one side by a thin gold film) were electrografted by ethyl acrylate (0.1M; reference sample) and PEG monoacrylate (1M) in a DMF solution containing TEAP (5×10^{-2}M). The sensorgrams showing the proteins' adsorption with time were compared for the hydrophobic poly(ethyl acrylate) (PEA) and hydrophilic poly(A-PEG) coatings. Figure 6 illustrates the adsorption of bovine serum albumin

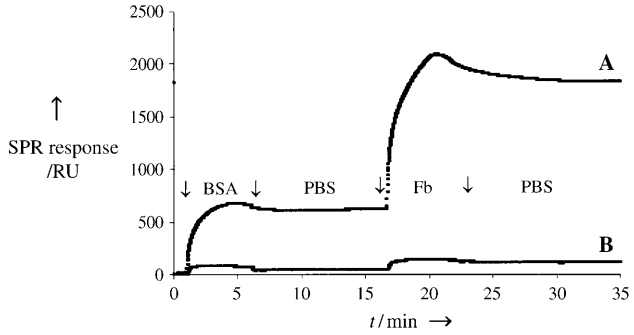

Figure 6. SPR adsorption profiles for bovine serum albumin (BSA; 0.5 mg mL^{-1}) and fibrinogen (Fb; 0.1 mg mL^{-1}) in a phosphate buffer (PBS; 10 mM, pH 7.4) on: A) electrografted PEA (0.1 M)[10] and B) electrografted A-PEG (M_n = 454, 1 M). Flow rate: 20 μL min^{-1}. Substrate: gold.

(BSA) (first injection) and fibrinogen (Fb) (second injection) on the two types of electrografted films. Curve B in Figure 6 is convincing evidence that electrografted PEG is very efficient in preventing protein adsorption. The electrografted PEG film decreases both the BSA and fibrinogen adsorption relative to the grafted PEA film: BSA adsorption is reduced by 93% (±3%), whereas fibrinogen adsorption is reduced by 92% (±2%). These results are highly reproducible, further indicating the homogeneous and reproducible coating process when using electrografting.

The one-step electrografting of PEG macromonomers is a technique that is very well suited for coating conductive substrates homogeneously with a strongly adhering hydrophilic coating. These electrografted chains, which have a comb-like architecture, have proven to be very efficient in preventing the adsorption of proteins. This very simple technique is thus highly promising in the field of coatings of biomedical devices and biosensors. In addition, the unreacted acrylic functions remaining in the PEG diacrylate films offer the opportunity of straightforward derivatization, for instance by Michael addition, with a variety of molecules of interest such as proteins. This opens the way to the easy elaboration of surfaces for the specific recognition of proteins or with sensor capabilities.

Experimental Section

PEG macromonomers (M_n = 258, 454, and 700; Aldrich) were dried by three azeotropic distillations with toluene. DMF and tetraethylammonium perchlorate (TEAP) were dried before use.[10] Electrochemical experiments were carried out in a three-compartment cell equipped with a Pt pseudo-reference electrode, a Pt counter-electrode, and a working electrode (1 cm^2) with a PAR EG&G potentiostat (Model 273A). All electrochemical experiments were carried out in a glovebox under a dry and inert atmosphere (N_2). All the electrografted films of PEG diacrylate and monoacrylate were prepared by, respectively, two or three scans of the potential between the initial potential of the open circuit and the potential at the top of the first peak, where it is kept for 5 s. The electrografted PEA films were prepared in the same way from ethyl acrylate (EA; 0.1M) solution in DMF/5×10^{-2}M TEAP, as reported previously.[10] This EA concentration was chosen such that the film thickness was similar to that prepared with the A-PEG macromonomer (1M). ATR-FTIR, Raman, and contact-angle measurements were carried out on the modified surface after rinsing with DMF to remove the monomer and the nongrafted polymer, and in acetonitrile to dry the coating. Ellipsometry was carried out with an Ellisel ellipsometer (Jobin-Yvon/Sofie instrument) operating at a wavelength of 632.8 nm. The film thickness was measured at 70.67° (incidence angle with respect to the normal of the substrate). At least five measurements were taken for each sample at different locations; the error in the thickness was about 10%. The refractive index for PEG was 1.506.[20] For thicker films (< 200 nm), the thickness was measured with a Sentech, SE 800 spectroscopic ellipsometer. SPR experiments were performed with a Biacore-X instrument equipped with an internal injection system (Hamilton syringe, 200 μL). The experiments were carried out with a phosphate buffer (10 mM, pH 7.4) at a flow rate of 20 μL min^{-1}. The concentration of the protein solutions was 0.5 mg mL^{-1} for bovine serum albumin (Sigma, Fraction V, 95%) and 0.1 mg mL^{-1} for fibrinogen (bovine, Sigma, Fraction I, 86%) rather than physiological protein concentrations. These low protein concentrations were chosen in order to avoid poisoning of the SPR signal by protein exchange phenomena. However, similar results were obtained for higher concentrations of fibrinogen (1 mg mL^{-1}). After each protein injection the SPR substrate was washed with a phosphate buffer (PBS). The percentage of absorbed protein was calculated based on the plateau levels after the PBS cleaning.

Received: February 21, 2005
Revised: June 13, 2005
Published online: July 29, 2005

Keywords: adsorption · electrochemistry · polymers · proteins · thin films

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