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End-grafting of (co)polyglutamates and (co)polyaspartates onto Si–OH containing surfaces

Michiel L. C. M. Oosterling, Edwin Willems and Arend Jan Schouten*
Laboratory of Polymer Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
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(Co)polyglutamates and (co)polyaspartates were grafted onto microparticulate silica and flat Si–OH containing surfaces, by initiating the *N*-carboxyl anhydrides of the corresponding α-amino acids with an immobilized primary amine. The copolymers were prepared by polymerization of mixtures of *N*-carboxy anhydrides. In the case of microparticulate silica, all the available monomer was converted into grafted polymer, whereas in the case of flat surfaces, non-grafted material was formed as well. The grafted products were identified with infra-red spectroscopy and X-ray photoelectron spectroscopy.

(Keywords: grafting; polypeptides; immobilized initiator)

INTRODUCTION

Polymer brushes of end-grafted chains are expected to have special properties due to their attachment to a surface. The conformations of the end-grafted chains are influenced by the presence of the surface, and at a sufficiently high surface coverage by the other grafted chains too. De Gennes presented a polymer segment density distribution of end-grafted polymer chains in both good and bad solvents using scaling laws[1], with perfectly flexible chains and ignoring interchain interactions and surface adsorption. Later models became more sophisticated as other authors used the self-consistent field theory to describe end-grafted polymer layers[2–16]. The effects of solvent quality[1,2,6,10], polydispersity[3], charged chains[5], compositional fluctuations[11] and surface shape[15,16] have been calculated and described.

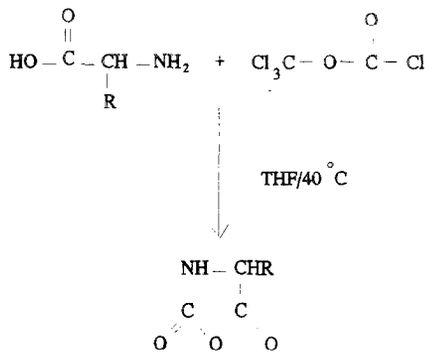
End-grafted layers can be made in various ways. One method is the reaction of polymer end-groups with suitable groups attached to the surface. Enriquez *et al.*[17] used poly(γ-benzyl L-glutamate) (PBLG) end-capped with a disulfide group for the self-assembly on gold substrates, resulting in end-grafted PBLG. Kurita *et al.*[18] and Tsubokawa *et al.*[19] started the poly(γ-methyl L-glutamate) (PMLG) polymerization at the surface of water-soluble chitin and carbon black with surface amine groups respectively.

High-molecular-weight polypeptides can be obtained by initiating the *N*-carboxy anhydrides (NCA) of α-amino acids, first described by Leuchs in 1906[20–22], and also known as Leuchs anhydrides, with tertiary amines. The usual method to obtain these *N*-carboxy anhydrides is the phosgenation of free amino acids[23–29] (*Scheme 1*). A large number of different *N*-carboxy anhydrides have been prepared., The polymerization mechanism has been investigated first by Bamford *et al.*[30–32]. In this mechanism, initiation takes place by deprotonation of the *N*-carboxy anhydride to give an activated monomer.

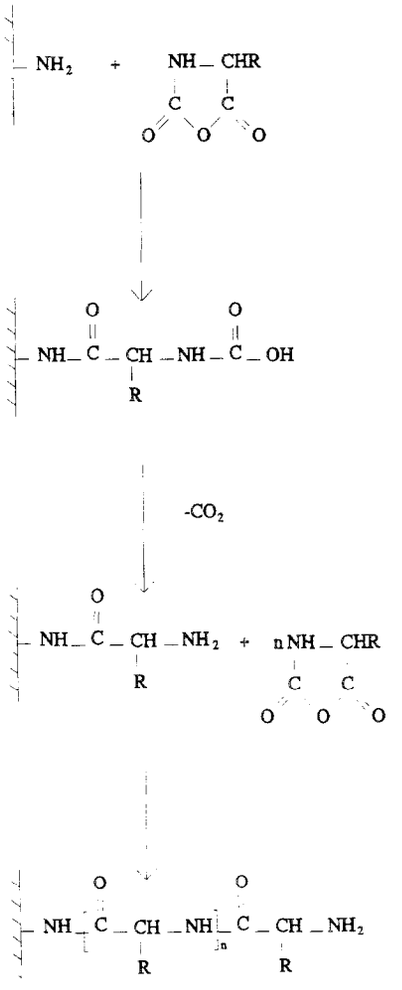
For grafting reactions starting at a surface, it is necessary that the initiator remains attached to the polymer. This can be achieved by starting the polymerization with a primary amine, in which case the polymerization mechanism is different. The amine reacts with the NCA by nucleophilic attack on the C5 atom. After ring opening and splitting off CO_2, an amine is formed again, which can be used to react with the next NCA. This 'amine mechanism' was investigated by Wessely *et al.*[33–38] and Waley *et al.*[39] (*Scheme 2*). A problem of polymerization according to this mechanism is the occurrence of termination steps. In the case of polyglutamates, the most likely termination step is the cyclization of the chain end, by reaction of the amine of the last monomeric unit with the carbonyl group of the ester of that same unit. Another possible termination reaction mechanism is given by Kopple[40,41], Seeney and Harwood[42] and Kricheldorf[43]. This concerns nucleophilic attack on the C-2, leading to an inactive chain end. In particular, increasing concentration and basicity of the primary amine favour these reactions. The formation of β-sheets may cause physical death to the growing chain of non-α-helix-forming polypeptides. Because of these termination steps the molecular weights of the polypeptides will remain relatively low ($DP_n \leqslant 150$).

Usually, copolyglutamates or copolyaspartates are made by transesterification of a preformed polymer with short side chains, for example poly(γ-methyl L-glutamate)[44–53]. However, we chose a direct copolymerization because transesterification leads to a decrease of the degree of polymerization as a result of scission of the main-chain amide bonds. The required monomers are

* To whom correspondence should be addressed



Scheme 1 Synthesis of an *N*-carboxy anhydride by phosgenation of an α-amino acid



Scheme 2 Initiation of an *N*-carboxy anhydride polymerization by an immobilized primary amine

not always commercially available, and were synthesized by a method described by Van Heeswijk *et al.*[54]. The α-amino acid side of L-glutamic acid or L-aspartic acid was protected by copper(II), after which esterification of the side-chain carboxylic group was carried out with an alkyl bromide. The obtained α-amino acids with different side groups could react with diphosgene to give the *N*-carboxy anhydride monomers.

The aim of this work was to study the grafting of copolyglutamates and copolyaspartates onto microparticulate silica and Si–OH-containing flat surfaces. We used the same strategy as Dietz *et al.*[55], who grafted poly(L-alanine) and poly(L-leucine) onto silicon dioxide. In our research the amino groups on the surface were introduced by using an amino-functional coupling agent, γ-aminopropyltriethoxysilane (γ-APS).

EXPERIMENTAL

Silica (Aerosil A200V, Degussa; average particle diameter 12 nm, specific surface area 200 $m^2 g^{-1}$) was dried at 120°C under vacuum conditions for two days. Glass microscope slides and silicon wafers (Topsil, 0.9 mm thickness, both sides polished) were cleaned by washing with a concentrated sodium hydroxide solution and thoroughly rinsing with water (Milli-Q quality, six times). Then the slides were treated with phosphoric acid, and again rinsed with water (six times) and isopropyl alcohol, after which they were dried under vacuum conditions at 120°C. Toluene and tetrahydrofuran (THF) were distilled from sodium wire under a nitrogen atmosphere. All other reagents and solvents were used without further purification unless stated otherwise.

Characterization

*FT*i.r. spectra were recorded on a Bruker IFS 88 or a Mattson Galaxy 6020 spectrophotometer. Monomers, copolymer composition and the amount of polymer per gram of silica were characterized by elemental analysis. ^{1}H n.m.r. spectra were recorded on a Varian VRX300.

Functionalization of silica and the glass slides and silicon wafers

γ-Aminopropyltriethoxysilane (γ-APS) was added (1.5 g per 100 ml toluene) to a 5 wt% silica suspension in toluene and the reaction mixture was refluxed for one hour. Then half the toluene was distilled off, and the product was isolated by centrifugation. The modified silica was washed with toluene (twice) and ether (twice) and dried at 100°C under vacuum conditions for two days. The product was characterized by *FT*i.r. and elemental analysis. The cleaned glass slides and silicon wafers were functionalized with γ-APS in the vapour phase, a method that is supposed to produce a uniform monomolecular silane layer at the surface[56]. The slides were placed above a refluxing solution of 10% of γ-APS in toluene. After a reaction time of 16 h the slides were washed successively with toluene and diethyl ether, and dried under vacuum conditions at 120°C for two days. The immobilization of the amino functionality was shown by X-ray photoelectron spectroscopy.

Synthesis of ω-alkyl L-glutamate and aspartate

ω-Alkyl L-glutamates were synthesized by a method described by Van Heeswijk *et al.*[54]. A copper salt complex was prepared by reaction of L-glutamic acid and copper(II) acetate in water. The complex salt was washed with water, ethanol and ether, and dried at 50°C under vacuum conditions. The L-glutamic acid complex salt (8.5 mmol) and L-glutamic acid (17 mmol) were suspended in dimethylformamide (15 ml) and water (2.5 ml), and *N,N,N′,N′*-tetramethylguanidine (34 mmol) was slowly added in 30 min. After dissolution of all solids (1–3 h) dimethylformamide was added (12 ml), and all at

once the alkyl halide was added to the dark blue solution of the complex salt (36 mmol, 12 g octadecyl bromide, 11 g hexadecyl bromide, or 8 g *p*-nitrobenzyl bromide). The reaction mixture was allowed to react for 24 h at 40°C, after which the obtained slurry was stirred with acetone until a fine blue precipitate was formed. After filtration and washing with acetone, the solids were suspended in water, and the product was isolated by filtration and washed with water. To remove the copper the products were vigorously stirred several times in a supersaturated aqueous solution of the disodium salt of ethylenediaminetetraacetic acid (EDTA) (Titriplex III, 5 g per 25 ml). In order to improve results, a little acetone was added (5 ml) and the suspension was slightly warmed ($T = 40$°C). Then the mixture was cooled, filtered and washed with cold water, and the product was dried under vacuum conditions. ω-Alkyl esters of L-aspartic acid were synthesized in a similar way. The products were characterized with *FT*i.r. and elemental analysis.

Synthesis of N-*carboxy anhydride (NCA)*

The *N*-carboxy anhydrides of the ω-alkyl L-glutamates and L-aspartates were synthesized using diphosgene[57] in tetrahydrofuran (THF) or tetrahydrofuran/dichloromethane (1/1 v/v) at 40°C. First, 1.5 ml of diphosgene was added to 100 ml of THF at 60°C. After 4 h the temperature was lowered to 40°C and the α-amino acid was added. After all the α-amino acid had been converted into its NCA, 85 ml of THF was carefully distilled off, and 20 ml of n-hexane was added, after which the reaction mixture was cooled until the NCA crystallizes. The NCAs were isolated by filtration, and were recrystallized at least four times. Just before polymerization, the monomers were recrystallized twice again. The monomers were characterized with *FT*i.r., ^{1}H n.m.r. after the first recrystallization cycle, and again with *FT*i.r. just before polymerization.

Graft polymerization

All graft polymerizations onto silica were carried out in THF at 40°C. The monomers were dissolved in THF, and then added to a silica suspension in THF. For the grafted copolymers the monomers were added in the desired ratio. After polymerization the reaction product was isolated by centrifugation and washed several times with dichloroacetic acid, a good solvent for polyglutamates and polyaspartates because it breaks up the hydrogen bonding that occurs in the possible secondary structures of these polymers, sometimes making them insoluble in other solvents like chloroform or tetrahydrofuran. After the last extraction the product was suspended in chloroform, precipitated into petroleum ether 40–60, filtered off and dried under vacuum conditions. Homopolymers that eventually might have been formed were precipitated from chloroform into petroleum ether 40–60 and dried under vacuum conditions. Products grafted onto Aerosil A200V were characterized with the DRIFT (diffuse reflectance infra-red Fourier transform) spectroscopic technique and elemental analysis. The other products were characterized by transmission *FT*i.r., elemental analysis and ^{1}H n.m.r. Graft polymerizations onto flat surfaces were carried out in tetrahydrofuran (THF) or dimethylformamide (DMF) at 40°C. The monomers were dissolved and added to the glass or silicon slide through a filter. If copolymers were to be grafted, the monomers were mixed in the desired ratio and added to the glass. After polymerization the reaction product was washed several times with dichloroacetic acid (DCA). Then the product was rinsed with chloroform and petroleum ether 40–60 and dried under vacuum conditions. If homopolymers were formed, they were precipitated in methanol, isolated by filtration and dried under vacuum conditions. The products were characterized by transmission (silicon wafers) and external reflection (both glass slides and silicon wafers) *FT*i.r. and by X-ray photoelectron spectroscopy.

RESULTS AND DISCUSSION

Functionalization of silica and the glass slides and silicon wafers

Aerosil could be functionalized with 0.45×10^{-3} mol amino groups per gram of Aerosil (elemental analysis). Assuming that all γ-APS molecules react with two surface hydroxyl groups[58], this meant that 90% of the hydroxyl groups of Aerosil ($10\,\text{min}^{-3}$ mol per gram[59] were converted into amino groups. The remaining hydroxyl groups were supposed to have no effect on the grafting reactions. Therefore, unlike the functionalized Aerosil for anionic polymerization[60], no hexamethyldisilazane was used to block these groups. A lot of attention was paid to the cleaning of the glass slides and especially the silicon wafers. Cleaning with chromic acid or fluoric acid (silicon wafers) turned out to be not sufficient. A vapour-phase coupling of γ-APS after cleaning the substrates this way resulted in multilayers of coupling agent, sometimes even visible as a haze over the glass slides. A.f.m. (atomic force microscopy) measurements confirmed that the treated surface was very rough, and showed a profile that was far greater than the dimensions of a single coupling agent molecule (*Figure 1*). Cleaning with sodium hydroxide solution and phosphoric acid improved these results considerably, and coupling of the γ-APS resulted in clear glass slides and a smoother surface. The immobilization of the amine groups can be seen in the X-ray photoelectron spectra showing a peak at 399 eV, originating from the nitrogen atom (*Figure 2*).

Synthesis of ω-alkyl L-glutamate and aspartate

The ω-alkyl esters of L-glutamic acid and L-aspartic acid were synthesized using stearyl bromide, cetyl bromide or *p*-nitrobenzyl bromide as an alkyl group.

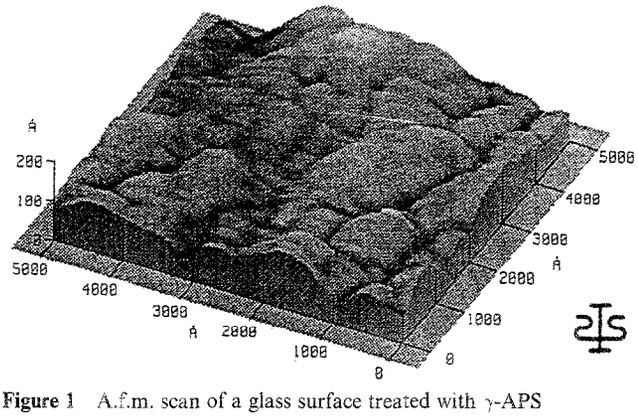


Figure 1 A.f.m. scan of a glass surface treated with γ-APS

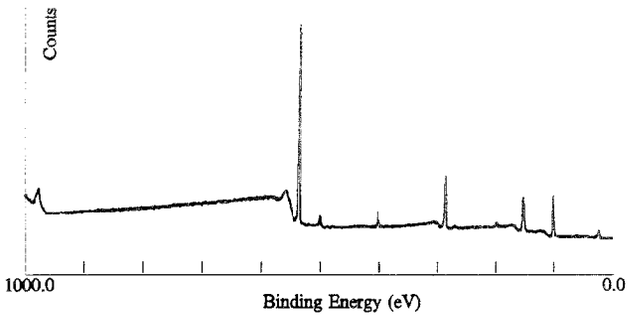


Figure 2 X.p.s. spectrum of an immobilized primary amine at the surface of a glass slide

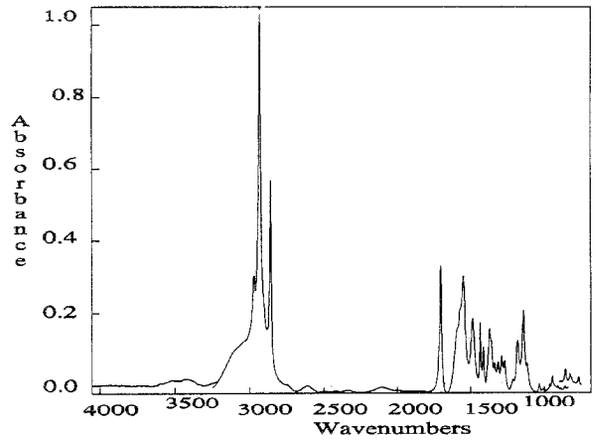
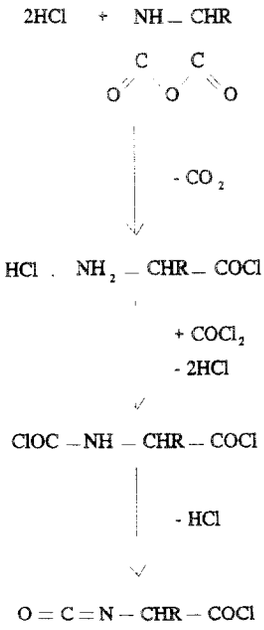


Figure 3 *FT* i.r. spectrum of stearyl L-glutamate (SLG) synthesized by the method of Van Heeswijk[54]

The copper complexes of both amino acids were characterized with elemental analysis. Experiment: Glu–Cu, C 24.4%, H 4.5%, N 5.7%, Cu 25.9%; Asp–Cu, C 20.6%, H 3.9%, N 6.0%, Cu 27.3%. Calculated: Glu–Cu, C 24.5%, H 4.5%, N 5.7%, O 39.3%, Cu 26.0%; Asp–Cu, C 20.8%, H 3.9%, N 6.1%, O 41.6%, Cu 27.6%. The reaction of the complexes with the alkyl bromides in DMF/water lasted for at least one day at 40°C. These reaction times and temperature were necessary to obtain favourable results. Removing the copper from the complex proved to be very difficult and the reaction products had to be washed many times with a supersaturated EDTA disodium salt solution at slightly elevated temperatures (40°C) to remove all the copper. The *FT*i.r. spectra (*Figure 3*) showed the appearance of the ester peak at about 1730 cm^{-1}, and after removing the copper a broad adsorption band between 1550 and 1680 cm^{-1} originating from the α-amino acid. Elemental analysis showed that small traces of copper were still present in the α-amino acids, even after washing repeatedly with EDTA. Experiment: SLG (γ-stearyl L-glutamate), C 69.0%, H 11.4%, N 3.5%, Cu 0.09%; SLA (β-stearyl L-aspartate), C 68.5%, H 11.3%, N 3.6%, Cu 0.11%. Calculated: SLG, C 69.2%, H 11.3%, N 3.5%, O 16.0%; SLA, C 68.6%, H 11.2%, N 3.6%, O 16.6%.

Synthesis of N-*carboxy anhydride*

The *N*-carboxy anhydrides were readily prepared from all the α-amino acids, using excess α-amino acid to avoid side reaction (*Scheme 3*). In the *FT*i.r. spectra (*Figure 4*) one can note the absence of the α-amino acid adsorption,



Scheme 3 Possible side reaction at the phosgenation of an α-amino acid

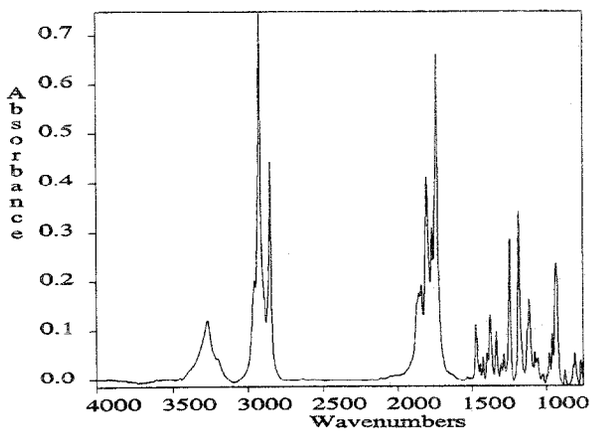


Figure 4 *FT*i.r. spectrum of the *N*-carboxy anhydride of stearyl L-glutamate

and the anhydride peaks at 1770, 1800, 1830 and 1855 cm^{-1}. The peaks at 1800 and 1835 cm^{-1} are caused by the five membered ring acid anhydrides, whereas the two other peaks might be ascribed to a linear acid anhydride[61]. Elemental analysis confirmed the presence of such linear acid anhydrides. In addition it revealed the presence of some copper (0.06%) and chlorine (0.1%) in the NCAs with long alkyl side chains. The linear acid anhydrides do not necessarily have a negative effect on the polymerization, but the copper and chlorine might interfere severely because of the formation of complexes with the immobilized amino groups, making them inactive as an initiator. Therefore, special care had to be taken to remove all the copper from the α-amino acids. The α-amino acids were cleaned more often with an EDTA solution in water. Especially at higher concentrations hydrochloric acid, originating from the reaction of an α-amino acid with diphosgene, can cause side reactions, in combination with unreacted phosgene. Therefore, the NCAs were now prepared in a solvent mixture of tetrahydrofuran and dichloromethane (1/1 v/v). By

using dichloromethane, which is a bad solvent for hydrochloric acid, and an excess of α-amino acid, this side reaction could be suppressed. The monomers were characterized with ^{1}H n.m.r. as well (*Figure 5*).

Grafting onto microparticulate silica

Most graft polymerizations were carried out in tetrahydrofuran at 40°C under a nitrogen atmosphere. Sometimes dimethylformamide, dioxane or dichloromethane were used, but the change in solvent had no effect on the results of grafting polymerization. The monomer was dissolved in the solvent (0.5 mmol l^{-1}), and added to a suspension of Aerosil A200V (1 g/10 ml). If sufficient care is taken to purify and dry all reagents, hardly any free polymer is formed. The products were washed several times with dichloroacetic acid (DCA) to remove all polymer that is not covalently bound but adsorbed to the surface, after which they were isolated by centrifugation. Particularly at higher polymer coverage the silica suspended very well in solvents like THF, DCA or chloroform; therefore centrifugation had to be carried out at high speeds and for longer periods of time because otherwise the grafted silica remained suspended. Washing experiments had shown that polypeptides adsorbed to the surface can be removed beyond detection by washing with DCA. After washing with DCA the Aerosil A200V with the grafted polymer was suspended in chloroform, and precipitated in petroleum ether 40–60. The product was isolated by filtration, and was dried at 50°C under vacuum conditions. The amount of polymer per gram glass and copolymer composition were determined by elemental analysis. *Figure 6* shows the *FT*i.r. spectrum of poly(γ-benzyl L-glutamate) (PBLG) grafted onto Aerosil A200V with a loading of 2.35 g of polymer per gram glass (elemental analysis). The amide I and amide II bands of the peptide bonds (1654 and 1548 cm^{-1} respectively) were easily detected. In order to graft copolymers the monomers were mixed in the desired ratio and dissolved in the THF. Grafted copolymers poly(γ-methyl L-glutamate-*co*-γ-stearyl L-glutamate) (P(MLG-SLG)) and poly(β-benzyl L-aspartate-*co*-β-stearyl L-aspartate) (P(BLA-SLA)) were synthesized this way. Again the polymerization led to an almost complete conversion into grafted polymer and very little free polymer was formed. By taking samples during the polymerization we found that at the beginning of the polymerization less monomer with long side chains was incorporated into the polymer than could be expected from the initial ratio of the two monomers used (elemental analysis, *FT*i.r.; *Figure 7*),

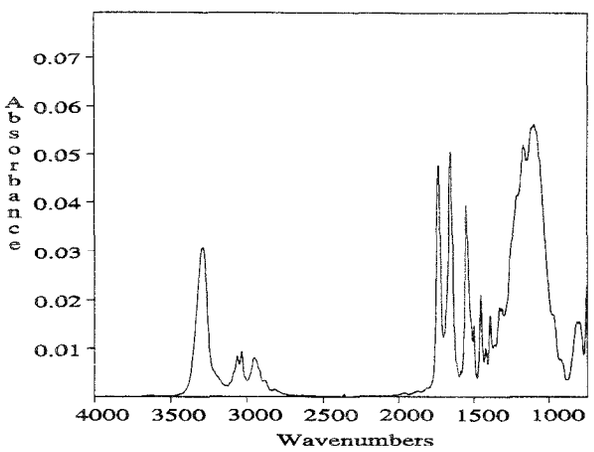


Figure 6 *FT*i.r. spectrum of poly(γ-benzyl L-glutamate) grafted onto microparticulate silica. Loading (amount of polymer per gram glass) = 2.35 g/g

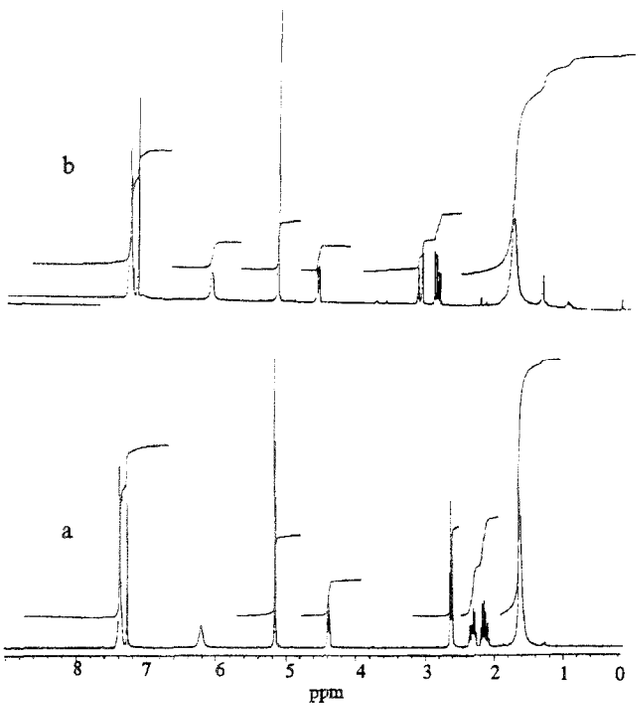


Figure 5 ^{1}H n.m.r. spectra of (a) benzyl L-glutamate NCA and (b) benzyl L-aspartate NCA

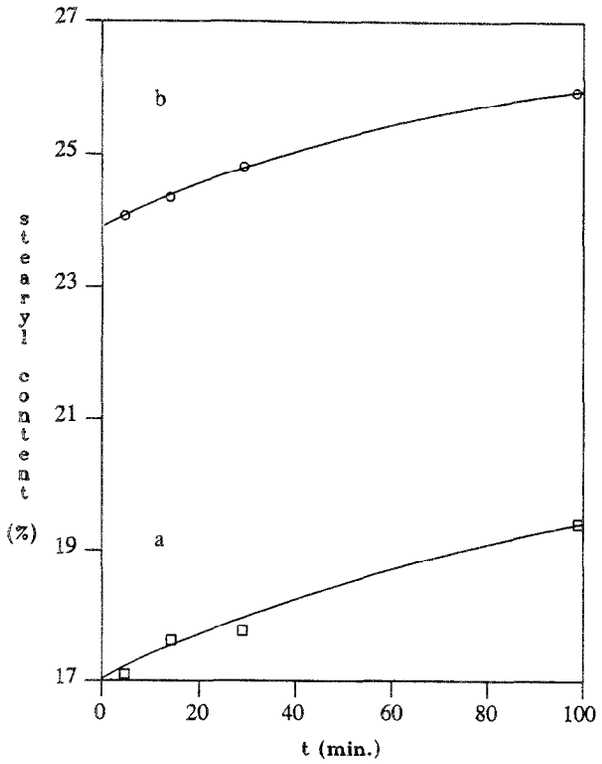


Figure 7 Content of repeat units with long alkyl side chains in (a) copolyglutamate (initial stearyl monomer content = 20%) and (b) copolyaspartate (initial stearyl monomer content = 30%)

meaning that the monomers were not equally reactive, which may be caused by changing the size of the side group, which in turn can have an effect on the reactivity of the C-5 atom, probably because of steric reasons[62], or by the existence of linear acid anhydrides. Polymerization was fast in all cases. It turned out that after about half an hour 90% of the total amount of monomer was polymerized. Attempts to dissolve the Aerosil A200V with a 20% hydrofluoric acid solution, as can be done with grafted methyl methacrylate (MMA)[63], were not successful, and did not result in a soluble product usable for g.p.c. measurements. Because the polymerization mechanism has a large effect on the resulting molecular weight of the polymers, and is strongly dependent on the type of initiator, it is unlikely that the free polymer that is formed has a similar molecular weight as the grafted polymer.

Grafting onto flat surfaces

The graft polymerizations onto flat surfaces were performed in THF at 40°C under a nitrogen atmosphere. The monomers were mixed in the desired ratio, dissolved (0.5 mmol l^{-1}) and added to the glass slides or silicon wafers. Sometimes insoluble products were formed in the monomer solution (α-amino acids or short polypeptide chains), and therefore the solution was added to the substrate through a clean and dry glass filter. After polymerization the slides were washed to remove all non-grafted material. The grafted layers were identified with X-ray photoelectron spectroscopy and *FT*i.r. spectroscopy, using the external reflection technique for glass slides and transmission measurements for the silicon wafers. In *Figure 8* the X.p.s. spectrum of poly(γ-benzyl L-glutamate) grafted onto a glass slide is shown. The amount of carbon (284 eV) has increased whereas the amount of silicon (100 and 149 eV) has decreased compared with the spectrum of a glass slide with just the coupling agent (*Figure 2*). The peak ratios are in good agreement with the expected values of pure poly(γ-benzyl L-glutamate). *Figure 9* shows the external reflection *FT*i.r. spectrum of poly(γ-benzyl L-glutamate) grafted onto a glass slide with the amide I (1653 cm^{-1}), amide II (1548 cm^{-1}) and ester (1735 cm^{-1}) absorption bands of the polypeptide. The grafting of copolyglutamates and copolyaspartates with the γ-stearyl L-glutamate and β-stearyl L-aspartate was less reproducible than the comparable graft polymerizations of the purchased monomers. A closer study of the *N*-carboxy anhydrides of γ-stearyl L-glutamate and β-stearyl L-aspartate with elemental analysis and *FT*i.r. spectroscopy showed the presence of a little copper (0.06%), chlorine (0.1%) and linear anhydrides. The copper can form complexes with the immobilized amine groups. The chlorine probably originates from hydrochloric acid produced during the NCA synthesis, and can form ammonium salts with the amine initiator. This has little effect on the graft copolymerizations onto microparticulate silica because there are enough initiator molecules available. With flat surfaces, however, the number of initiator sites is so small (about 4×10^{-6} mmol per slide, assuming the same surface coverage of coupling agent per square centimetre as with Aerosil A200V: 0.45 mmol per 2000000 cm^2, determined by elemental analysis) that even the smallest traces of these impurities have a large effect on the grafting reactions. Chlorine was detected at the surface of glass slides with X-ray photoelectron spectroscopy after several unsuccessful graft polymerizations (*Figure 10*; chlorine peaks at 200 and 270 eV). By taking special care of monomer preparation and purification, it was possible to graft both poly(γ-methyl L-glutamate-*co*-γ-stearyl L-glutamate) (P(MLG-SLG)) and poly(β-benzyl L-aspartate-*co*-β-stearyl L-aspartate) (P(BLA-SLA)) onto glass slides and silicon wafers, as can be seen from the X-ray photoelectron spectroscopy (*Figure 11*; increased carbon peak at 284 eV and decreased silicon peaks at 100 and 149 eV compared to the spectrum of immobilized coupling agent), and *FT*i.r. spectroscopy (*Figure 12*; amide I at 1653 cm^{-1} (a) and 1667 cm^{-1} (b), amide II at 1548 cm^{-1} (a) and 1555 cm^{-1} (b), and ester at 1740 cm^{-1}

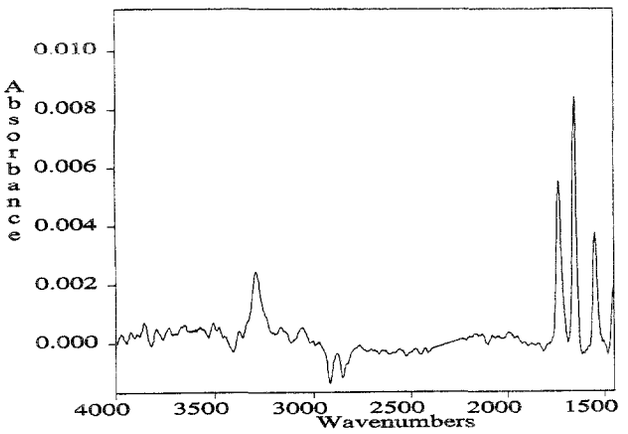


Figure 9 External reflection *FT*i.r. spectrum of poly(γ-benzyl L-glutamate) grafted onto a glass slide

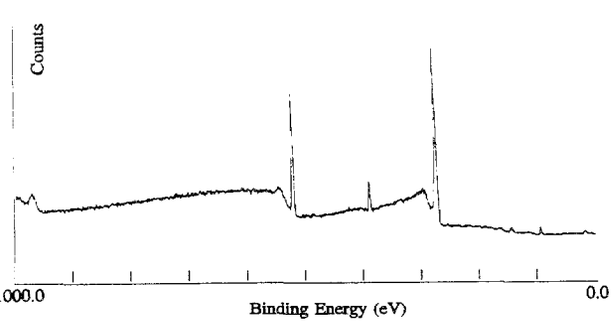


Figure 8 X.p.s. spectrum of poly(γ-benzyl L-glutamate) grafted onto a glass slide

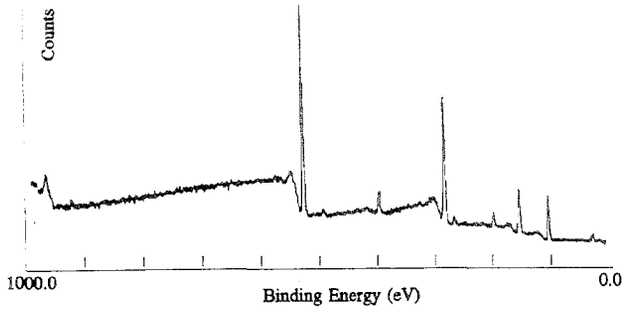


Figure 10 X.p.s. spectrum showing the presence of chlorine (200 and 270 eV) at the surface after an attempted graft polymerization at the surface of a glass slide

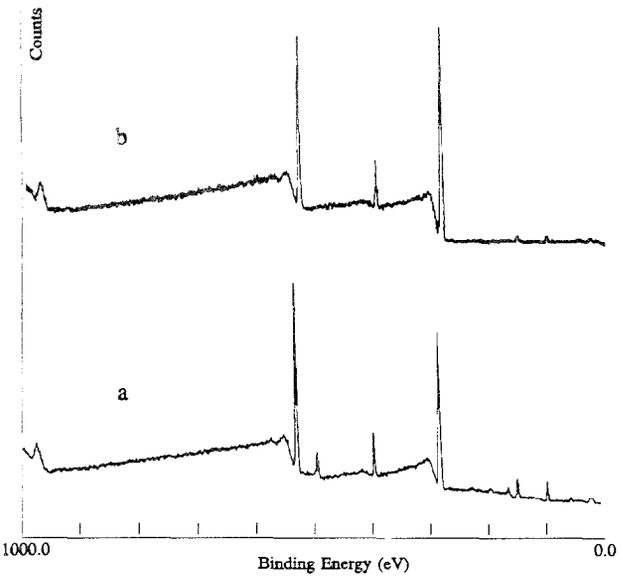


Figure 11 X.p.s. spectra of (a) P(MLG-SLG) at the surface of a silicon wafer and (b) P(BLA-SLA) at the surface of a quartz slide

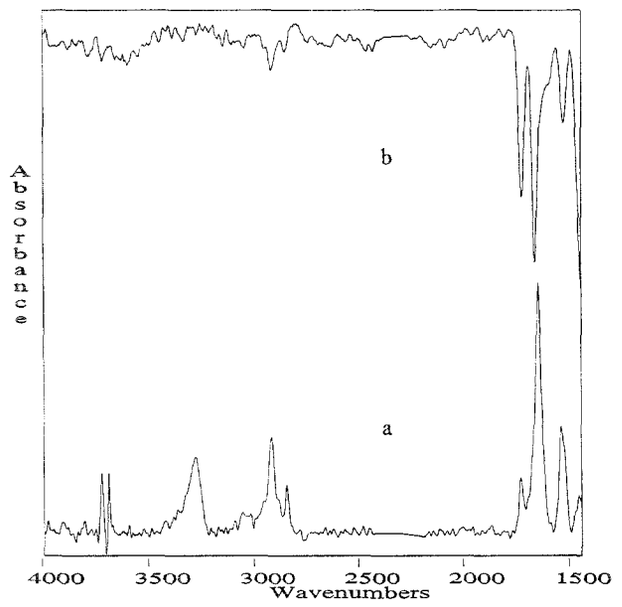


Figure 12 *FT*i.r. spectra of (a) P(BLA-SLA) at the surface of a quartz slide (external reflection) and (b) P(MLG-SLG) at the surface of a silicon wafer (transmission)

(a) and 1742 cm^{-1} (b)). At longer reaction times (more than 2 h) the glass slides and silicon wafers were covered with an opaque polymer film that consisted of the polypeptide in various conformations. These layers could not be removed by washing with chloroform, but washing with dichloroacetic acid resulted either in a complete removal of all polymer beyond detection with X-ray photoelectron spectroscopy or *FT*i.r. spectroscopy, or in a clear film that consisted of polypeptides in just one detectable conformation, indicating that the polypeptide in the other conformation was not covalently attached to the surface. The occurrence of the opaque polymer films was probably caused by the polymerization of the monomers initiated by free initiators, for example traces of water if the solvent was not absolutely dry. This problem could be avoided by taking special care of the solvent purification and reducing the reaction times. The grafting of poly(γ-methyl L-glutamate) (PMLG), poly(γ-benzyl L-glutamate) (PBLG) and poly(β-benzyl L-aspartate) (PBLA) could be done with reasonable reproducibility this way. The thickness of the grafted layers after extensive washing can be estimated using the ester carbonyl band of the side groups at 1735 cm^{-1}. Comparing the adsorption of this band with the adsorption calculated with the computer spectrum simulation program described by Boven *et al.*[64] results in an estimated thickness of about 50–100 Å. With a helix pitch of 5.41 Å, 3.61 monomers per helix run (PBLG) and an expected degree of polymerization of 150[38], the helices are 224 Å long. These results indicate either that the degree of polymerization is lower than expected, which might be caused by the occurrence of linear acid anhydrides in the monomers, or that the helices are not perpendicular to the surface.

CONCLUSIONS

Our studies have shown that it is possible to graft high amounts of polypeptides onto the surface of Aerosil A200V. γ-APS has been immobilized at the surface of the glass in order to be used as an initiator. Adding the *N*-carboxy anhydride monomers has resulted in end-grafted polymers and copolymers. Virtually the total amount of monomer has been converted into grafted polymers, and hardly any free polymer has been formed. The monomers with long alkyl side chains reacted somewhat slower than the others, which was probably caused by steric hindrance. The existence of linear acid anhydrides in the monomers with long side chains can cause termination resulting in a lower overall stearyl content as well. The (co)polymerization of several *N*-carboxy anhydrides onto flat Si–OH containing surfaces was investigated as well. The polymerizations were initiated by an amine group, immobilized at the surface by the vapour-phase coupling of γ-APS. Both (co)polyglutamates and (co)polyaspartates could be covalently attached to the glass slides and silicon wafers using this immobilized amine for an initiator. Good cleaning of both the glass slides and silicon wafers and the monomers proved to be essential for successful polymerizations.

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