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Solvent free vapour phase photografting of acrylamide onto poly(methyl methacrylate)

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Abstract

Poly(methyl methacrylate) (PMMA) sheets were surface grafted with acrylamide under reduced pressure in a solvent free vapour of acrylamide and benzophenone. The grafting was initiated with UV irradiation and no pre-treatment of PMMA by impregnation or sorption of reactants was required. Characterization of grafted samples by ESCA and contact angles showed that the nitrogen content increased with grafting time and temperature. The surface bound amide groups obtained were converted into amine groups by the Hofmann reaction and used in coupling reactions with a *p*-nitrophenylcarbonate terminated PEG and 4,4,4-trifluoro butyraldehyde.

Keywords: Photografting; Functional surface; Hofmann reaction

1. Introduction

In a previous publications [1,2] we have studied the photografting of acrylamide (AAm) or maleic anhydride (MAH) in a solvent free vapour phase onto poly(ethyl terephtalate) (PET). In case of grafting of AAm the amide groups could be transformed into amine groups by the Hofmann reaction [3]. Both the amine and the carboxylic groups obtained from the surface grafting with AAm and MAH, respectively, were used in modification by chemical coupling reactions [1,2]. The fact that the grafting could be performed in absence of solvent and under relatively mild process conditions increases the range of polymeric substrates, which can be used. This may also give a potential for the physical and chemical modification of polymeric materials with surface structures in the micron or nano scale. Particularly for grafting onto solid poly(methyl methacrylate) PMMA the range of organic solvents for monomers and catalysts to which PMMA is inert is limited whether they would occur in the liquid or vapour phase.

PMMA and related polyacrylates are important as implant materials specifically in ophthalmic applications [4] and in artificial joints [5]. Direct sub micron patterning of PMMA by electron beam [6] or in recent years by imprint technology [7] are methods which may be used in the creation of cell interactive surfaces. Due to the present and future use of PMMA in biosciences, there is a current interest for the chemical and physical surface modification of this polymer.

In the present study, we investigated the UV initiated surface grafting of acrylamide (AAm) onto solid PMMA in a solvent free vapour phase at reduced pressure with or without activation by benzophenone (BP). In analogy with the previous study [1], the surface bound amide groups initially obtained were transformed into amine groups by the Hofmann reaction and their functionalities were confirmed by coupling reactions with a fluoro-substituted aliphatic amine, 4,4,4-trifluorobutyraldehyde. Coupling reactions were also made with a telechelic polyethylene glycol derivative, methoxy-poly(ethylene-glycol)-*p*-nitrophenylcarbonate.

2. Experimental

2.1. Materials and reagents

PMMA sheets, thickness 0.5 mm, VOS, Notz AG Switzerland, were cut to 1×2 cm^2 pieces sonicated in 99.5% ethanol and dried under vacuum. Acrylamide (AAm) (Acros

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164855000 electrophoresis grade), benzophenone (BP) (Acros 219680500) sodium hypochlorite solution 1 M in 0.1 M NaOH (BDH 230393L), 4,4,4-trifluorobutyraldehyde (TFBA) (ABCR F07291S), sodium cyanoborohydride, $NaCNBH_3$ (Aldrich 15615-9), methoxy-poly(ethyleneglycol) paranitrophenyl carbonate M_w 5000 (*m*-PEG-npc 5000) (Shearwater), 2,4,6-trinitrobenzenesulfonic acid (TNBS) (=picryl sulfonic acid, Sigma P2297), glycin (Fluka 50046) potassium tetraborate tetrahydrate ($B_4K_2O_7 \cdot 4H_2O$) (Fluka 60542), ethanol 99.5% (Kemetyl) and argon (AGA, OTC50, $O_2 < 3$ ppm) were used as received. All water used unless stated otherwise was deionized and sterilized from a MilliQ Synthesis® unit.

2.1.1. Grafting reactor

The glass reactor was described in detail in a previous publication [1]. In short it had two chambers one reactor chamber fitted with a quartz window and connected to a second chamber containing the grafting reagents. The reactor was connected to a vacuum line and immersed in a thermostated water bath. Grafting was initiated by irradiating UV rays emitted in the range of 300–420 mm from an Osram Vitalux 300 W lamp.

2.2. Characterization

ESCA(XPS) spectra were obtained from two spectrometers. One was a Physical Electronics Quantum 2000 Photoelectron Spectrometer at the Ångström Laboratory in Uppsala with a monochromatic Al K_α X-ray source operated at 21.1 W. With this equipment ESCA spectra were recorded at 45° take off relative to the sample surface at a pass energy of 58.7 eV and at a pressure of 10^{-9} Torr.

The other equipment was a Kratos-HS X-ray photoelectron spectrometer at the Surface Chemistry Institute in Stockholm. In this case, the monochromatic Al K_α X-ray source was operated at 15 kV at a pressure below 10^{-8} Torr and measurements were made at a take off angle of 90°.

Contact angle measurements were made on 10 μl droplets of MilliQ water on the sample surface with a Rame-Hart instrument or with a sessile drop method by Dahlgren et al. [8]. The contact angle data are averages of 4–6 measurements on each sample and were made at a relative humidity of 40–50%. Measurements were made 1 min after the drop application.

2.3. Grafting with acrylamide

The PMMA sheet samples $1 \times 2\ cm^2$ were placed on a perforated teflon disc mounted horizontally 4 cm under the quartz window in the glass reactor. AAm 3.5 g (50 mmole) and BP 0.9 g (5 mmole) were separately ground to fine powders in a mortar mixed together on a vortex shaker and transferred to the reagent chamber of the reactor. The glass vessel was then connected to a vacuum line evacuated and refilled with argon 2 times. At a final vacuum of 3×10^{-4} mbar the reactor was closed and immersed in a thermostated water bath at 23 or 60 °C with the quartz window 3 cm below the water surface. The grafting was initiated by UV irradiation from the Osram Vitalux lamp positioned 12 cm above the quartz window. After predetermined irradiation times the reactor was reconnected to the vacuum line refilled with argon and opened. All samples were thoroughly washed in running warm tap water and in MilliQ water over night including sonication for 20 min. All samples were finally dried under vacuum.

2.4. Hofmann rearrangement

The amide groups bound to the surfaces by the photografting of AAm were converted to primary amine groups by the Hofmann rearrangement following the procedures developed in a previous article on heparinized surfaces [9]. In summary aqueous sodium hypochlorite and sodium hydroxide solutions were cooled separately in an ice bath and mixed at 0 °C with vigorous stirring. Two solutions were tested, one with 0.01 M NaOCl and 0.01 M NaOH (Hofmann I) and the other with 0.02 M NaOCl and 0.045 M NaOH (Hofmann II). After thermostatting to 23 °C AAm grafted PMMA sheets were immersed in the respective solution for 1 h then carefully washed in a warm tap water jet and kept in MilliQ water over night and dried in vacuo.

The concentration of the stock solution of NaOCl (BDH 23039) was controlled by titration: 5 ml of the stock solution was diluted to 300 ml with water. To 50 ml of the diluted solution was added 15 ml 10% aqueous potassium iodide solution, 1 ml 30% HCl and 1 ml of aqueous starch solution as indicator and titration was made with 0.1 N $Na_2S_2O_3$.

2.5. Quantification of amine groups

The amount of primary amine formed on the PMMA surfaces by the Hofmann reaction groups was determined by reacting them with an excess of 2,4,6-trinitrobenzene sulfonic acid (TNBS). Then the excess was determined by measuring the UV absorption of the complex formed between unreacted TNBS and glycine [11]. Each grafted and Hofmann reacted sample was immersed in a solution of 250 μl of 0.004 M TNBS diluted to a final volume of 10 ml with borate buffer, 0.1 M $K_2B_4O_7 \cdot 4H_2O$, and reacted at 37 °C under agitation. The same solution without sample was run in parallel. After 2.5 h, 2 ml of each solution was withdrawn and diluted with 5 ml borate buffer and 250 μl of 0.010 M glycine was added. Also internal standards were prepared for each sample and the reference solution by diluting 2 ml with 5 ml of borate buffer but replacing the glycine with 250 μl of water.

After 45 min at room temperature 5 ml of cold methanol was added and the absorbance of each sample was measured versus its own blank at 346 nm. The concentration of the amino groups was determined from the difference of the

absorbances of the reference and sample using a molar absorption coefficient of 12,400 l/mole cm [11]. For the analysis eight samples of 1×1 cm^2 grafted with AAm at 60 °C for 20 min and from the same batch were used. Six of them were aminized by the Hofmann reaction keeping two unreacted grafted samples as references. Only surfaces aminized by the Hofmann II solution were analysed, by the TNBS method.

2.6. Coupling with poly(ethylene glycol)

Poly(ethylene glycol) (PEG) coupling to the Hofmann reacted samples was made by reacting the amine groups on the substrate surface with 0.5 g (0.1 mmole) *m*-PEG-npc 5000 in 10 ml, 0.05 M NaH_2PO_4 at pH 8.0 for 20 h at room temperature. The samples were washed in MilliQ H_2O over night and sonicated for 5 min and then repeatedly washed and dried under vacuum.

2.7. Coupling with fluorosubstituted aldehyde

Samples grafted with AAm and aminized by the Hofmann reaction were exposed to TFBA in aqueous solution at two different pH and in methanol. TFBA 0.1 g (0.79 mmole) and 0.07 g (1.1 mmole) $NaCNBH_3$ were added to 30 ml of 0.1 M citric acid buffer solution adjusted to pH 3.7 and to 30 ml of 0.1 M NaH_2PO_4 buffer solution adjusted to pH 7.0, all pH adjustments made with 1 M NaOH. The coupling reaction was also performed in methanol with the same concentrations of TFBA and $NaCNBH_3$. All reactions were carried out at room temperature over night under agitation.

The samples from aqueous as well as alcoholic solutions were washed in a hot water jet and methanol followed by sonication in methanol for 10 min then washing in methanol and finally MilliQ H_2O over night. After drying under vacuum the samples were analysed by ESCA.

3. Results and discussion

As discussed in the previous articles [1,2] the amounts of grafting reagents in the vapour phase in the evacuated and sealed reactor are determined by the temperature. Under the conditions used [1] the equilibrium concentrations of AAm were calculated from its vapour pressure data [12] to be 18.7 and 0.5 μM at the water bath temperatures of 60 and 23 °C, respectively. For BP the corresponding concentrations will be about one magnitude lower [13]. At these low monomer concentrations there is a question whether ceiling temperatures are exceeded in which case only monomolecular or very short grafts would be formed. In a recent publication by Yasutake et al. [14] the vapour phase polymerization of methylmethacrylate (MMA) was studied in pure saturated monomer vapour at 40 °C and 106.6 Pa upon free radical initiation by UV on a surface prepared with photoinitiator. After irradiation times of 1–4 h, M_n values in the range 2700–7600 were obtained. From the monomer vapour pressure of 106.6 Pa, a MMA concentration of 41 μM could be calculated which is about twice the AAm concentration at 60 °C. A comparison of polymerization kinetics between MMA and AAm in the vapour phase could not be found in the literature where only solution and bulk polymerization data have been published. Also comparisons of data from polymerizations and copolymerizations of MMA and AAm give ambiguous results [15,16] due to the varying hydrogen bonding and aggregation of AAm in different solvents. One way of making a relative comparison of the propagation between MMA and AAm is to calculate the copolymerization ratios by using the Alfrey Price Q-e scheme [17] which only takes into account the propagating reactants and not the surrounding media. With MMA as M_1 and AAm as M_2 the reactivity ratios are obtained as $r_1=3.2$ and $r_2=0.32$ Although the data obtained by the Q-e scheme are not in general quantitatively correct [17] such a large difference in *r*-values gives a strong implication that MMA will homopropagate to a higher extent than AAm. For the comparison of homopolymerizations between MMA and AAm in their respective pure vapour phases at low concentrations the differences in propagation and monomer concentration would rather give lower M_n for AAm than the 2700–7800 obtained for MMA [14].

For the present purpose, oligomeric or even monomolecular grafting would actually be an advantage as long as sufficient chemical functionality is obtained since very high molecular weight grafts could eventually disturb or even devour a nanopattern.

ESCA and contact angle measurements were the analytical tools used for the characterization of the surfaces obtained. FTIR–ATR techniques were hampered by the strong carbonyl absorbtion in PMMA and the thin surface layer affected by the grafting.

The high resolution ESCA spectra for C1s in Fig. 1 show that the C1s envelope curve for PMMA in Fig. 1(a) changes to a polyacrylamide resembling shape [18] in Fig. 1(b)

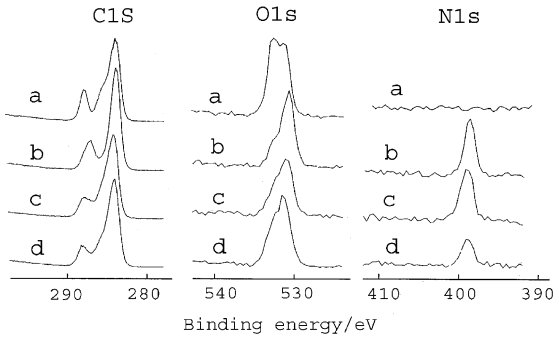

Fig. 1. High resolution ESCA C1s, O1s, N1s spectra measured at ESCA take off angle of 45°. Ungrafted PMMA (a), after AAm/BP grafting for 45 min at 60 °C (b), after Hofmann rearrangement (c), after PEG coupling (d). The nitrogen contents corresponding to the N1s spectra are (a) 0.0 at.%, (b) 10.9 at.%, (c) 12.3 at.%, (d) 5.9 at.%.

where the shoulder from the ether carbon at 287 eV has disappeared. Also the twin peak from ether and carbonyl oxygen in the envelope curve of the O1s spectrum of PMMA in Fig. 1(a) has changed into a single peak for O1s at 531.5 eV in Fig. 1(b) although with a shoulder remaining at about 533 eV from the ether oxygen of the PMMA substrate. Finally the most obvious difference upon grafting is the appearance of a N1s peak at 399 eV in Fig. 1(b). The C1s, O1s and N1s spectra in Fig. 1(c) and (d) will be further commented under the coupling reactions below.

The ESCA data measured at take off angles 45 and 90° for the samples in Fig. 1 are compiled in Table 1. The apparent changes upon grafting when comparing PMMA with PMMA–AAm are—apart from the appearance of nitrogen—a substantial decrease in carbon and oxygen contents as expected. The slightly higher carbon and lower oxygen contents for ungrafted PMMA compared to the theoretical values of 71.4 at.% carbon and 28.6 at.% oxygen (hydrogen excluded in ESCA) may depend on the composition of the commercial PMMA used. For AAm the theoretical contents for ESCA are 60 at.% carbon and 20 at.% each for oxygen and nitrogen. For a surface layer containing on the average 55% of AAm as indicated by the nitrogen content of 11 at.% the theoretical contents would be 65 and 24 at.% for carbon and oxygen, respectively. Also here the slightly higher C/O ratios for PMMA–AAm in Table 1 may be related to the higher C/O contribution from the PMMA substrate. On the whole the similarity of data in Table 1 for PMMA–AAm obtained at 45 and 90° indicates a high graft yield for a grafting time of 45 min. The other data in Table 1 will be commented below under the Hofmann rearrangement and coupling reaction with *m*-PEG-npc.

Fig. 2 shows the nitrogen contents expressed as atomic percent versus grafting time for some different grafting conditions as obtained by ESCA. Although there is a scatter in data the influences of temperature and UV initiation are evident. For grafting at 60 °C the nitrogen content reaches a plateau averaging in the range 10–12 at.% after about 25 min. The corresponding curve when the grafting reactor is thermostatted at 23 °C—hereafter referred to as room temperature grafting—increases slowly to about 4% after 90 min. This difference in grafting rate and yield reflects the difference in vapour pressures and thus the concentrations of the reagents in the gaseous phase at the two temperatures. It is also seen that in grafting experiments without UV

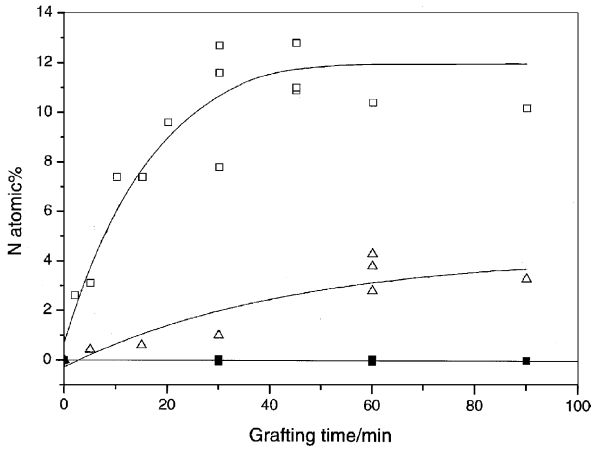

Fig. 2. Atomic% nitrogen from ESCA as function of time for grafting with AAm/BPO at 60 °C with UV (□) and at 60 °C without UV irradiation (■) and at 23 °C with UV(△).

irradiation under otherwise identical conditions at 60 °C no significant amount of nitrogen is obtained by ESCA indicating that surface modification is due to photografting and not adsorption.

In Fig. 3 contact angle measurements are shown where the data points represent individual samples. As expected the increase in nitrogen content in Fig. 2 corresponds to a decrease in contact angle in Fig. 3. For photografting at 60 °C the advancing contact angle decreases from 76° for ungrafted PMMA to about 55° after 10 min and levels out to an average of 40° after 30 min. The receding contact angle indicates almost complete wetting even for the shortest grafting time of 10 min. Also in Fig. 3 the contact angle data are shown for grafting at 60 °C without UV source as well as for grafting with UV at room temperature which confirm the results from the ESCA data for nitrogen in Fig. 2. Without UV irradiation there is no significant change in surface properties under otherwise identical conditions. Room temperature grafting shows a decrease in contact angle which is much smaller in rate and amount compared to grafting at 60 °C. An interesting result is shown for grafting at 60 °C without the hydrogen abstractor BPO. As indicated by the decrease in advancing contact angle grafting still occurs but at a rate and yield similar to those obtained at room temperature. Self initiation in photografting with monomer as single reactant although in solution has been observed for AAm [19] and also for maleic anhydride [20]

Table 1
ESCA data measured at a take off angle of 90° for PMMA and PMMA–AAm after grafting with AAm for 45 min at 60 °C and subsequent Hofmann rearrangement and coupling with *m*-PEG-npc 5000

Samples	ESCA 45°			ESCA 90°		
	C	O	N	C	O	N
PMMA	73.5	26.4	0.1	72.1	27.9	0.0
PMMA–AAm	67.7	21.2	11.0	65.9	23.2	10.9
Hofmann II before coupling	70.4	15.7	14.0	70.3	18.0	12.2
Hofmann II + *m*-PEG-npc 5000	71.2	22.5	5.9	69.0	21.4	9.8

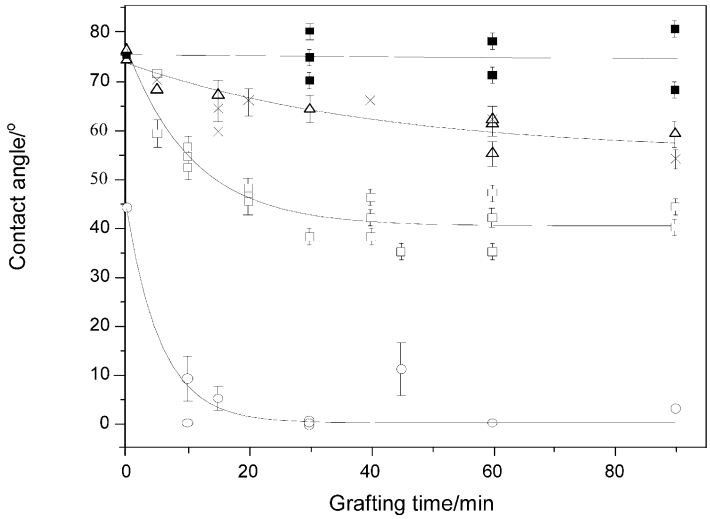

Fig. 3. Advancing contact angle as function of time for grafting with AAm/BPO at 60 °C with UV irradiation (□), at 60 °C without UV (■) and at 23 °C with UV(△). Grafting with UV without BPO at 60 °C (×). Receding contact angles for AAM/BPO grafting at 60 °C with UV irradiation (○).

and styrene [21]. Considering that ESCA measures a surface layer thickness in the order of 50–100 Å and the hydrophilicity from the contact angle measurements the results indicate that a surface layer with a high concentration of AAm or polyacrylamide has been formed on the PMMA substrate. This raises the question of the density of grafted chains and the average chain length obtained. As discussed earlier the system may well be close to or even establish depropagation conditions due to the low monomer concentration in the gaseous phase. If the grafting by AAm in the vapour phase would give oligomeric or even monomolecular grafts a high density of grafting sites is required to obtain the surface modification observed when grafting at 60 °C.

3.1. Functionalization

As reviewed in a previous article [1], biological applications of surfaces depend on surface bound chemical functionalities for the attachment of bio-inert molecules like poly(ethylene glycol) or bioactive agents like heparin and various cell interacting peptides. In case of AAm grafted surfaces, the amide groups initially obtained are not very reactive although direct derivatizations of the amide groups have been made as reviewed earlier [1].

Primary amines are among the most versatile groups for selective and efficient coupling reactions and they may be obtained from amides through the Hofmann rearrangement. Since this reaction has been successfully applied in the conversion of polyacrylamide to polyvinylamine segments in high yields [22,23], we have used this method for the aminization of AAm grafted substrates. In our case the method was modified by making the reaction at room temperature to shorten the reaction time while maintaining a conversion of about 70 mole%. In the present investigation, AAm grafted PMMA samples were aminized by the modified Hofmann reaction following the optimization results obtained earlier [9,10]. In these investigations, the highest conversions were found for two different concentrations and ratios of NaOH and NaClO. Both were tested in coupling reactions with TFBA as Hofmann I and Hofmann II surfaces which were specified in Section 2.

Quantification of the amine functionality obtained was made only for the Hofmann II surfaces by TNBS analysis as described in Section 2. The average concentration and standard deviation of primary amine groups for samples grafted with AAm/BP at 60 °C for 20 min was obtained as $1.8 \pm 0.4 \times 10^{-8}$ mole/cm^2 while no amine groups could be detected in AAm grafted PMMA analysed prior to the Hofmann aminization.

3.2. Coupling reactions

Coupling reactions with amino reactive ligands provide the ultimate tests in the evaluation of the efficiency and versatility of the functionalized surfaces. Two common coupling reactions with amine groups were tested using ligands with *p*-nitrophenol carbonate and aldehyde groups, respectively. In the former case a higher molecular weight ligand in the form of PEG of molecular weight 5000 with a *p*-nitrophenyl carbonate group at one chain end and an unreactive methoxy group at the other (*m*-PEG-npc 5000) was tested for attachment to the aminized PMMA substrates. This experiment represents the coupling of a biologically useful ligand in the aqueous phase. The *p*-nitro carbonate end groups react with unprotonated amines to give stable urea links [24–26] and the reaction proceeds within a couple of hours at room temperature. The reaction is often carried out over night when coupling larger molecules due to their low concentration of chain ends. Since hydrolysis of the *p*-nitrocarbonate groups increases

rapidly above pH 9 the reaction is usually carried out at pH 8–8.3.

The ESCA data measured at both 45 and 90° for the aminized surface obtained by conversion of the grafted PMMA–AAm by the Hofmann II route and after subsequent coupling with *m*-PEG-npc 5000 are given in Table 1. After the Hofmann reaction there is an increase in nitrogen from 11 to 14 at.% and from 10.9 to 12.2 at.% for ESCA angles of 45 and 90°, respectively. This indicates formation of ethyl or ethylene amine groups which contain 33 at.% nitrogen compared to 20 at.% for the AAm grafts. The differences obtained from the ESCA angles show an increased conversion in the grafted layer when approaching the surface. Considering the partial hydrolysis of amide groups and urea formation which occur as bi-reactions in the Hofmann rearrangement [22,23] the increase in nitrogen indicates a substantial yield of amine groups.

After coupling the *m*-PEG-npc 5000 the most significant difference in Table 1 is the decrease in nitrogen from 14 to 5.9 at.% at an ESCA angle off 45°. However, when measured at 90° the decrease is much less or from 12.2 to 9.8 at.% indicating that the coupling is localized to a thin surface layer. Also for the ESCA angle of 45° the oxygen content of 22.5 at.% is lower than the theoretical 33 at.% for PEG but still represents a significant increase compared to the 15.7 at.% obtained for the preceding Hofmann II surface.

By comparing the high resolution ESCA spectra for O1s in Fig. 1(c) and (d) it is seen that the contribution from ether oxygen at 533 eV seen as a left shoulder in Fig. 1(d) has increased in comparison to the precursors in 1b and 1c. Since the spectra in Fig. 1 are obtained at an ESCA angle of 45° the decrease in nitrogen content from 14 to 5.9 at.% correspond to the difference in N1s spectra in Fig. 1(c) and (d).

For the aldehyde coupling a fluoro substituted aldehyde TFBA was used as a probe since fluorine gives a strong signal in ESCA. The coupling efficiency was compared between the two-aminized surfaces obtained by the Hofmann I and II procedures. An aldehyde reacts with a surface bound amino group to form an imine known as a Schiff base which upon reduction with $NaCNBH_3$ gives a stable secondary amine link. Samples which had been grafted with AAm/BP at 60 °C for 20 min and aminized with the Hofmann I and II solutions were used in the coupling reactions with TFBA. The coupling was performed in aqueous solution, i.e. in citric acid buffer at pH 3.7 and in phosphate buffer at pH 7.0. Although TFBA is sparingly soluble in water no visible phase separation occurred at the low concentrations used. For comparison the coupling was also performed in methanol being a good solvent for TFBA as well as $NaCNBH_3$ and a non-solvent for PMMA [27].

After purification and drying of the samples as described in the experimental part the surface composition was obtained by ESCA. In this case the data given in Table 2 were all measured at 90°. The nitrogen contents for the PMMA–AAm and derived surfaces are lower in Table 1 due to the shorter grafting time of 20 min compared to 45 min in Table 2.

The ESCA data from the coupling in buffered aqueous solutions give fluorine contents in the range 2.0–4.8 at.%. There is a tendency to give lower yields at pH 3.7 compared to pH 7.0 for the Hofmann I surface and to a lesser degree for the Hofmann II surface. Lower yields at pH 3.7 would be in agreement with the general pH dependence of reduction with $NaCNBH_3$ [28]. Below pH 4, the reduction of the aldehyde carbonyl group becomes competitive with the reduction of the imine initially formed between amine and aldehyde whereas at pH 7 the carbonyl reduction is negligible and a higher coupling yield is expected. For the samples in Table 2, this pH dependence although consistent seems to be moderate for the present systems especially for the Hofmann II surface.

From Table 1, it is also seen that the Hofmann I surface gives lower fluorine yields than Hofmann II when coupling is performed at the same pH. The difference between the Hofmann I and II surfaces is a higher molar ratio of NaOH to NaClO for Hofmann II as well as higher concentrations. In general, this will give a higher amount of amide conversion to amine but with increased chain scission of the polyacrylamide [20,21]. However, in the present modification of the Hofmann aminization using a short reaction time the comparatively high reaction temperature should be the main factor for chain scission and this factor is equal for Hofmann I and II. Then a higher amount of amino groups may be expected for the Hofmann II surface due to the higher concentrations and ratio of NaOH/NaClO.

When the coupling reactions were made in methanol to assure a homogeneous solution of TFBA the yield of 4.5 percent fluorine is comparable to that obtained in the buffered aqueous solution at pH 7. Also in this case the Hofmann II surface gives the highest coupling yield expressed as fluorine content. Considering the proton

Table 2
ESCA data for grafting of PMMA with AAm for 20 min at 60 °C with subsequent Hofmann rearrangements and coupling with TFBA in aqueous and methanol solutions

Sample	ESCA 90°			
	C	O	N	F
PMMA	72.1	27.0	0.0	0.0
PMMA–AAm	70.6	22.0	8.3	0.0
Hofmann II before coupling	68.5	21.5	9.7	0.0
Hofmann II +TFBA				
pH 3.7	66.9	21.4	7.7	4.0
pH 7.0	67.0	21.8	6.4	4.8
Hofmann I +TFBA				
pH 3.7	68.6	21.6	7.7	2.0
pH 7.0	67.0	21.7	7.8	3.0
Blank PMMA–AAm+TFBA	69.0	23.6	7.4	0.1
HofmannII+TFBA MeOH	70.4	17.7	7.4	4.5
Hofmann I+TFBA MeOH	70.8	19.8	6.4	3.0
PMMA–AAm+TFBA	71.0	21.2	7.8	0.2

dependence of the $NaCNBH_3$ reduction a higher yield may in principle be expected by acidification of the methanol balanced towards predominant Schiff base reduction. This is, however, beyond the scope of this investigation where coupling in aqueous phase has priority for application with ligands of interest in biological systems. The blank samples of PMMA–AAm with TFBA in aqueous buffer as well as in methanol gave 0.2 at.% fluorine or less which indicates that unspecific binding to the PMMA substrate is insignificant compared to the yields obtained by coupling.

The total nitrogen contents in Table 2 represent amine groups from the Hofmann reaction and also remaining amide groups and to a lesser extent urea groups which may occur as byproducts [22,23]. By comparing the fluorine and nitrogen contents an estimate of the coupling efficiency can be made since each coupling of TFBA gives an atomic ratio of fluorine to nitrogen of 3 to 1. From Table 1, the highest yields which are obtained for the Hofmann II surface give F/N ratios of 0.75 in aqueous buffer at pH 7.0 and 0.61 in methanol. This corresponds to coupling yields of 25 and 20%, respectively, counted on the total amount of nitrogen, i.e. between four or five of all nitrogen containing groups or segments will have TFBA ligands. Since not all the nitrogen will represent amine the yield of the actual coupling reaction is higher. However, even a minimum yield of 25% in relation to an amino content of 1.8×10^{-8} mole/cm^2 for the Hofmann II surface as determined by the TNBS analysis the TFBA coupling would give 4.5×10^{-9} mole/cm^2 corresponding to 2.7×10^{15} sites/cm^2. This is between 3 and 6 magnitudes higher than the concentrations needed for surface bound peptides in cell interactions [29,30] but is required for a dense terminal coupling of larger ligands like PEG. Overview ESCA spectra of the Hofmann II surface are shown in Fig. 4. The ungrafted PMMA gives C1s and, O1s peaks at binding energies of 284 and 532 eV, respectively. For the Hofmann II surface a nitrogen peak appears at 399 eV and also the C/O ratio is higher. After coupling with TFBA a fluorine peak from F1s appears at 686 eV which is clearly seen in Fig. 4(a).

The continued developments will focus on the modification of homogeneous and nano-patterned surfaces for cell interactions.

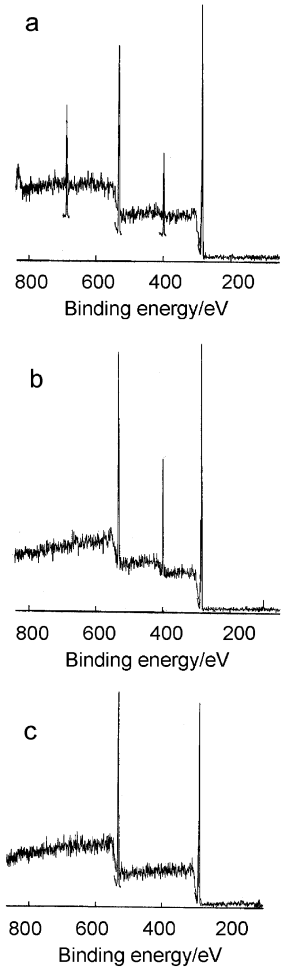

Fig. 4. ESCA overview spectra of PMMA (c), PMMA grafted with AAm/BPO at 60 °C for 20 min and Hofmann II reaction (b) and after subsequent coupling with TFBA at pH 7 (a).

4. Conclusions

PMMA could be surface grafted in a solvent free vapour phase of acrylamide and a hydrogen abstractor benzophenone under mild conditions using a low energy UV source. Self-initiation of acrylamide in absence of benzophenone is indicated but the yield is higher in presence of benzophenone. The grafting rate and yield as measured by ESCA and contact angle also increase with temperature as higher concentrations of reactants is obtained in the vapour phase. The surface bound amide groups may be converted by the Hofmann reaction into amine groups, which could be used in coupling reactions for further modifications of the surface. The amine functionality of the surfaces was confirmed by coupling reactions with a poly(ethylene glycol) with a *p*-nitrophenylcarbonate chain end and an aldehyde compound.

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