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Covalent attachment of fluorescence probes on the PEEK–OH film surface

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Abstract

7-Amino-4-(trifluoromethyl)-coumarin and 1-aminopyrene were fixed on the PEEK–OH film surface by direct substitution of the hydroxyl functions in acidic medium. The resulting materials, called PEEK–coumarin and PEEK–pyrene, were analyzed by XPS, ToF-SIMS and fluorescence spectroscopy. This last method was not suitable for quantitative assays.

Keywords: Poly(ether ether ketone); PEEK film; Surface wet-chemistry

1. Introduction

Poly(ether ether ketone) (PEEK) is a high-performance industrial thermoplastic, recently considered as a potential biomaterial for new medical [1,2] and biotechnological applications [3,4]. Therefore, the designed chemical modification of the polymer surface [5,6] has emerged as an important strategy for improving the material biocompatibility. A crucial point remains the characterization [7] of the modified surface from the point of view of the accessibility of functions and their chemical reactivity [8]; indeed, reactive groups can be further used for linking biologically active molecules such as proteins [4,9]. For this purpose, the covalent attachment of molecular probes onto the polymer surface has been developed as a valuable analytical method [9].

In our laboratory, the reactivity of various functional groups (OH, CO_2H, NH_2, NCO) displayed on the surface of poly(ethylene terephthalate) membranes [10–12] and PEEK films [3,13,14] has been assayed by covalent derivatization with radioactive labels (^{3}H-molecules) followed by liquid scintillation counting (LSC) of the sample-associated radioactivity. This method has been combined with the X-ray photoelectron spectroscopic (XPS) analysis of the F/C atomic ratio, in the case of using radioactive labels equipped with a fluorine tag [15,16]. Beside these quantitative assays, a qualitative analysis of surface-functionalized PEEK films has been obtained by time-of-flight secondary ion mass spectrometry (ToF-SIMS) [17].

The application of fluorescence (spectroscopy and microscopy) to study chemical and analytical problems is well documented, particularly in the biomedical and chromatographic sectors [18,19]. The method has been more recently applied to study polymeric materials, in homogeneous solution, in suspension in a liquid phase, as well as in the solid state [20,21]. Yet, the analysis of solid materials (usually powders) appears more difficult, and requires special detection techniques [19,22,23].

This paper reports an attempt to assay surface hydroxyl groups on PEEK film by using the covalent derivatization with fluorescence probes and their quantification by fluorescence spectroscopy. To our knowledge, only one paper deals with the fluorescence analysis of PEEK surface [24]: sulfonated terbium salt of powdered PEEK has been assayed by measurement of the fluorescence intensity of Tb^{3+}.

2. Experimental

2.1. Chemistry on model compounds

2.1.1. Materials and methods

The reagents were of analytical grade and purchased from Aldrich (Bornem, Belgium) and Acros Chimica (Beerse, Belgium). The solvents were dried and distilled as usual. Merck silica gel 60 (70–230 mesh ASTM) was used for column chromatography. The R_F values were determined

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on Merck TLC 60 F_{254} plates with a thickness of 0.2 mm (visualization with UV).

Melting points are uncorrected (digital melting point apparatus, Electrothermal, UK). The IR spectra were taken with a Perkin–Elmer 1710 instrument and calibrated with polystyrene. The NMR spectra were recorded on Varian Gemini-300 and Brucker AM-500 spectrometers with tetramethylsilane as internal standard. The mass spectra were obtained with a Finnigan MAT TSQ-70 instrument. The UV spectra were taken with a SLM-Aminco DW 2000 UV–VIS spectrophotometer. The fluorescence spectra were recorded on a SLM-Aminco 48 000 apparatus. The microanalyses were performed at the University College (London, UK).

2.1.2. N(4,4′-dimethoxybenzhydryl)-7-amino-4-(trifluoromethyl) coumarin (4)

A solution of 4,4′-dimethoxybenzhydrol **1** (53.3 mg, 0.218 mmol, 1 equiv.) and 7-amino-4-(trifluoromethyl) coumarin **2** (100 mg, 0.436 mmol, 2 equiv.) in acetic acid (5 ml) was stirred overnight at room temperature. The mixture was poured into ice-cold water (20 ml). Extraction with CH_2Cl_2 (3×10 ml), washing with 5% $NaHCO_3$ (3×10 ml), drying over $MgSO_4$ and column chromatography on silica gel (cyclohexane–CH_2Cl_2, 10:90) gave pure adduct **4** (73 mg, 74% yield) as a yellow solid. $R_F = 0.5$; m.p. 182.8–183.9°C; MS (EI) *m/e* 455.2 (M^+), 228.2, 227.2; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 6H), 4.97 (d, $J = 4.5$ Hz, 1H), 5.56 (d, $J = 4.5$ Hz, 1H), 6.43 (s, 1H), 6.44 (s, 1H), 6.60 (d, $J = 8.9$ Hz, 1H), 6.92 (d, $J = 8.8$ Hz, 4H), 7.25 (d, $J = 8.8$ Hz3, 4H), 7.48 (d, $J = 8.9$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) (in ppm) 55.3, 61.34, 99.64, 104.28, 109.2, 111.96, 114.46, 121.9, 126.04, 128.45, 133.49, 141.8, 151.23, 156.87, 159.34, 160.02; IR (film) ν 2964, 1724, 1625, 1607, 1260, 1172 cm^{-1}; UV (MeOH, $c = 10^{-5}$ M) $\lambda_{max} = 388.6$ nm; Fluorescence (MeOH, $c = 10^{-6}$ M) $\lambda_{emission} = 496$nm; Anal. Calcd for $C_{25}H_{20}F_3NO_4$ (455.43); C, 65.93; H, 4.42; N, 3.07—Found: C, 65.55; H, 4.20; N, 2.82.

2.1.3. N-(4,4′-dimethoxybenzhydryl)-1-aminopyrene (5)

A solution of 4,4′-dimethoxybenzhydrol **1** (50 mg, 0.2 mmol, 1 equiv.) and 1-aminopyrene **3** (89.8 mg, 0.4 mmol, 2 equiv.) in acetic acid (2 ml) was stirred overnight at room temperature. The mixture was worked-up as before. Column-chromatography (SiO_2; cyclohexane–CH_2Cl_2, 70:30) gave pure adduct **5** (65 mg, 73% yield) as a green solid (to be stored under argon atmosphere and in the dark, at −20°C). R_F (cyclohexane–CH_2Cl_2, 50:50) = 0.33; MS (APCI) *m/e* 443.2 (M), 442.1, 307.9, 306.9; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.81 (s, 6H), 5.23 (br s, 1H), 5.81 (s, 1H), 6.91 (d, $J = 8.7$ Hz, 4H), 7.16 (d, $J = 8.6$ Hz, 1H), 7.38 (d, $J = 8.7$ Hz, 4H), 7.76 (d, $J = 9.2$ Hz, 1H), 7.85 (d, $J = 9.2$ Hz, 1H), 7.91 (m, 2H), 7.97 (d, $J = 9.2$ Hz, 1H), 8.01 (d, $J = 9.2$ Hz, 1H), 8.03 (d, $J = 7.6$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) (in ppm) 55.15, 61.78, 110.41, 114.1, 116.44, 119.35, 123.0, 123.18, 123.24, 123.78, 125.54, 125.71, 125.76, 126.04, 127.58, 128.51, 131.48, 132.21, 134.88, 141.23, 158.84; IR (film) ν 2957, 2923, 2852, 1602, 1510 cm^{-1}; UV (MeOH, $c = 10^{-5}$ M) $\lambda_{max} = 237$, 282, 357 nm; Fluorescence (MeOH, $c = 10^{-6}$ M) $\lambda_{emission} = 429$ nm ($\lambda_{excitation} = 360$ nm).

2.2. Chemistry on polymer films

2.2.1. Materials and methods

Amorphous PEEK film (Stabar K200; thickness of 25 μm) received from ICI (UK) was surface-reduced according to Refs. [13,14,25,26]. The amount of hydroxylated monomer units was determined by XPS considering the C=O/C–O and the O=C/O–C atomic ratios in the fine structures of the C_{1s} and O_{1s} peaks [17]. The percentage of surface reduction was about 80% for the samples used in this study. The PEEK–OH disks (1.12 cm^2) and squares (1 cm^2 or 4 cm^2) used for the surface derivatizations were cut off a large PEEK–OH sample (rectangle of 30×15 cm^2).

Water used for the rinsing of the modified polymer samples was of HPLC grade and obtained with a Milli-Q system (Millipore, Bedford, MA). The other solvents were of analytical grade and purchased from Acros Chimica (Beerse, Belgium).

The XPS spectra were obtained with an SSI X-probe (SSX-100/206) spectrometer from Fisons (Surface Science Laboratories, Mountain View, CA), equipped with an aluminium anode (10 kV, 20 mA) and a quartz monochromator. The direction of photoelectron collection made angles of 55 and 75° with the normal to the sample and the incident X-ray beam, respectively. The electron flood gun was set at 6 eV. The vacuum in the analysis chamber was 2.5×10^{-7} Pa. The binding energies of the peaks were determined by setting the C_{1s} component due to carbon bound only to carbon and hydrogen at a value of 284.8 eV. The peak areas were determined with linear background subtraction. Intensity ratios were converted into atomic concentration ratios by using the SSI ESCA 8.3D software package. The XPS experimental technique was fully described in Refs. [14,17].

The ToF-SIMS measurements were carried out with the Charles Evans and Associates TFS-4000 MMI system using a $^{69}Ga^+$ (15 keV) liquid metal ion source. In this system, the secondary ions are accelerated up to a 3 keV energy before being deflected 270° by three electrostatic hemispherical analysers (TRIFT^{TM}). A 5 keV post-acceleration was used before the detector to increase the detection efficiency of the high-mass ions. A 500 pA d.c. current was pulsed at a 5 kHz repetition rate with a 15 ns pulse width. The analyzed area was about 97×97 μm^2 to 196×196 μm^2. The acquisition time was 5 min for each spectrum, corresponding to a total ion fluence of $<10^{12}$ ions cm^{-2} and ensuring static conditions. Charge compensation was performed with a pulsed electron gun operated at 20 eV and a non-magnetic

Table 1
XPS analysis of PEEK samples treated with coumarin (**2**) and pyrene (**3**)

	Sample	Conditions[a]	Atomic ratios (10^2)		Calcd% modified units[b] from		
		%[c] (label); solv.	N/C	F/C	N/C	F/C	(corr.)
1	PEEK–OH[d]	1.0 (**2**); HOAc	0.94	3.23	20	23	
2	PEEK–OH[d]	2.0 (**2**); HOAc	0.77	3.13	17	22	
3	PEEK–OH[d]	3.0 (**2**); HOAc	1.22	5.47	26	42	
4	PEEK–OH[e]	0.5 (**2**); HOAc	1.05	2.51	22	18	(18)
5	PEEK[e]	0.5 (**2**); HOAc	0	0	0	0	
6	PEEK–OH[e]	0.5 (**2**); EtOAc	0	0	0	0	
7	PEEK–OH[e]	1.125 (**2**); HOAc	2.28	8.75	56	77	(70)
8	PEEK[e]	1.125 (**2**); HOAc	0.59	1.01	12	7	
9	PEEK–OH[e]	1.125 (**2**); EtOAc	0	0	0	0	
10	PEEK–OH[e]	1.75 (**2**); HOAc	1.56	7.09	35	59	(51)
11	PEEK[e]	1.75 (**2**); HOAc	1.01	1.06	20	8	
12	PEEK–OH[e]	1.75 (**2**); EtOAc	0.99	1.11	20	8	
13	PEEK–OH[d]	2.0 (**3**); HOAc	1.65	–	43	–	
14	PEEK–OH[d]	3.0 (**3**); HOAc	1.23	–	30	–	
15	PEEK–OH[d]	4.0 (**3**); HOAc	2.36	–	72	–	

[a] The samples were immersed in the reactive solutions, with shaking, for 3 days at 20°C.
[b] For example of calculation, see text (corrected value = subtraction of the non-covalent contribution considering the F/C ratios).
[c] Concentrations (%) are given in weight/volume.
[d] Sample of 2×2 cm^2.
[e] Sample of 1×1 cm^2.

stainless-steel grid (2 mm apertures) was put onto the surface. The ToF-SIMS experimental technique was fully described in Ref. [27]. In order to make a quantitative comparison between spectra, each absolute intensity was normalized to the intensity of a reference peak. The reproducibility of the normalized intensities was estimated from the variance obtained for at least four independent measurements [17].

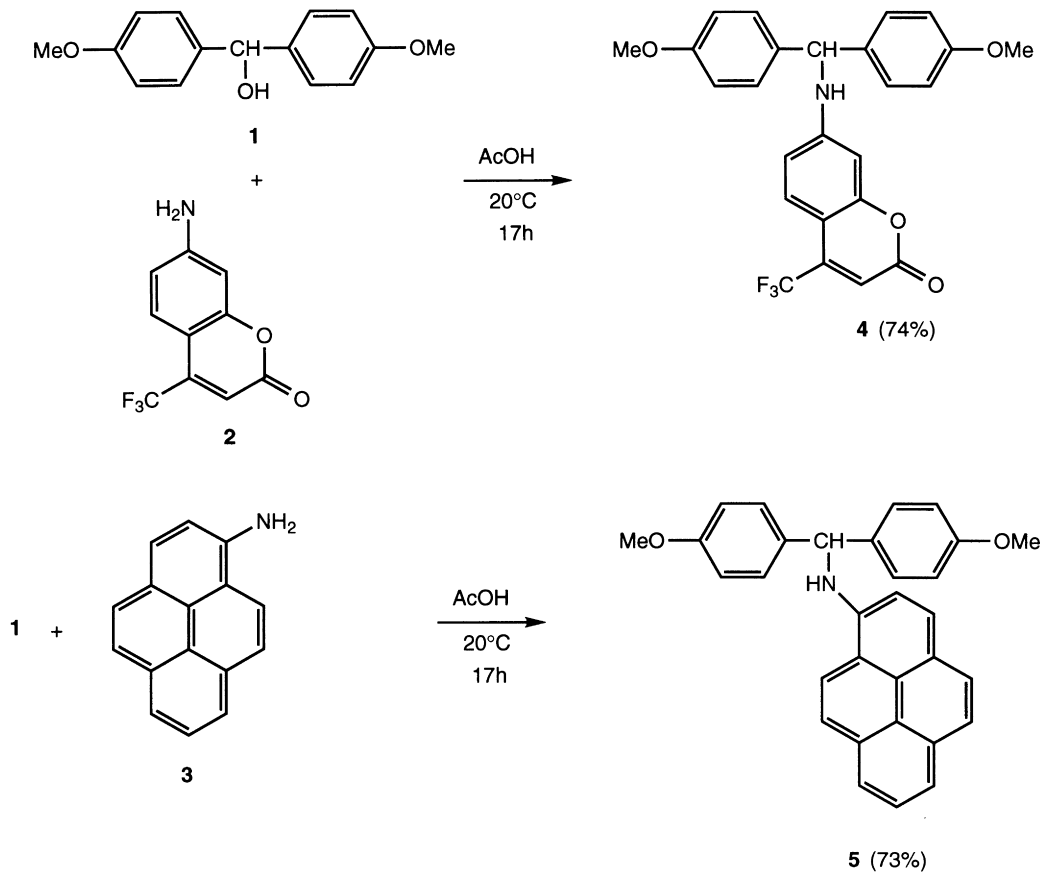
The fluorimetric analyses were realized with a SLM-Aminco 48000 apparatus equipped with a front face configuration holder (SLM Instruments, Inc., Front Surface Accessory). In this device, the film could be fixed between two plates with an aperture of 1×2 cm^2. The measures were recorded under normal atmospheric conditions. Usually, excitation beam and measured emission beam make angle of 90°, i.e. $\theta = 0°$ for the surface accessory. After optimisation for a maximum of detection intensity, the angle of analysis has been fixed at $\theta = 24°$ [20,28]. The surface sensitivity obtained by using the Front Surface Accessory was not specified by the manufacturer (this would most probably depend not only from the equipment, but also from the polymer film nature). A home-made sensitivity test has been performed as follows: samples of PEEK–OH film (squares of 1 cm^2) were immersed into methanolic solutions of 1-aminopyrene (8 ml) of various concentrations (10^{-3}–10^{-10} M), air dried and then analyzed by fluorescence spectroscopy. The limit of detection of the physisorbed label was reached for the 10^{-9} M solution of 1-aminopyrene. Assuming that all the dissolved label has been adsorbed on the PEEK–OH film, this would correspond to a maximum value of 8 pmol/cm^2. We have previously calculated [6,14] that an interface domain of one molecular layer of PEEK film "covered" by 1 cm^2 of surface contains about 200 pmol of repetitive monomer units. Considering a range of 30–60% of surface derivatization, the amount of labels fixed in a monolayer should be around 60–120 pmol/cm^2, a value really accessible by the technique. However, we were unable to establish a quantitative calibration graph since the reproducibility of the measurements was poor.

2.2.2. Preparation of PEEK–coumarin

Two PEEK–OH samples (film pieces of 2×2 cm^2) were immersed into a solution of 7-amino-4-(trifluoromethyl) coumarin **2** (0.1 g) in dry acetic acid (10 ml) and shaken over 72 h at room temperature. The samples were removed from the reactive solution and successively rinsed with HOAc (3×10 min), water (3×10 min) and acetone (3×10 min), then dried under vacuum at 60°C. The samples were stored in the dark. XPS analysis (entry 1): C_{1s} (284.8–291.7 eV), 82.83%; O_{1s} (533.3–540.2 eV), 13.73%; N_{1s} (399.7 eV), 0.78%; F_{1s} (688.5 eV), 2.68%.

2.2.3. Preparation of PEEK–pyrene

Two PEEK–OH samples (disks of 1.2 cm of diameter) were immersed into a solution of 1-aminopyrene **3** (0.24 g) in dry acetic acid (12 ml), shaken for 72 h at 20°C, and then treated as above. XPS analysis (entry 13): C_{1s} (284.8–287 eV), 88.24%; O_{1s} (533.3–540 eV), 10.28%; N_{1s} (399.5 eV), 1.46%.



Scheme 1.

XPS data of Table 1

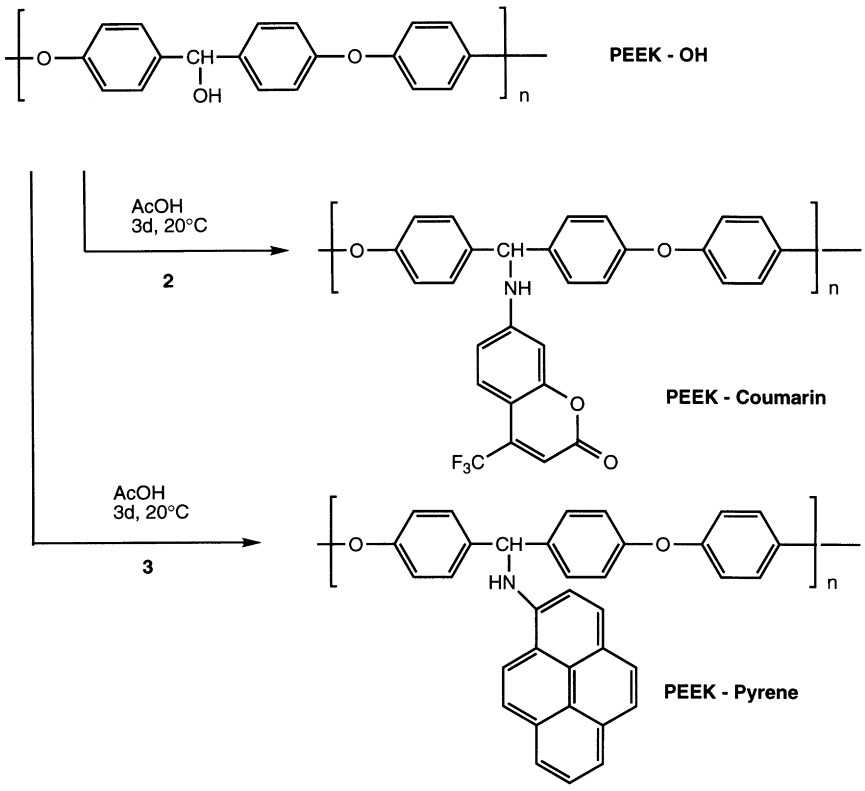
Entry 1	C, 82.83%; O, 13.73%; N, 0.78%; F, 2.68%.
Entry 2	C, 83.47%; O, 13.29%; N, 0.64%; F, 2.61%.
Entry 3	C, 82.02%; O, 12.49%; N, 1.0%; F, 4.49%.
Entry 4	C, 81.24%; O, 15.88%; N, 0.85%, F, 2.04%.
Entry 5	C, 84.56%; O, 15.44%.
Entry 6	C, 84.16%; O, 15.84%.
Entry 7	C, 74.61%; O, 17.17%; N, 1.70%; F, 6.53%.
Entry 8	C, 79.11%; O, 19.63%; N, 0.47%; F, 0.80%.
Entry 9	C, 85.24%; O, 14.76%.
Entry 10	C, 77.59%; O, 15.69%; N, 1.21%; F, 5.51%.
Entry 11	C, 78.87%; O, 19.49%; N, 0.80%; F, 0.84%.
Entry 12	C, 80.02%; O, 18.29%; N, 0.80%; F, 0.89%.
Entry 13	C, 88.24%; O, 10.28%; N, 1.46%.
Entry 14	C, 88.47%; O, 10.45%; N, 1.09%.
Entry 15	C, 89.12%; O, 8.78%; N, 2.1%.

3. Results and discussion

We have previously developed a wet-chemical approach for the selective surface functionalization of a thin film of PEEK [6]. The method is based on the film surface reduction [13] to produce the so-called PEEK–OH film displaying reactive hydroxyl functions [3,25]. These could be directly substituted with various aniline, amide and carbamate reagents [14,26]. The reactions were performed at the solid–liquid interface, using acetic acid as the liquid phase which allows dissolution of the reagents on the one hand, and good wetting and chemical activation of the immersed PEEK–OH solid samples, on the other hand.

Our present objective is to covalently fix fluorescence probes on the PEEK–OH film surface, and to analyze the resulting materials by fluorescence spectroscopy combined with other well-established methods. Indeed, the fluorescence spectroscopy is not a usual surface spectroscopic technique, and the quantitative measurement of label concentrations appears somewhat difficult [20,29]. Also, the sampling depth of the method was not reported. Therefore, the classical assay of the samples, by XPS for instance, should provide reliable references.

We selected two probes for this study, namely the coumarin **2** and the pyrene **3** derivatives (Scheme 1). 7-Amino-4-(trifluoromethyl) coumarin **2**, although scarcely used in surface analysis, has been chosen because its CF_3 group will provide an excellent internal XPS tag. The pyrene motif is the most useful label for the characterization of silica [30], and organic polymers [31–33].



Scheme 2.

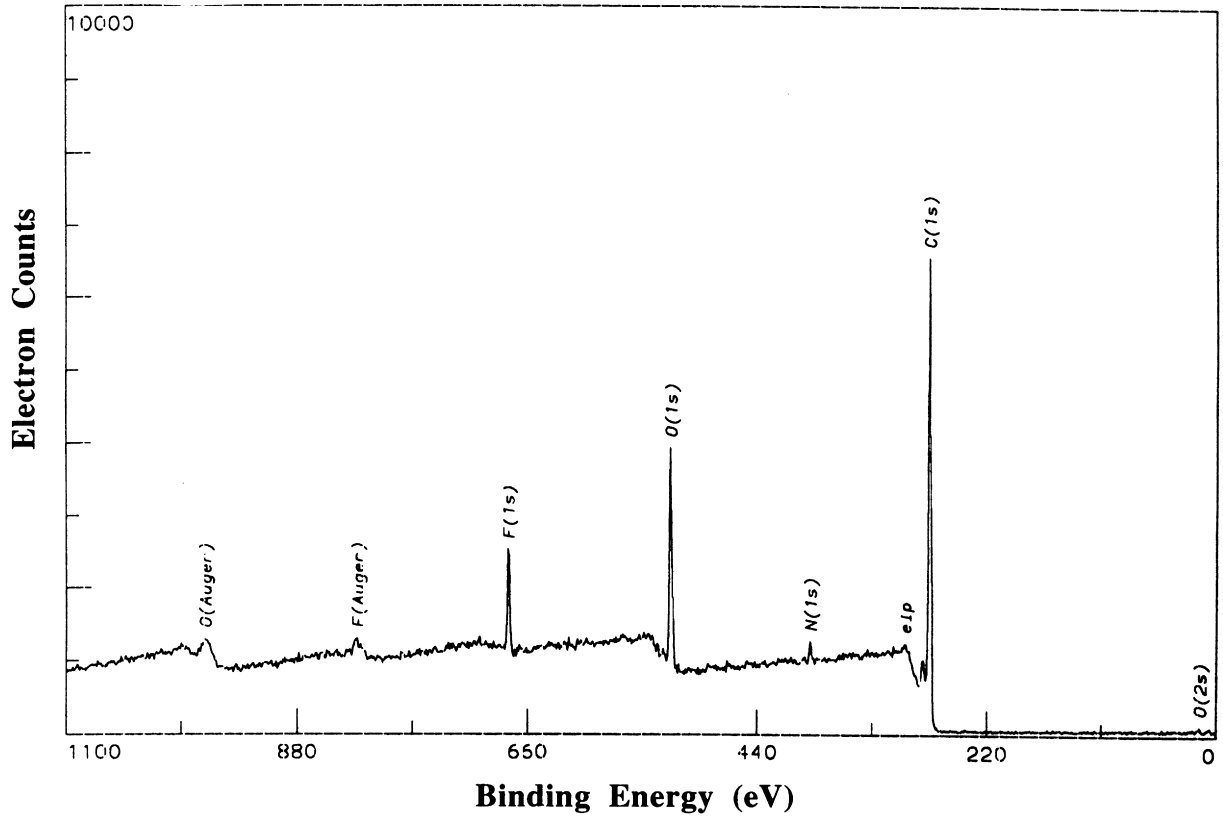


Fig. 1. XPS spectrum of PEEK–coumarin.

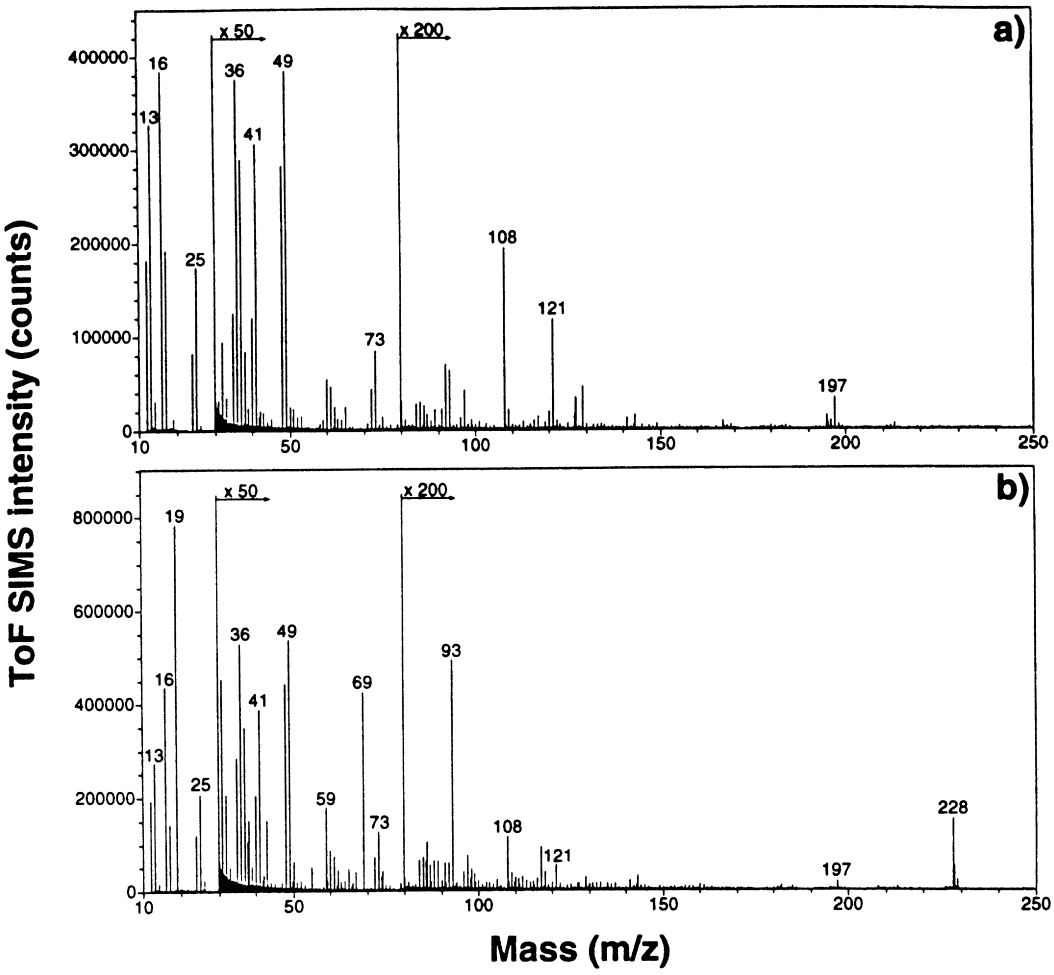


Fig. 2. ToF-SIMS analysis in negative mode of (a) PEEK–OH and (b) PEEK–coumarin.

Pyrene-functionalized polyethylene, prepared via the process of entrapment functionalization, has been particularly well studied by the Bergbreiter's group [34–36]; semi-quantitative information about the mobility, solvent accessibility, two-dimensional morphology and reactivity of functional groups at the polymer-solvent interface could be recorded (samples analyzed under a liquid phase).

3.1. Study of model compounds

Our synthetic plan for the PEEK–OH surface covalent grafting with molecules **2** and **3** was based on the direct substitution of the hydroxyl groups with the aniline derivatives. This strategy was first examined in homogeneous solution, with 4,4′-dimethoxybenzhydrol **1** considered as a good mimic of the reduced PEEK monomer unit [37]. A mixture of **1** and the aniline, **2** or **3**, in acetic acid solution was left for 17 h at room temperature. The substitution proceeded smoothly, without any added catalyst, and furnished the corresponding substitution product, **4** or **5**, in good yield, after precipitation with water and chromatographic purification (Scheme 1). The substituted anilines **4** and **5** were fully characterized as usual (see Section 2); typical fluorescence emission was observed in solution (MeOH) at 496 and 429 nm, respectively, for excitation at 389 and 360 nm.

This validated strategy has been applied to the polymer surface functionalization.

3.2. Study of PEEK–coumarin films

3.2.1. Preparation of the samples

PEEK–OH film samples with an average of 80% of hydroxylated monomer units (obtained by the surface reduction of PEEK film with $NaBH_4$ in dimethylsulfoxide at 120°C for 3 h [13,14,25,26]) were immersed into solutions of **2** in acetic acid during three days at room temperature, under gentle shaking, to give the PEEK–coumarin samples (Scheme 2). Various reagent concentrations were examined, from 0.5 to 3% (w/v), in order to prepare polymer samples with different densities of surface grafting. The samples were adequately rinsed for desorbing at best the excess of non-fixed reagent (checking of the washing

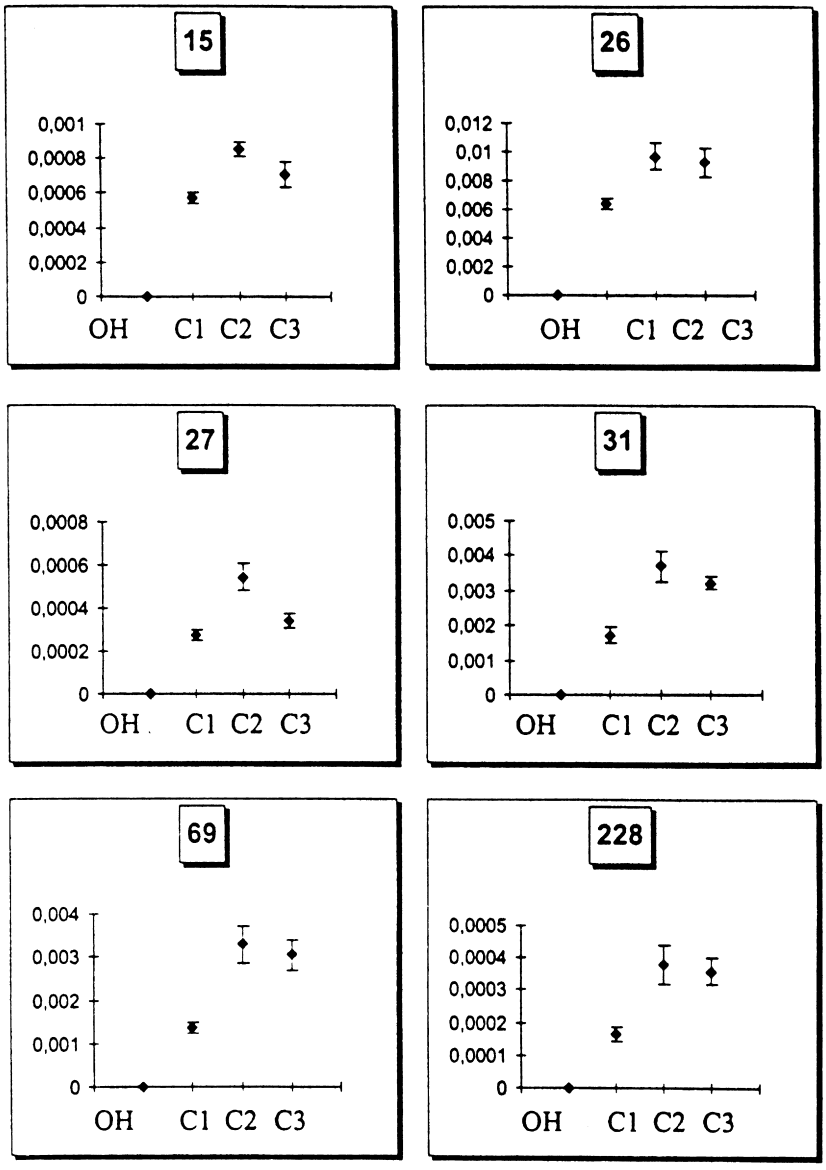


Fig. 3. Relative intensities of $m/z = 15$ (NH^-), 26 (CN^-), 31 (CF^-), 69 (CF_3^-) and 228 ($C_{10}H_5O_2F_3N^-$), normalized to the total intensity; OH = 0%, C1 = 0.5%, C2 = 1.125% and C3 = 1.75% of **2** (w/v).

solutions by fluorescence spectroscopy), and then analyzed by XPS (Fig. 1).

3.2.2. XPS analyses (Table 1)

A first set of experiments (samples of 4 cm^2) revealed that the label fixation occurs in 20–40% yield (entries 1, 2 and 3), depending on the concentration of **2** in the acetic acid solution (1–3%). From the experimental N/C and F/C atomic ratios, we could determine the percentages of modified monomer units as follows (entry 1, for instance): considering a theoretical monomer unit consisting of $[(PEEK + PEEK–OH)_x + (PEEK–coumarin)_y]$, i.e. $[(C_{19}O_3)_x + (C_{29}NO_4F_3)_y]$, where $x + y = 1$, we calculated for $x = 0.8$ and $y = 0.2$, a N/C atomic ratio of $0.2/(29 \times 0.2 + 19 \times 0.8) = 0.0095$ (experimental value = 0.0094). Similarly, we calculated for $x = 0.77$ and $y = 0.23$, a F/C atomic ratio of $3 \times 0.23/(29 \times 0.23 + 19 \times 0.77) = 0.0324$ (experimental value = 0.0323).

In another set of experiments (samples of 1 cm^2), we tried to find the optimal concentration of **2** (entries 4, 7 and 10); this was 1.125% of **2** in HOAc, giving 56–77% of derivatized monomer units according to whether the calculations are performed from the N/C or F/C atomic ratios. Control experiments were realized in order to determine the level of non-covalent fixation, i.e. the physisorption of the label **2**. Samples of native PEEK film (absence of reactive hydroxyl functions) were treated as above (entries 5, 8 and 11): the nitrogen and fluorine atoms could not be detected for the 0.5% treatment, but the XPS analyses revealed maximum values of 12 and 20% of adsorbed **2** for the 1.125 and 1.75%

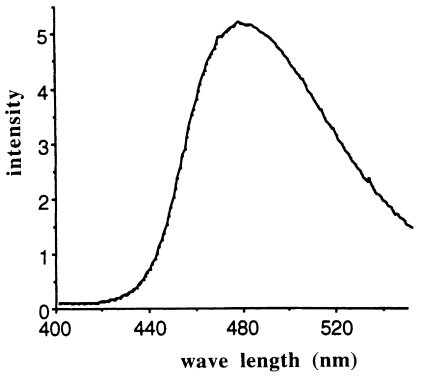


Fig. 4. Surface fluorescence spectrum of PEEK–coumarin.

treatments, respectively. Treatment of PEEK–OH samples by immersion into a non-acidic solvent (i.e. ethyl acetate) containing the fluorescent label did not give rise to detectable physisorption of **2** for concentrations inferior to 1.75% (entries 6, 9 and 12). This confirms the activating role of the solvent (HOAc) for promoting the PEEK–OH substitution, most probably via the formation of benzhydryl cationic intermediates from the protonated benzhydrol moieties. By subtracting the non-covalent contribution, the corrected PEEK–OH surface derivatization values were 18% (entry 4), 70% (entry 7) and 51% (entry 10) of modified monomer units, corresponding respectively to treatments with 0.5, 1.125 and 1.75% of coumarin **2** in acetic acid solution. Thus, the conditions of the entry 7 illustrate the best compromise between high level of covalent fixation and low level of physisorption of the label.

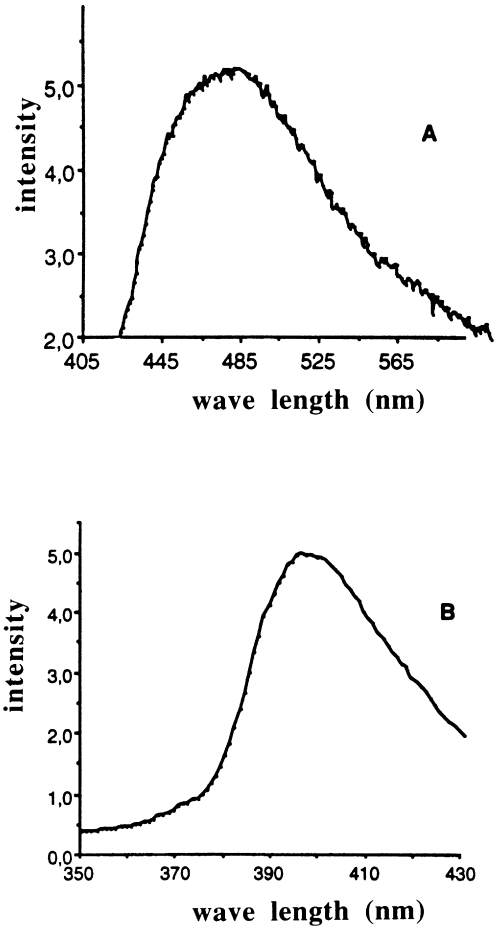


Fig. 5. (A) Emission and (B) excitation spectra of PEEK–pyrene.

3.2.3. ToF-SIMS analyses

The time-of-flight secondary ion mass spectrometric analysis of the different PEEK–coumarine samples (entries 4, 7 and 10), and their related blank references, qualitatively confirmed the XPS results. The spectra recorded in negative mode (Fig. 2) were the most informative ones. Characteristic nitrogen- and fluorine-containing fragments appeared at $m/z = 15$ (NH^-), 26 (CN^-), 31 (CF^-), 69 (CF_3^-) and 228 ($C_{10}H_5O_2F_3N^-$), this last fragment corresponding to the whole coumarin molecule. The peak intensities, normalized to the total intensity, showed the same functionalization profile as determined by XPS (Fig. 3), i.e. a maximum of label fixation for a solution concentration of 1.125% of **2** in acetic acid. Traces of coumarin ($m/z = 228$) were detected on the outermost surface of all the blank samples (entries 5, 8, 9, 11 and 12), except one (entry 6).

3.2.4. Fluorescence analyses

The PEEK–coumarin samples (entries 1–3) were examined by fluorescence spectroscopy (under air atmosphere) using a special device for film analysis allowing to place the samples in a reproducible manner into the spectrometer. Emission was detected under an angle of 24° of the excitation light [21]. Under such conditions, the emission peak of the grafted coumarin was observed at 478 nm, for an excitation at 380 nm (Fig. 4). In solution (10^{-6} M, MeOH), the model compound **4** (Scheme 1) gave an emission peak at 496 nm for an excitation at 388 nm.

Disappointingly, no significant differences could be detected in the fluorescence spectra of film samples functionalized with 20 or 40% of labels from XPS. It appeared also quite difficult to record reproducible results for a same film sample.

3.3. Study of PEEK–pyrene films

3.3.1. Preparation of the samples and XPS analyses

PEEK–pyrene samples (squares of 4 cm^2) were prepared by immersing PEEK–OH pieces into acetic acid solutions of **3** with various concentrations (from 2–4% (w/v); Scheme 2). According to the XPS analyses, the films contained 30–70% of functionalized monomer units (Table 1, entries 13, 14 and 15). From the experimental N/C atomic ratio of 0.0165 (entry 13, for instance), we calculated $x = 0.57$ and $y = 0.43$, considering a theoretical monomer unit consisting of $[(\text{PEEK} + \text{PEEK–OH})_x + (\text{PEEK–pyrene})_y]$, i.e. $[(C_{19}O_3)_x + (C_{35}NO_2)_y]$, where $x + y = 1$. Since blank samples were not prepared in this case, the XPS results

could not be corrected; they involve the both contributions of covalent and non-covalent fixation of the aminopyrene **1**.

3.3.2. Fluorescence analyses

The fluorescence spectrum of PEEK–pyrene was observed at 470 nm (emission) for an excitation at 404 nm (Fig. 5). This emission value around 470 nm as a broad featureless band is typical of excimer fluorescence [30,34,38,39], due to the surface proximity of the labels. Indeed, the required distance between two pyrene species is 3.5 A° to form an excimer, i.e. the interaction of an excited pyrene with a well-orientated pyrene in its ground state [34]. Such an interaction was not observed in the fluorescence spectrum of the soluble model compound **5** diluted at 10^{-6} M in methanol; emission was recorded at 429 nm for an excitation at 360 nm.

Unfortunately, all the PEEK–pyrene samples showed very similar fluorescence spectra, and the excimer was the only visible species. As in the case of PEEK–coumarin, a quantitative analysis could not be performed.

4. Conclusion

Two fluorescence probes, namely 7-amino-4-(trifluoromethyl) coumarin **2** and 1-aminopyrene **3**, have been fixed on the surface of the PEEK–OH film, most probably via covalent linkage on the benzhydryl moiety, as ascertained by the coupling reactions performed with 4,4′-dimethoxybenzhydrol **1** considered as a representative model of the polymer reactivity. Different PEEK–coumarine and PEEK–pyrene samples were prepared by immersing PEEK–OH pieces in acetic acid solutions of various label concentrations. The percentages of surface derivatization were calculated from the N/C or F/C atomic ratios provided by the XPS analysis. In some cases, the results obtained were somewhat different according to whether the N/C or F/C values are considered. Since the nitrogen detection is always around the limit of detection of the XPS method (<1%), we assume that the results obtained from the fluorine concentrations (>1%) are the most reliable ones. The effect of the reagent concentration in solution on the level of surface modification appears to be typical of the polymer wet-chemistry process [6,8]. Indeed, the surface modification is a delicate compromise between surface chemical transformation and etching (progressive dissolution) of the resulting modified interface. Accordingly, reagent solutions with high concentrations are generally not the best surrounding media to perform surface wet-chemistry.

The quantitative assays provided by the XPS measurements were qualitatively confirmed by the ToF-SIMS analysis of the outermost surface. This suggests that the modified polymer interface is almost homogeneous within the domain usually explored by XPS, i.e. 50–70 A° in depth, or about 10 molecular layers [17].

Our initial objective to quantitatively correlate XPS and fluorescence measurements could not be reached. Indeed, the fluorescence measurements recorded on the various PEEK–coumarin and PEEK–pyrene samples were not strictly reproducible for a same sample, and not significantly different from one sample to another one. Thus, in our hands, quantitative analysis of surface-fixed labels could not be performed by fluorescence spectroscopy [20]. However, the fluorescent motifs were qualitatively detected in all cases.

As a result of our selected strategy for coupling fluorescent labels on the PEEK–OH film surface, we needed aniline reagents, and, therefore, the modified surfaces displayed *N*-substituted aniline derivatives of the labels. Due to the well-known quenching effect of aromatic amines [34,35], the pyrene's vibronic emission spectrum of PEEK–pyrene was devoid of interesting fine structures. The only recorded information was the presence of excimers, as expected in the case of high level of surface derivatization and/or mobility of the surface chains allowing local proximity of the labels.

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