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Surface carboxylation of PEEK film by selective wet-chemistry

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Surface carboxylated PEEK films were obtained from a common key-intermediate, the PEEK-OH film, resulting from the surface reduction of amorphous PEEK film. Substitution of the hydroxyl groups, under mild acidic conditions, with 4-aminobenzoic acid and succinamic acid gave PEEK–Ph–CO_2H and PEEK–$(CH_2)_2$–CO_2H in 38% and 70% yields, respectively. The sample surfaces were analysed by X-ray photoelectron spectroscopy. The reactivity of PEEK–$(CH_2)_2$–CO_2H was assayed by activation with WSC, $SOCl_2$ or CDI, followed by coupling to ^{3}H-lysine and liquid scintillation counting of the radioactivity associated with the samples. © 1997 Elsevier Science Ltd.

(Keywords: poly(ether ether ketone); PEEK film; surface wet-chemistry)

INTRODUCTION

The surface chemical nature of polymeric materials impacts on numerous applications by governing a wide range of interfacial properties, such as wettability, hydrophobicity, adhesion and biocompatibility[1,2]. As part of a program aiming at the discovery of new substrates for the *in vitro* mammalian cell cultivation[3–5], we became interested in the tailor-made surface modification of poly(aryl ether ether ketone) (PEEK) film in order to investigate the role played by selected chemical motifs in mediating cellular adhesion, growth and differentiation[6]. The chemically modified surfaces could be further used as starting materials for the development of active biocompatibilization strategies involving the covalent grafting of various biological signal mediators (proteins, peptides, peptidomimetics,...), able to specifically interact with cell receptors.

PEEK is a high-performance thermoplastic[7] exhibiting excellent mechanical properties, thermal stability, environmental resistance, and passive biocompatibility, i.e. absence of toxicity and biological inertness[8–10]. However, the native PEEK film was found to be a very poor substrate of cell cultivation, extremely reluctant to allow cellular adhesion[11,12]. Improvement of the bioadhesion properties could possibly result from an increase of the surface hydrophilicity, for instance by the introduction of carboxyl groups[13,14]. Moreover, such reactive functions should be excellent anchorage points for the immobilization of bioactive molecules[5,15].

This article deals with the development of original procedures for the PEEK film surface carboxylation, the characterization of the modified surfaces by spectroscopic methods, and the assay of their chemical reactivity by the coupling of a radioactive label followed by counting of the sample-associated radioactivity[16–18]. The technique used for the PEEK surface functionalizations and the radiochemical assays is the wet-chemistry[19,20]. Indeed, organic reactions carried out at the solid–liquid interface under mild conditions restrict the changes to specific surface groups and do not alter the structure of the polymer bulk. Moreover, the use of selective reagents can readily produce surfaces with well-defined chemical compositions[21].

The surface reduction of PEEK film by wet-chemistry has been previously studied by Franchina and McCarthy[21], and ourselves[22]. The resulting surface (PEEK-OH) displays hydroxyl functions in high amounts, the reactivity of which has been established by direct labelling with ^{3}H-acetic anhydride[22], activation with *p*-nitrophenyl chloroformate followed by coupling to ^{3}H-lysine or trifluoroethylamine[22], and substitution with diethylaminosulfur trifluoride[23] and 4-trifluoromethylbenzamide[23]. In this paper, we further take advantage of the remarkable PEEK-OH reactivity for the construction designed surfaces.

EXPERIMENTAL

Polymer sample

Amorphous PEEK film was received from ICI (UK); the sample was Stabar K200 (reference number K0310902; thickness 25 μm). The contact angle of water was 71.2° (±1.5°), and the X-ray photoelectron spectroscopy (XPS) analysis revealed an O/C atomic ratio of 0.20 (theoretical value for $C_{19}H_{12}O_3$: O/C = 0.158). This results from an industrial surface treatment by corona discharge (information disclosed by the supplier). The modified interface (mainly due to oxidative fragmentations) appeared to be somewhat fragile and unstable; it could be easily removed by etching (i.e. interface dissolution). Thus, native PEEK film was obtained as follows: (i) immersion of Stabar K200 in refluxing acetone for 48 h; (ii) rinsing twice with acetone; (iii) drying under vacuum (1 mmHg) for 3 h at 60°C. After this treatment, the contact angle of water was 82° (± 1.2°).

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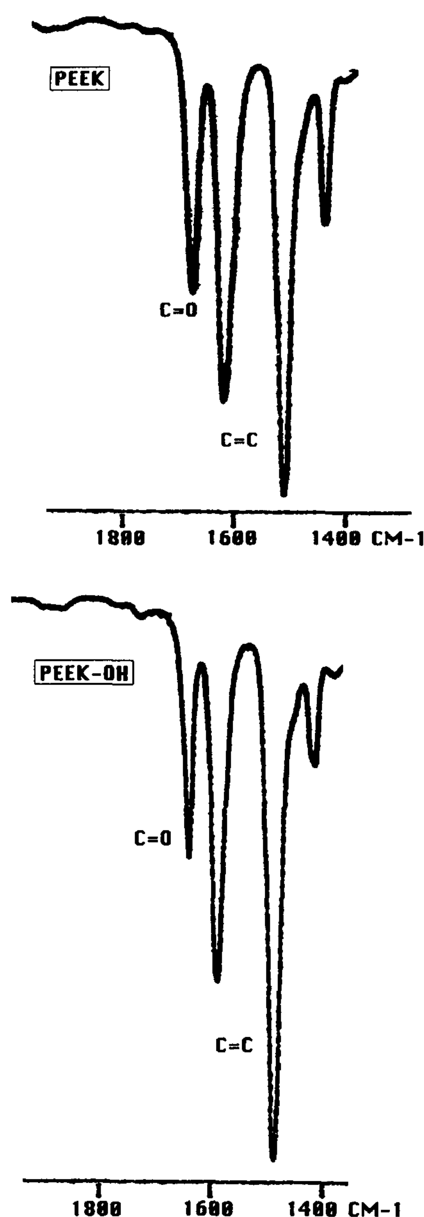


Figure 1 MIR analysis of PEEK and PEEK-OH film

From the XPS analysis we found an atomic O/C ratio of 0.148, close to the theoretical value; C_{1s} (87.13%): 284.8 eV (C–C, C–H), 286.4 eV (C–O), 287.1 eV (C = O), 291.5 eV (π–π*); O_{1s} (12.87%): 531.5 eV (O = C), 533.3 eV (O–C). MIR analysis (*Figure 1*): $\nu_{1650}\,cm^{-1}/\nu_{1490}\,cm^{-1} = 0.67$[22].

Reagents and solvents

Organic reagents (99^{+}% purity) were purchased from Acros Chimica (Beerse, Belgium) or Aldrich (Bornem, Belgium) and used as received. Inorganic reagents (analytical grade) were obtained from U.C.B. (Braine l'Alleud, Belgium) or Merck (Darmstadt, Germany) and used as received. The radiolabeled reagent was provided by Amersham (Little Chalfont, UK): the activity of (L)-[4,5 – ^{3}H]lysine monohydrate in aqueous solution was 82 Ci mmol^{-1}. Dimethylsulfoxide (DMSO) was distilled before use. Acetone was dried over $CaSO_4$ and distilled. Ethanol was dried over Mg and distilled. Toluene was dried over Na and distilled. Tetrahydrofuran (THF) was dried over Na and distilled. Water (HPLC grade) was obtained with a Milli-Q system (Millipore, Bedford, MA). The phosphate buffered saline (PBS, pH 7.2) was prepared from NaCl (4 g), KCl (0.1 g), KH_2PO_4 (0.1 g) and Na_2H-$PO_4.2H_2O$ (0.71 g) dissolved in water (500 ml). The MES buffer (pH 3.5) was obtained from 2-(*N*-morpholino)-ethane sulfonic acid hydrate (MES, 10.66 g) dissolved in water (500 ml).

Methods

The contact angles of water were measured at room temperature using the sessile drop technique and an image analysis system (CCD camera of MXR 5010 type and contour processor PIO-12 with computer monitor 80 from Electronish Ontwerp Bureau De Boer, Holland). The values given in the tables are the average of ten measurements. The standard deviation is indicated in parentheses.

The surface infra-red (i.r.) spectra (MIR (multiple internal reflection) mode) were recorded on PE 580 and PE 1760 spectrometers using an optical deviation system from Perkin-Elmer and a thallium bromide–iodide crystal KRS-5 (incidence angle: 45°); the instrument was coupled with a PE 3600 computer (sampling depth of about 1–5 μm).

The scanning electron microscopy (SEM) was performed using a Hitachi (Tokyo, Japan) S-570 system with an accelerating voltage of 15 kV and a working distance of approximatively 5 mm. The samples were gold coated in a Balzers Union SCD 040 vapour disposition unit, at 15 mA, for a period of 120 s.

The XPS analyses (sampling depth of about 50–80 Å) were performed with a SSI X-Probe (SSX-100/206) photoelectron spectrometer from Fisons, interfaced with a Hewlett Packard 9000/310 computer allowing instrument control, data accumulation and data treatment. This spectrometer used a monochromatized Al–K_α X-ray radiation (1486.6 eV) focused onto the sample. The direction of photoelectron collection made angles of 55° and 75° with the normal to the sample and the incident X-ray beam, respectively. A survey analysis was performed and the individual peaks were recorded in detail. For survey analysis, the Al anode was powered at 10 kV and 20 mA; the spot size was 1000 μm. The analysed area, corresponding to the irradiated zone, was about 1.37 mm^2. The constant pass energy in the hemispherical analyser was about 150 eV and the detector range was about 18 eV, shared between 128 channels. In these conditions, the full width at half maximum (FWHM) of the $Au_{4f7/2}$ peak was about 1.66 eV. The following conditions were selected for individual peak analysis: Al anode powered at 10 kV and 10 mA, spot size of 600 μm corresponding to an irradiated zone of 0.49 mm^2, analyser pass energy of 50 eV giving a detector range of about 6.30 eV. In these conditions, the FWHM of the $Au_{4f7/2}$ peak was about 0.98 eV. The charge created by photoejection was compensated by a flood gun (6 eV) with a nickel grid (384 μm side square mesh, wire of 10 μm diameter) placed 2–3 mm above the sample surface. During the analyses, the vacuum in the chamber was between 2.5×10^{-6} and 2.4×10^{-7} Pa. The data treatment was performed with the ESCA 8.3D software provided by the spectrometer manufacturer. The peak areas were determined by linear background subtraction. The atom concentration ratios *R* of elements X and Y were computed directly with the spectrometer software from the peak areas normalized on the basis of acquisition parameters *I*, according to: $R_{X/Y} = (I_x/i_x).(i_Y/I_Y)$ where i_X is the sensitivity factor proportional to the Scofield cross section and to $(E_{kX})^{0.7}$, with E_{kX} being the kinetic energy of photoelectrons of element X; this implies the assumption that the transmission function of the spectrometer is constant over

the whole binding energy range. In the energy range considered here, these sensitivity factors have been proved to be reliable: the XPS C/O ratios on standard polymers (as PET (poly(ethylene terephthalate)) or PC (polycarbonate of bisphenol-E)) are close to those expected from the stoichiometry[3,24]. The atom fraction of the different elements, excluding hydrogen, was deduced from the atom concentration ratios. The peaks were decomposed using the software least squares fitting routine with a Gaussian/Lorentzian ratio 85/15. The data treatment involved first a decomposition of the carbon peak C_{1s}. The binding energy scale was calibrated by setting the component due to carbon only bound to carbon and hydrogen at 284.8 eV. The peak decomposition process of C_{1s} consisted of two main steps: (1) initial values for binding energies, FWHM and intensities of four to seven components were introduced, and curve fitting was performed without constraint; (2) the FWHM of all components of a given peak was set equal to the FWHM of component at 284.8 eV. If the FWHM of one component was to wide, a new component was added and a new fitting was performed with this constraint. Step 2 had sometimes to be repeated, with adjusted FWHM or binding energy values, in order to improve the Chi-square value.

The amount of ^{3}H-lysine associated with the PEEK samples (small disks) was measured by liquid scintillation counting (LSC)[16–18]. The disks were individually placed in 20 ml polyethylene vials (Milli-Q 20, Packard, San Diego, CA) and 5 ml of Aqualuma cocktails (Lumac, Basel, Switzerland) was added in each vial. A Tri-Carb 1600 TR liquid scintillation analyser (Packard) was used. The experimental counts per minute (CPM) were converted in disintegrations per minute (DPM) using the relationship DPM = CPM/counting efficiency. The results are expressed in pmol per surface unit (cm^2); each value is the average of, at least, five independent measurements performed with five samples similarly treated. Since the PEEK samples were totally insoluble in the scintillation cocktails, surface quenching could probably occur, leading to some under-evaluation of the counting; the corrective factor should be 2, as a maximum.

Surface chemistry

A large PEEK film sample (rectangle of 30 cm in length and 15 cm in width) was fixed on a home-made glass cylinder (height: 16 cm; diameter: 8.5 cm) and placed into a 1.5 l wide neck (diameter: 12 cm) reaction flask (Sovirel glassware) containing the reactive solution for the reduction. The neck was fitted, with a large diameter flange joint, in a lid with a two-socket neck equipped with a reflux condenser and a drying tube. The external side of the film was marked and considered for the surface analyses.

The PEEK-OH derivatizations and the radiochemical assays were performed on small film samples (disks of 1.2 cm in diameter) cut off the large film sample precursor.

After chemical treatment and suitable rinsing, the film samples were dried under vacuum ($p \leq 1$ mmHg) at 60°C. We used a Gallenkamp oven and an Edwards E2M5 pump, connected through two vapour-condensers cooled with liquid air.

Preparation of PEEK-OH film [22,23]. DMSO (1.2 l) and sodium borohydride (2.4 g) were introduced in the reaction flask and heated at 120°C under stirring (dissolution occurred). The PEEK film sample (30 cm × 15 cm) fixed on the glass cylinder was totally immersed into the reactive solution for 3 h at 120°C, then removed from the support and rinsed successively with methanol (15 min), water (10 min), 0.5 N HCl (10 min), water (10 min) and ethanol (5 min). After drying under vacuum (3 h, 60°C), the sample (PEEK-OH) was stored, in the dark, in a polystyrene box (damp and dust proof).XPS analysis; C_{1s} (85.16%): 284.8 eV (C–C, C–H), 286.4 eV (C–O), 287.1 eV (C = O), 291.6 eV (π); O_{1s} (14.84%): 531.5 eV (O = C), 532.4 eV (O–H), 533.3 eV (O–C), 539.8 eV (π-π*).MIR analysis (*Figure 1*): diminution of the C = O band at 1650 cm^{-1}; ν1650 cm^{-1}/ν1490 cm^{-1} = 0.55[22].

Preparation of PEEK-Ph-CO_2H film. A solution of 4-aminobenzoic acid (0.9 g) in acetic acid (30 ml) was introduced into a 100 ml round-bottomed flask. Five disks of PEEK-OH film were immersed in the reactive solution and stirred, at room temperature, for 3 days. The disks were taken off with tweezers and rinsed successively with acetic acid (2 × 10 min), water (3 × 10 min) and acetone (2 × 10 min) The samples (PEEK-Ph-CO_2H) were dried under vacuum (3 h, 60°C).

Preparation of PEEK-$(CH_2)_2$-CO_2H film. A solution of succinamic acid (3 g) and conc. H_2SO_4 (0.5 g) in acetic acid (100 ml) was introduced into a 250 ml round-bottomed flask. Fifteen disks of PEEK-OH were immersed in the reactive solution and stirred, at room temperature, for 3 days. The samples were rinsed and dried as described above.

Blank samples were prepared as above, but omitting the catalyst (H_2SO_4) in the reactive solution.

Activation of PEEK-$(CH_2)_2$-CO_2H. Method a: Five disks of PEEK-$(CH_2)_2$-CO_2H were immersed into a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide, WSC, 0.04 g) in 0.1 N MES buffer (40 ml), and shaken for 1 h at 20°C with an Edmund Bühler stirrer (model KL-2). The disks were taken off the reactive solution with tweezers and rinsed, under shaking, successively with 0.1 N MES buffer (1 × 10 min) and water (2 × 10 min). The samples were used directly in the radioactivity assays.

Method b: Five disks of PEEK-$(CH_2)_2$-CO_2H were immersed into a hot solution of $SOCl_2$ (0.05 ml) in dry toluene (50 ml), under argon atmosphere. The disks were stirred for 3 h at 110°C, then taken off and rinsed successively with dry toluene (1 × 10 min) and acetone (1 × 5 min), under argon atmosphere. The samples were directly used in the radioactivity assays.

Method c: Five disks of PEEK-$(CH_2)_2$-CO_2H were immersed into a solution of 1,1'-carbonyldiimidazole (CDI, 0.04 g) in dry THF (40 ml), under argon atmosphere. The disks were stirred for 15 min. at 20°C, then taken off and rinsed successively with dry THF (1 × 10 min) and acetone (1 × 5 min), under argon atmosphere. The samples were directly used in the radioactivity assays.

Coupling to [4,5-^{3}H]-lysine (lys).* To a 10^{-3} M solution of lysine in PBS buffer (50 ml) was added Lys* (375 μL of the commercial solution, specific activity of 82 Ci mmol^{-1}). The activated samples, prepared according to the methods a, b or c (disks of 1.2 cm in diameter), were individually treated by immersion in the radioactive solution distributed in small tubes (2 ml per tube). The disks were shaken for 3 h at 20°C, then taken off the solution with tweezers and rinsed three times with PBS buffer containing 0.1% Triton X-100 (2 ml per disk, 10 min). The

disks were drained over filter paper and directly assayed by LSC.

RESULTS AND DISCUSSION

Due to the insolubility of PEEK in the majority of organic solvents, modifications of the polymer bulk cannot be readily achieved. Recently, the soluble methyl-PEEK, obtained by the polycondensation of methylhydroquinone with 4,4'-difluorobenzophenone[25], was used as a key-intermediate for the introduction of acid functions. Bromination gave bromomethyl-PEEK which was further substituted with cyanide and hydrolysed into carboxy-methyl-PEEK[26]. From the dibromomethyl-PEEK, hydro-carbonyl- and carboxyl substituted PEEKs were easily prepared[27]. Another approach towards carboxyl-containing PEEKs was based on the polycondensation of 4,4-bis(*p*-hydroxyphenyl)pentanoic acid with 4,4'-difluorobenzo-phenone[28].

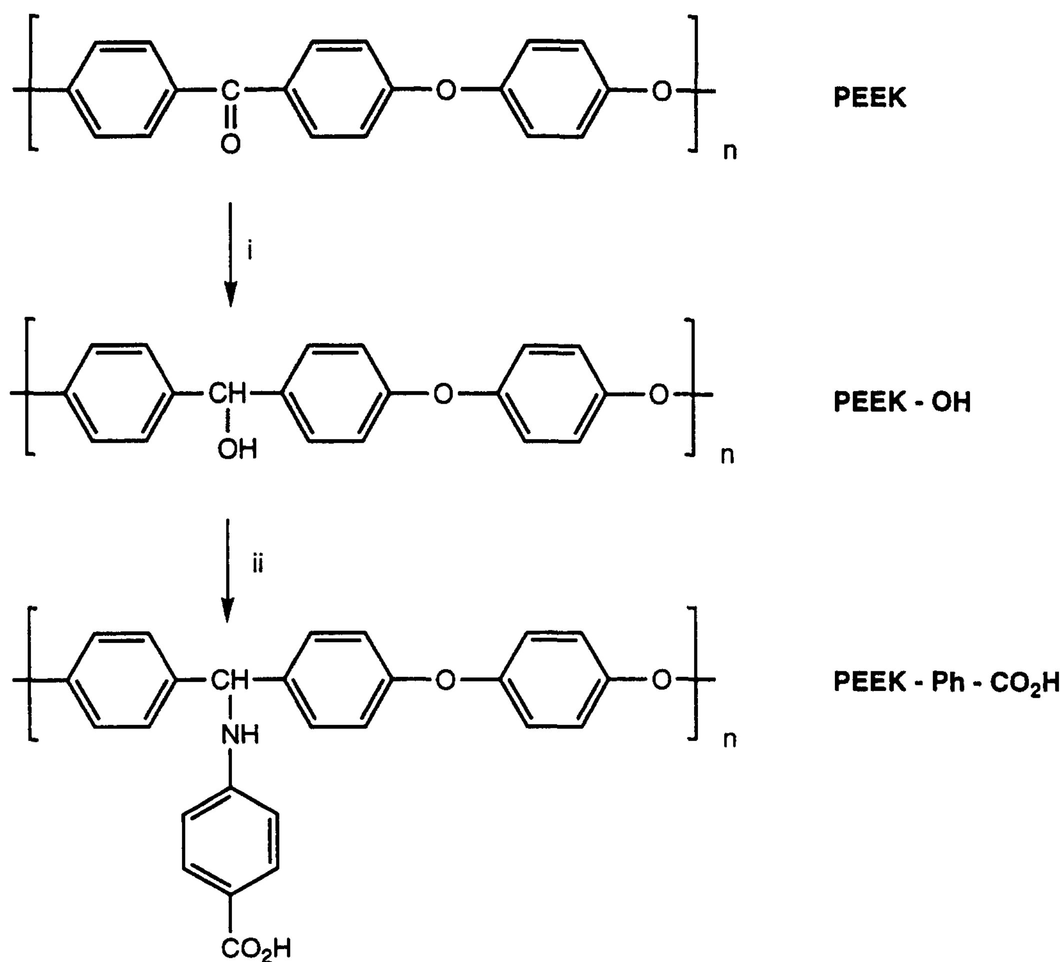
Surface carboxylation of PEEK film appears moderately documented in the previous literature. Some aggressive, non selective, reactions involving polymer chain cleavages were performed at the solid–gas interface, i.e. irradiation[29] with He^{+2}, photooxidations with oxygen[30,31] or ozone[32,33], and plasma treatments[34–36]. One non-selective reaction carried out at the solid–liquid interface was mentioned: the oxidation with potassium chlorate in aqueous sulfuric acid[37].

We have already established that the surface reduction of PEEK film furnishes a practical and versatile intermediate, the PEEK-OH film (*Scheme 1*), which can be further used in various substitution reactions[22,23]. In a model study considering the 4,4'-dimethoxybenzhydrol as a PEEK-OH mimic[38], we found that the hydroxyl group is easily substituted by the aniline function of 4-aminobenzoic acid, and by the amide function of succinamic acid, in acetic acid solution at room temperature. The first reaction can be readily performed without any catalyst, while the second substitution requires the presence of a strong acid catalyst, like sulfuric acid. These two reactions have been now successfully applied to the surface carboxylation of the PEEK-OH film.

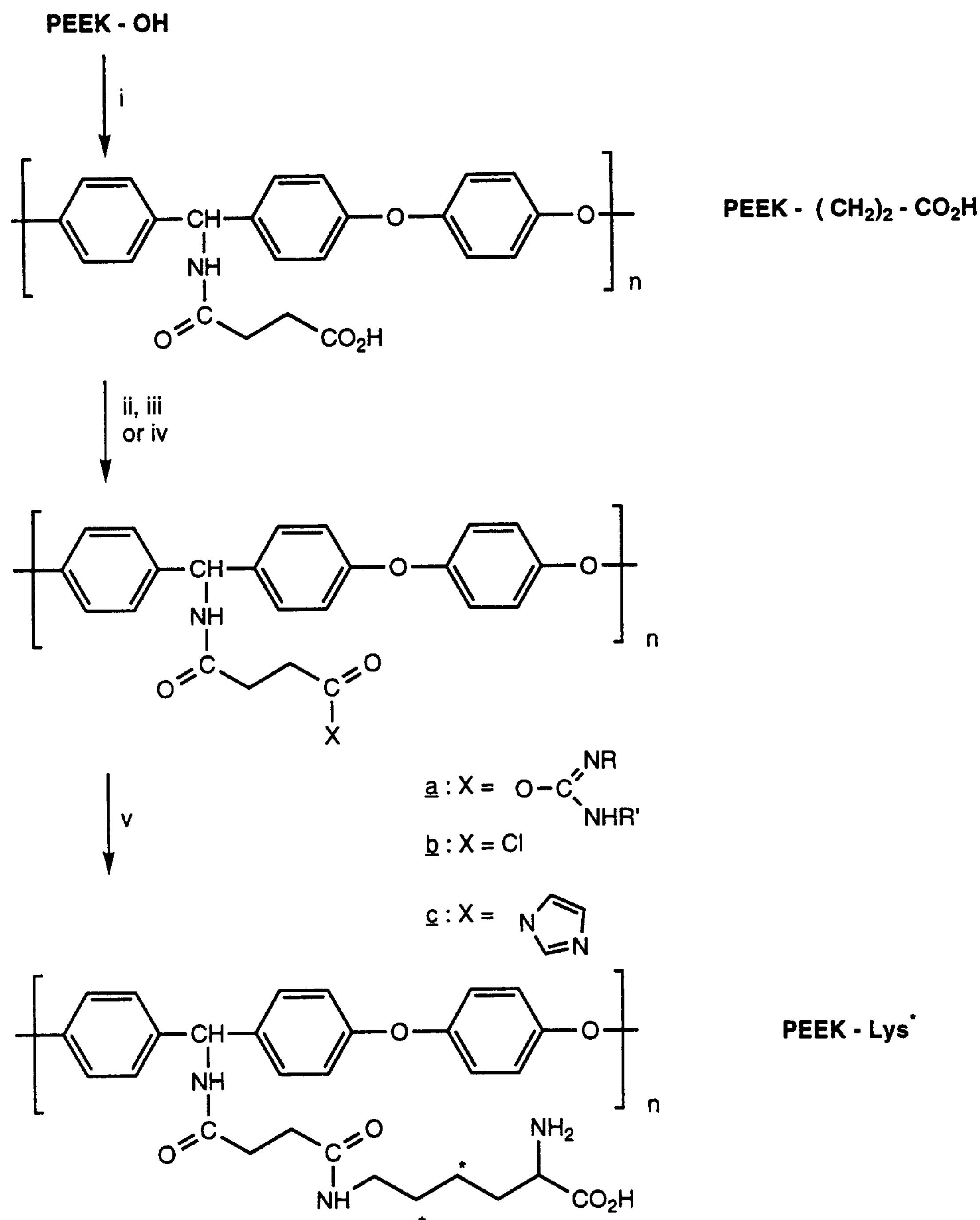
Reaction of PEEK-OH with 4-aminobenzoic acid

The PEEK-OH starting material was prepared, as previously described[22,23], by surface reduction of the amorphous PEEK film with sodium borohydride dissolved in hot dimethylsulfoxide. The amount of reduced monomer units depends on the temperature: for treatments at 100°C, 110°C and 120°C, during 3 h, the percentages of surface hydroxylation determined by XPS were respectively of about, 60%, 70% and 80%. We measured the $\underline{C}=O/\underline{C}-O$ and the $\underline{O}=C/\underline{O}-C$ atomic ratios in the fine structures of the C_{1s} and O_{1s} peaks[22].

The PEEK-OH film (60% of reduction) was immersed for 72 h at room temperature into a solution of 4-aminobenzoic acid (3%, w/v) in pure acetic acid, then suitably washed and dried. The XPS analysis of the sample clearly showed the presence of nitrogen atoms at 400 eV, due to the hydroxyl substitution with the aniline motif (*Scheme 1*). The



Scheme 1 Preparation of PEEK-OH and PEEK-Ph-CO_2H. Reagents and conditions: (i) $NaBH_4$, DMSO, 120 °C, 3 h, then 0.5 N HCl; (ii) 4-aminobenzoic acid (3%, *w/v*), HOAc, 20 °C, 72 h.



Scheme 2 Preparation of PEEK-$(CH_2)_2$–CO_2H, activation and coupling to lysine. Reagents and conditions: (i) H_2NCO–$(CH_2)_2$–CO_2H (3%, *w/v*), H_2SO_4 (0.5%, *w/v*), HOAc, 20°C, 72 h; (ii) WSC (0.1%, *w/v*), 0.1 N MES buffer, 20°C, 1 h; (iii) $SOCl_2$ (0.1%, *w/v*), toluene, 110°C, 3 h; (iv) CDI (0.1%, *w/v*), THF, 20°C, 15 min; (v) 10^{-3} M lysine, PBS buffer, 20°C, 3 h.

experimental N/C × 100 atomic ratio was 1.12, corresponding to 23% of derivatization* (corrected yield = 38% taking into account the ratio of initial reduction). The chemical structure of the PEEK-Ph-CO_2H surface was further confirmed by the analysis of the fine structure of the C_{1s} peak: as compared to the PEEK-OH precursor, a new component was found at 289.16 eV, attributed to the carboxyl carbon atom.

In order to improve the grafting of 4-aminobenzoic acid on the PEEK-OH surface, we tried to catalyse the reaction with sulfuric acid[38]. Addition of 0.5% (w/v) H_2SO_4 into the reactive solution caused some precipitation of the 4-aminobenzoyl reagent from the acetic acid solution. Immersion of the PEEK-OH sample in this heterogeneous medium during 72 h at 20°C gave a PEEK-Ph-CO_2H film showing a N/C × 100 atomic ratio of 0.33, from the XPS analysis. This result corresponds to 7% of derivatization (see footnote 1, corrected yield = 12%). Thus, we were unable to improve the coupling of 4-amino-benzoic acid to PEEK-OH by catalysing the reaction. The coupling of succinamic acid was therefore preferred.

Reaction of PEEK-OH with succinamic acid

Succinamic acid was coupled to PEEK-OH under various experimental conditions (*Scheme 2*, route i); we examined different reagent concentrations in acetic acid (2%, 3% and

* We considered a theoretical monomer unit consisting of [(PEEK-OH)$_x$ + (PEEK-Ph-CO_2H)$_y$], i.e. [$(C_{19}O_3)_x$ + $(C_{26}O_4N)_y$], where $x + y = 100$. For $x = 77$ and $y = 23$, the calculated N/C × 100 atomic ratio was 1.11 (experimental value = 1.12). For $x = 93$ and $y = 7$, the calculated N/C × 100 atomic ratio was 0.35 (experimental value = 0.33).

Table 1 Preparation of PEEK-$(CH_2)_2$-CO_2H films

Entry	Condition				XPS analysis			SEM analysis
	PEEK-OH (% OH)	Succinamic acid (%, w/v)	H_2SO_4 catalyst (%, v/v)	Time (h)	N/C × 100	Yield (%)	Corrected yield (%)	
1	71	3	0.1	48	0.51	10	14	Smooth surface
2	71	3	0.1	72	0.84	17	24	Smooth surface
3	71	3	0.1	96	1.49	30	42	Surface erosion
4	60	2	0.5	72	0.89	17	28	Smooth surface
5	60	3	0.5	72	1.94	40	66	Smooth surface
6	60	4	0.5	72	1.22	25	41	Smooth surface
7	77	3	1.0	72	3.75	80	~100	Surface erosion
8	85	3	0.5	72	2.81	60	70	Smooth surface
9	78	3	0.5	72	2.23	46	60	Smooth surface
10	62	3	0.5	72	2.01	42	67	Smooth surface

4%), different catalyst concentrations (0.1%, 0.5% and 1% H_2SO_4) and different reaction times (1 day to 4 days) (*Table 1*). In all cases, we prepared blank samples, i.e. PEEK-OH films treated in the selected conditions, but omitting the acid catalyst. In the corresponding XPS analyses, nitrogen atoms could not be detected; thus, the substitution reaction with succinamic acid well requires a strong acid catalyst for activating the PEEK-OH surface. The various treated samples were analysed by SEM; some surface erosion was visible for samples treated during 4 days (entry 3), and with 1% of H_2SO_4 catalyst (entry 7). Otherwise, the surfaces of the PEEK-$(CH_2)_2$-CO_2H samples appeared smooth and clean. The percentages of surface derivatization were calculated from the XPS spectra (*Figure 2*), by measuring the N/C × 100 atomic ratios.† The yields covered a range of 10–80%, depending on the experimental conditions. We selected, as standard conditions, the reaction of PEEK-OH with 3% (w/v) succinamic acid in acetic acid containing 0.5% (v/v) of conc. sulfuric acid during 72 h at room temperature (entry 5), followed by the suitable washings and drying. This procedure was fully reproducible, starting from PEEK-OH samples displaying 60–85% surface hydroxyl groups (entries 8, 9 and 10); the corrected yields were within 60–70%.

The PEEK-$(CH_2)_2$-CO_2H surface (sample of the entry 8) showed a water contact angle of 75.3° (± 0.8); in the MIR spectrum (*Figures 1, and 3*), we observed new bands, at 1740 cm^{-1} corresponding to the carboxyl function, and at 1648 cm^{-1} due to the amide function. The fine structure of the C_{1s} peak, in the XPS spectrum (sample of the entry 7) revealed the presence of $\underline{C}O_2H$ motifs (289.35 eV, 4.05%), O = $\underline{C}$–N motifs (288.17 eV, 3.61%), and $\underline{C}$–N motifs (285.67 eV, 3.92%), in almost the same concentration (*Figure 2*). We performed an angular-dependent XPS study (sample of the entry 6): the N/C atomic ratios were not significantly different for photoemission take-off angles of 15°, 35° and 90°. We can therefore consider that the PEEK derivatization is homogeneous throughout the usual XPS sampling depth (~50–80 Å).

Reactivity-assay of PEEK-$(CH_2)_2$-CO_2H

One of our objectives was to further use the surface carboxyl groups as anchorage points for the immobilization of various molecules of interest. For that purpose, we have established the best conditions of surface activation.

In classical organic synthesis, carboxyl functions are routinely activated by reaction with a carbodiimide reagent; this method has been readily applied in the case of polymer surfaces[3,15,16,18,39]. Thus, the PEEK-$(CH_2)_2$-CO_2H film was immersed during 1 h at 20°C into a solution of WSC (0.1%) in MES buffer (*Scheme 2*, route ii, product *a*). The efficiency of this activation step was controlled by coupling with ^{3}H-lysine under the standard conditions that we previously established for the labelling of the carboxyl chain ends of poly(ethylene terephthalate) membranes[3]. LSC of the radioactivity associated with the resulting PEEK-Lys* sample (*Scheme 2*, route v) gave a corrected value (subtraction of the blank value) of ± 30 pmol cm^{-2} of fixed label (*Table 2*, entry 1). Appropriate washings allowed to remove most of the unreacted ^{3}H-lysine; thus the measured sample radioactivity could be correlated with the amount of covalently fixed label. Nevertheless, some irreversible adsorption or diffusion of the radioactive label into the polymer interface could not be avoided. This contribution of non specific fixation of lysine was estimated by the counting (LSC) of blank samples prepared by omitting the WSC activation reagent in the previously described procedure (*Table 2*, entry 2).

The selectivity of the WSC activation towards the carboxyl functions was controlled by treating as above the PEEK-OH film; the level of radiolabelling was significantly diminished (± 20 pmol cm^{-2}; *Table 2*, entry 3), proving some chemoselectivity in favour of the carboxyl functions. However, the amount of aminoacid that could be fixed on WSC-activated PEEK-$(CH_2)_2$-CO_2H film is rather low. This could result from a poor wetting of the polymer surface by the aqueous solution of carbodiimide.

Therefore, we examined the possibility of activating the PEEK-$(CH_2)_2$-CO_2H film in dry organic medium. Oxalyl chloride[28] and thionyl chloride[40–42] have been used to form chlorocarbonyl motifs on the surface of carboxyl-enriched polymers. The treatment of PEEK-$(CH_2)_2CO_2H$ in hot toluene containing 0.1% of thionyl chloride (*Scheme 2*, route iii, structure *b*) was followed by the coupling to ^{3}H-lysine under the standard conditions. From LSC, we determined that about 170 pmol cm^{-2} (corrected value) of lysine have been immobilized (*Table 2*, entries 5 and 6). By similarly treating the PEEK-OH film, we obtained a value of 130 pmol cm^{-2} (corrected value) of fixed lysine (*Table 2*, entries 7 and 8). Thus, this activation is not highly chemoselective; both the carboxyl and the hydroxyl functions were transformed into chlorocarbonyl and chloride functions[21,37] respectively, susceptible to further

† We considered a theoretical monomer unit consisting of $[(\text{PEEK-OH})_x + (\text{PEEK-(CH}_2)_2\text{-CO}_2\text{H})_y]$, i.e. $[(C_{19}O_3)_x + (C_{23}O_5N)_y]$, where $x + y = 100$.

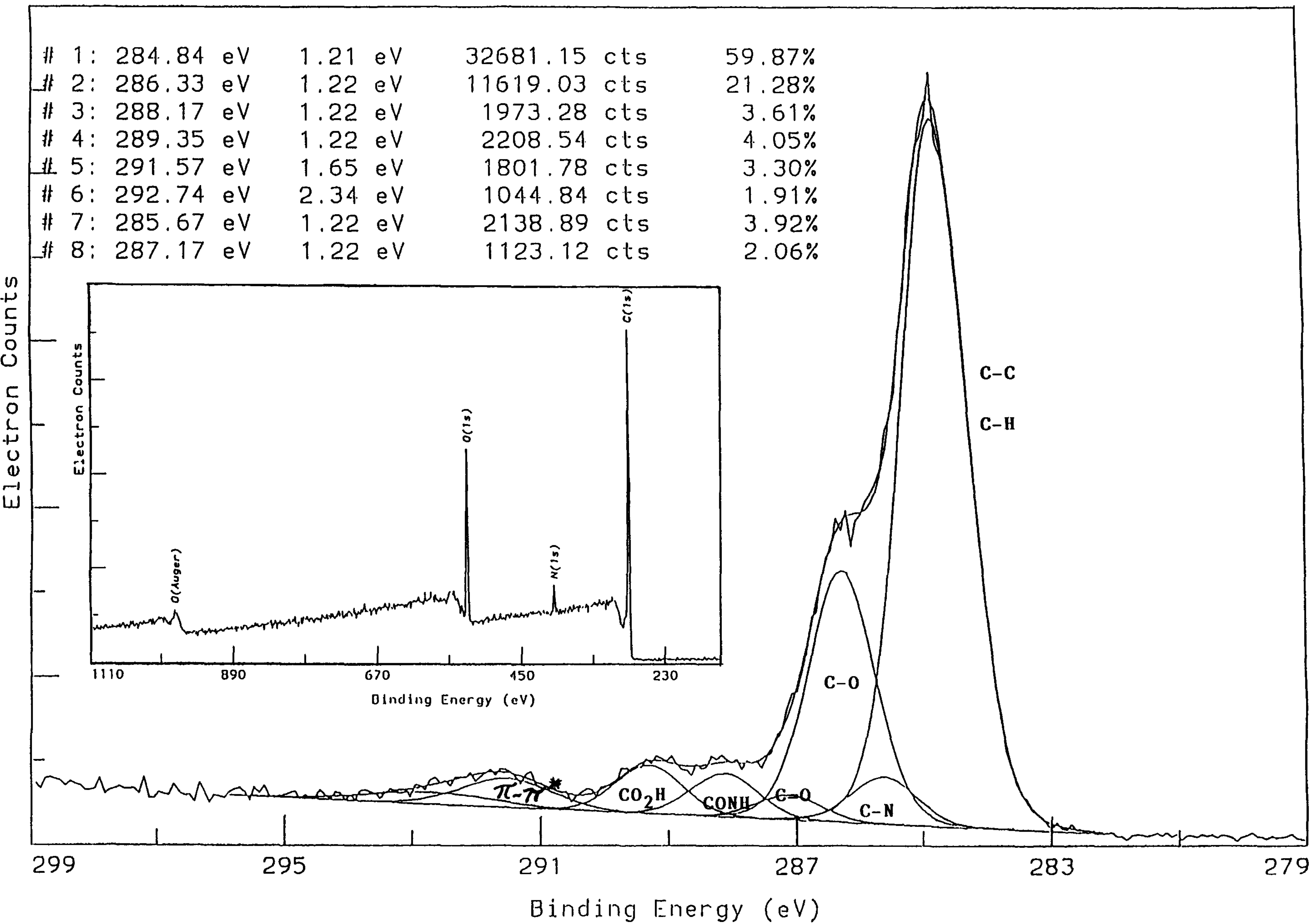


Figure 2 XPS analysis of PEEK-$(CH_2)_2$-CO_2H film: (insert) general spectrum; (main figure) detailed C_{1s} peak

reaction with lysine by nucleophilic substitution. The same results were collected by using oxalyl chloride in the activation step.

Last, we considered the CDI[43] as a selective activating reagent towards the carboxyl functions displayed on the surface of the PEEK-$(CH_2)_2$-CO_2H film. The sample was immersed into a solution of CDI (0.1%) in toluene for 30 min at room temperature (*Scheme 2*, route iv, structure *c*), then labeled with ^{3}H-lysine (standard procedure). We recorded a value of about 80 pmol cm^{-2} (corrected value) of fixed lysine (*Table 2*, entries 9 and 10). Under similar conditions, the PEEK-OH film was not labeled, proving the complete chemoselectivity of CDI (*Table 2*, entries 11 and 12). This last route was therefore selected as a model strategy for the coupling of biological signals on the PEEK film. We have further optimized this route by examining the effect of the reagent concentration, the solvent nature and the reaction time.

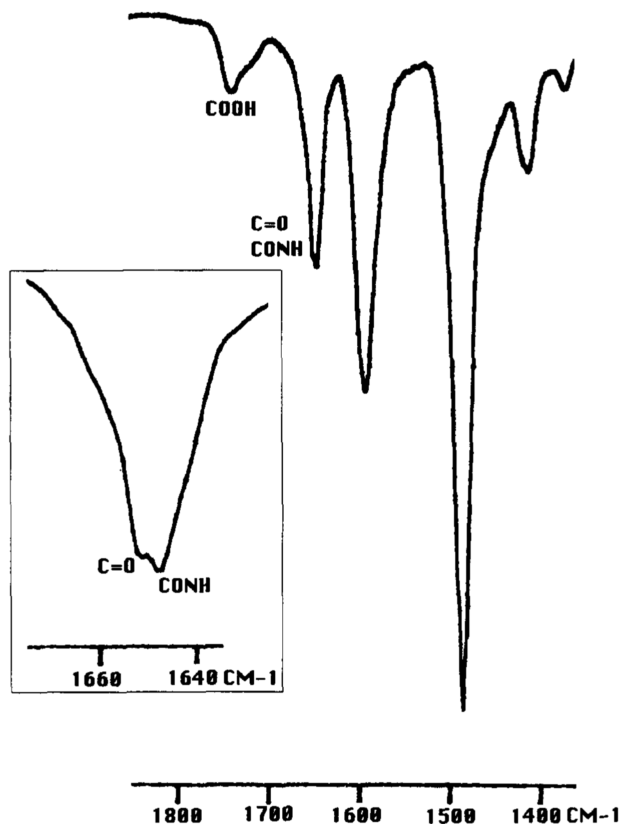


Figure 3 MIR analysis of PEEK-$(CH_2)_2$-CO_2H film

In a first set of experiments, the CDI concentration in toluene was increased from 0.05 to 0.5% (*Table 3*, entries 1 to 3), and the duration of the treatment was increased from 15 to 120 min (*Table 3*, entries 4 to 7); these modifications induced negative effects, i.e. a diminution of the radiolabelling. This could probably result from some erosion (partial dissolution)[19] of the modified polymer interface. In a second set of experiments, we used THF (*Table 3*, entries 10 to 13) and dioxane as solvents (*Table 3*, entries 14 and 15). We observed an enhancement of the radiolabelling as compared to similarly treated samples in toluene (*Table 3*, entries 8 and 9). We conclude that THF and dioxane should possess good wetting properties towards the PEEK-$(CH_2)_2$-CO_2H surface.

The selected conditions for aminoacid grafting were thus: activation with 0.1% CDI in THF for 15 min at 20°C followed by coupling with the label at 10^{-3}M in PBS buffer for 3 h at 20°C and washing with PBS containing 0.1% Triton X-100. This mild procedure gave a ratio of surface derivatization of about 180 pmol cm^{-2} (corrected yield obtained by subtraction of the blank).

Generally, the sampling depth reached by radiochemical assays strongly depends on the experimental conditions used for the label fixation[16,17]. Working in water (10^{-3} M ^{3}H-lysine in PBS buffer), a poorly wetting solvent of PEEK, we should most probably affect the outermost layers of the polymer suface. Assuming[22] that one PEEK monomer unit should occupy a volume of 6.24×10^{-22} cm^3, we calculated‡ that an interface domain of 65 Å depth (usual

Table 2 Selectivity of PEEK-$(CH_2)_2$-CO_2H activation

Entry	Origin of sample	Activation	Fixed [Lys*]^a (pmol cm^{-2})
1	PEEK-CO_2H, *Table 1*, entry 8	WSC (0.1%), MES buffer, 1 h, 20°C	35 (± 10)^b
2	PEEK-CO_2H, *Table 1*, entry 8	blank^d	7 (± 2)^b
3	PEEK-OH (60%)	WSC (0.1%), MES buffer, 1 h, 20°C	19 (± 5)^b
4	PEEK-OH (60%)	bank^d	2 (± 1)^b
5	PEEK-CO_2H, *Table 1*, entry 10	$SOCl_2$ (0.1%), toluene, 3 h, 110°C	210 (± 26)^c
6	PEEK-CO_2H, *Table 1*, entry 10	blank^d	44 (± 11)^c
7	PEEK-OH (62%)	$SOCl_2$ (0.1%), toluene, 3 h, 110°C	150 (± 30)^c
8	PEEK-OH (62%)	blank^d	23 (± 8)^c
9	PEEK-CO_2H, *Table 1*, entry 10	CDI (0.1%), toluene, 15 min, 20°C	135 (± 38)^c
10	PEEK-CO_2H, *Table 1*, entry 10	blank^d	63 (± 11)^c
11	PEEK-OH (62%)	CDI (0.1%), toluene, 15 min, 20°C	11 (± 0.9)^c
12	PEEK-OH (62%)	blank^d	13 (± 1)^c

^{a}10^{-3}M lysine, PBS buffer, 3 h, 20°C
^{b}washing with PBS buffer and Triton X100
^{c}washing with water, 10^{-2}M HCl and 10^{-2}M Na_2CO_3
^{d}non activated samples treated with 3H-lysine

‡ Volume of one monomer unit: 6 Å × 13 Å × 8 Å = 6.24×10^{-22} cm^3. Volume of eight atomic layers (65 Å depth) covered by 1 cm^2 = 6.5×10^{-7} cm^3. This volume contains 6.5×10^{-7} cm^3/6.24×10^{-22} cm^3 = 1.04×10^{15} monomers units, corresponding to $1.04 \times 10^{15}/6.03 \times 10^{23}$ = 0.1725×10^{-8} mol of $C_{19}H_{12}O_3$, or 1725 pmol.

Table 3 Conditions of CDI activation (20°C)

Entry	Origin of the sample PEEK-$(CH_2)_2$-CO_2H	Conditions			Fixed[Lys*][a] (pmol cm^{-2})
		CDI concentration	Solvent	Time	
1	30% Carboxyl, from PEEK-OH (62%)	0.05%	Toluene	30 min	196 (±) 38[c]
2	30% Carboxyl, from PEEK-OH (62%)	0.1%	Toluene	30 min	225 (±) 23[c]
3	30% Carboxyl, from PEEK-OH (62%)	0.5%	Toluene	30 min	109 (±) 25[c]
4	30% Carboxyl, from PEEK-OH (62%)	0.1%	Toluene	15 min	280 (±) 31[c]
5	30% Carboxyl, from PEEK-OH (62%)	0.1%	Toluene	45 min	148 (±) 24[c]
6	30% Carboxyl, from PEEK-OH (62%)	0.1%	Toluene	75 min	115 (±) 43[c]
7	30% Carboxyl, from PEEK-OH (62%)	0.1%	Toluene	120 min	96 (±) 27[c]
8	*Table 1*, entry 10	0.1%	Toluene	15 min	135 (±) 38[c]
9	*Table 1*, entry 10	0% (blank)	Toluene	15 min	63 (±) 11[c]
10	*Table 1*, entry 10	0.1%	THF	15 min	350 (±) 62[c]
11	*Table 1*, entry 10	0% (blank)	THF	15 min	131 (±) 22[c]
12	43% Carboxyl, from PEEK-OH (77%)	0.1%	THF	15 min	219 (±) 47[b]
12	43% Carboxyl, from PEEK-OH (77%)	0% (blank)	THF	15 min	44 (±) 4[b]
14	*Table 1*, entry 10	0.1%	dioxane	15 min	206 (±) 24[c]
15	*Table 1*, entry 10	0% (blank)	dioxane	15 min	120 (±) 19[c]

Footnotes as in *Table 2*

sampling depth reached by XPS; ± 8 atomic layers) covered by 1 cm^2 of surface contains about 1700 pmol of repetitive units. Approximately 40% of these units are equipped with the carboxylated arm (XPS analysis), corresponding to 680 pmol of units (maximum value) susceptible to fix the radioactive label. Thus, the experimental LSC value of 180 pmol cm^{-2} means that, at least, two atomic layers (15 Å depth) have been reached by the assay, considering a quantitative chemical yield of labelling. Now, competition between nucleophilic substitution with ^{3}H-lysine and hydrolysis of the activated carboxyl functions (*Scheme 2, c*) would certainly occur during the labelling process in aqueous solution. Therefore, we could assume that the LSC sampling depth is within 15–65 Å.

CONCLUSION

Using the wet-chemistry technique, we could purposely and selectively modify the PEEK film surface. Carboxyl functions were introduced according to an original two-step sequence: $NaBH_4$ reduction produced an hydroxylated surface (60–80% yield), the hydroxyl groups of which were readily substituted by the amide function of succinamic acid under acidic conditions (60–70% yield). The PEEK-$(CH_2)_2$-CO_2H surface was fully characterized by θ_w, SEM, MIR and XPS. The reactivity of the carboxylated PEEK film was finely controlled by radiochemical assays with ^{3}H-lysine, after appropriate activation. The mild experimental conditions of labelling, carried out in aqueous medium, mimic at best the grafting of bioactive molecules like proteins and peptides. Accordingly, the present analytical work has established the quantitative bases required for the further development of active biocompatibilization strategies.

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