Synthesis and adsorption of a poly(N-acetylethyleneimine)-polyethyleneoxide-poly (N-acetylethyleneimine) triblock-copolymer at a silica/solution interface. Influence of its preadsorption on platelet adhesion and fibrinogen adsorption

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The synthesis of a triblock copolymer poly-(N-acetylethyleneimine)-polyethylenoxide-poly(N-acetylethyleneimine) includes two successive steps: the first is the functionalization of a poly(ethyleneglycol) precursor by creating sulfonic esters at its chain ends, the second uses these esters to initiate the cationic polymerization of 2-methyl-2-oxazoline. Homopolymers appear in the raw product; hence successive selective extractions of the copolymer with benzene and dioxane are necessary. The final yield in pure copolymer was 11%. The copolymer was characterized by UV and ^{1}H-NMR spectrometry and light scattering. Adsorption isotherms were determined on silica, for varying pH and salt concentration. Optimum conditions for coating silica with the polymer were determined. The efficiency of this precoating to reduce the adsorption of fibrinogen was very high (99.2% reduction with respect to bare silica). Steric exclusion chromatography of a variety of proteins gave a satisfactory calibration curve. Platelet accumulation on copolymer precoated glass was reduced to 10–20% of its value on bare glass, a result superior to that obtained by albumin passivation of the same glass surface.

INTRODUCTION

The interaction between artificial materials and aqueous suspensions such as blood or solutions of proteins leads to a complex series of events, the first one being the adsorption of various low-molecular-weight solutes. This first step is followed by displacement by higher-molecular-weight compounds,

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which initiates a cascade of reactions, resulting in thrombus formation in the presence of whole blood, unless the interface possesses antithrombogenic properties.[1,2] Such an undesirable result has to be avoided with synthetic materials aimed to be in contact with flowing blood. Among blood proteins, fibrinogen has been extensively studied, since it has been shown to increase, through its preferential adsorption, the thrombogenicity of artificial surfaces.[3] However, at high plasma concentrations, decrease of fibrinogen adsorbance versus time and plasma concentration was described as the Vroman effect. The ability of fibrinogen to adsorb on a new surface is therefore a first hint of the surface thrombogenicity. Additionally, blood platelet adhesion, and the possible subsequent modification or/and activation of the adherent platelets, represents an indication of blood-material interaction.[2,4]

Before considering synthesis and large-scale manufacture of a new biomaterial, the potentialities of such a new material may be seen by adsorbing or grafting it onto a solid surface, and studying the interactions between the new interface and protein solutions or blood components.

Coating procedures on solid supports like beads could likewise lead to improvements in separation processes such as steric permeation chromatography in aqueous medium. The mechanical resistance of the support associated with a nonadsorbing interface would permit the application of high pressures, not possible with soft hydrophilic gels. We present in this article the synthesis of a block copolymer with a polyethyleneoxide (PEO) sequence linking two sequences of poly(N-acetylethyleneimine) (PAEI). The PEO is designed to improve blood compatibility,[5] while the PAEI should anchor the copolymer on the solid silica surface through potential strong hydrogen–bond interactions between the silanols and the tertiary amide functions.

The adsorption isotherms on silica are determined by a chromatographic technique. The method is described in some detail since it is not generally used: it does not involve desorption to determine amounts of adsorbed polymer.

The adsorbances of fibrinogen on bare and polymer precoated silica surfaces are then compared and calibration curves from chromatography of proteins are presented. Finally, we show the efficiency of such a polymer precoating for the reduction of interfacial platelet accumulation.

MATERIALS

Solvents, reagents, monomer, and polymer precursor

All solvents and the monomer 2-methyl-2-oxazoline (MOXZ) were purified by distillation over appropriate drying reagents: tetrahydrofuran (THF) over disodium benzophenone complex; acetonitrile (ACN) over P_2O_5; pyridine, MOXZ, and dimethylacetamide (DMAC) over CaH_2 (vacuum distillation for DMAC). The solvents were stored in Schlenk vessels over molecular sieves 4 Å under argon.

Benzenesulfonyl chloride was distilled under vacuum just before use, and *p*-toluenesulfonylchloride (TsCl, Aldrich 99%) was used without further purification.

Polyoxyethyleneglycol (PEO H-20,000, Hoechst) was purified by precipitation from $CHCl_3$ solution into a 10-fold excess of diethylether. GPC measurements (H_2O, NaCl 0.1 M, Sephacryl columns, calibration with Toyo Soda PEO standards) lead to $\overline{M}_n = 2.43 \times 10^4$ daltons, $\overline{M}_w/\overline{M}_n < 1.1$.

Silica

Silica was purchased from Rhône-Poulenc under the reference Spherosil XOC 005. Specific area was 10 m^2/g and mean diameter 300 nm. Sphere pearl diameter lay in the range 100–200 μm. Suspensions were carefully degassed before experiments.

Fibrinogen

Fibrinogen was purchased from Kabi (grade L, 90% clottable). One-milliliter aliquots at 1% (w/w) in distilled water, were stored at −30°C. Before use it was quick thawed at 37°C. Distilled water was prepared with a deionizing and filtering device Super-Q-Millipore. ^{125}I-labeling was carried out following the ICl method described by Krohn et al.[6]

Polymer solutions

The polymer was dissolved in 20 mL of water under gentle agitation at concentration 1% (w/w) until complete dissolution. Afterward, the solutions were carefully degassed under reduced pressure.

METHODS

Synthesis of the PEO macroinitiators and of the block copolymers

All experiments were carried out under a slight pressure of argon in an all Pyrex glass double-wall reactor fitted with a magnetic or mechanical stirrer, allowing the use of vacuum and argon sweeping cycles; temperature was monitored by an external thermostat regulated to ±0.1°C.

PEO α,ω-benzenesulfonate through PEO sodium alkoxide

A 10% (w/w) solution of PEO in THF was titrated at room temperature with a solution of naphtalene–sodium in THF (#0.5N), the endpoint being visualized by the persistance of a characteristic slight green coloration[7]

(POE-OH → POE-O^-, Na^+ + a slight excess of the metallizing reagent). Benzene sulfonylchloride ($[C_6H_5SO_2Cl]/[Na^+] = 1.2$) was added dropwise to the very viscous (almost gelified) alkoxide solution: The viscosity dropped immediately and stirring was maintained overnight at room temperature. After separation of insoluble NaCl by centrifugation, the polymer was recovered by precipitation into a 10-fold excess of diethylether; it was further purified by solubilization in benzene, centrifugation and reprecipitation; the PEO sulfonate was dried at 35°C under 10^{-2} mm Hg. Yield #80%.

PEO α,ω-tosylate according to a Schotten-Bauman condensation process[8]

A strong excess of TsCl ([TsCl]/[OH] = 4) was added to a solution of PEO in pyridine (20% w/v precooled to 0°C) and stirring is maintained overnight at 0°C. The polymer was recovered by slow precipitation of the milky solution into a 10-fold excess of diethylether precooled to −20°C; it was purified by four successive crystallizations in ethanol (dried over molecular sieves 3 Å): solubilization at 45°C followed by crystallization at 0°C for 15 h, and finally washing of the polymer with diethylether. This multistep purification was necessary to obtain the PEO tosylate free of pyridinium hydrochloride, as checked by thin-layer chromatography. Yield # 75%.

PEO–PAEI block copolymers

The polymerization of MOXZ initiated by PEO α,ω-disulfonate was carried out either in homogeneous solution (ACN or DMAC) or in bulk between 80 and 105°C, as detailed in Table I. The reaction medium was diluted in hot methanol and the copolymer was recovered by precipitation into a 10-fold excess of diethylether; it was further washed with diethylether in a Waring

TABLE I
Cationic Polymerization of 2-Methyl-2-Oxazoline Initiated by PEO α,ω-Aromatic Sulfonates

	Synthesis I	Synthesis II	Synthesis III	Synthesis IV
Initiator				
PEO-SO_3Ar	Ar = phenyl	Ar = phenyl	Ar = phenyl	Ar = p-tolyl
$\overline{M}_n$ = 24700	m = 6.81 g	m = 4.20 g	m = 7.75 g	m = 50.4 g
Monomer				
2-methyl-2-OXZ	13.3 g	8.5 g	15.7 g	129 g
Solvent	CH_3CN 70 mL	DMAC 60 mL	Bulk	Bulk
Temperature	80°	90°	95°C	105°C
Reaction time	17 h	17 h	17 h	17 h
Monomer conversion (w%)	42	28	82	85

blendor and dried at 50°C under 10^{-2} mm Hg. Fractionation of the crude copolymer was performed by two successive selective extractions (vigorous stirring of 10% w/v copolymer suspension at 60°C overnight) first in benzene, and then in dioxane for the benzene insoluble fraction.

Physicochemical characterization of the polymers

^{1}H-NMR spectra were recorded at room temperature on a Perkin-Elmer apparatus using D_2O solution (chemical shifts, δ ppm, were measured with respect to HMDS as external reference).

Ultraviolet measurements were carried out on a Beckman Acta V Spectrometer. The refractive index increments were measured on a Brice-Phoenix BP-1000 differential refractometer for $\lambda = 632$ nm; $dn/dc = 0.154$ and 0.202 mL $\cdot$ g^{-1} for PEO and PAEI, respectively, in methanol solution at 40°C.

Light scattering experiments were performed on a Fica PGD 420.0M apparatus under the same temperature conditions in order to avoid aggregation phenomena which occur for PEO solution in methanol at room temperature.[9]

Chromatographic method to determine isotherms

The batch technique, widely used in the field of polymer adsorption to determine equilibrium isotherms, consists of measuring the depletion of the supernatant concentration in presence of the adsorbant. The decrease in polymer solution concentration corresponds to the excess amount adsorbed. However, there exist some limitations of the technique, especially with an aqueous medium. Various causes lead to rather disperse results. Among these is the mechanical fragility of the adsorbant and the difficulty of stabilizing the properties of the suspensions, pH for instance. Moreover, for the polydisperse systems encountered with synthetic polymers, the results are dependent on the ratio adsorbant area/volume of solution.[10] Thus we were led to propose a chromatographic technique.

To avoid stirring of fragile particles, these particles can be fixed inside a column which constitutes the flow path for the solution of adsorbing molecules. The variations of the pertinent parameters are examined on the column itself (radioactivity γ)[11–13] or at the exit of the column by any technique suitable to measure a volume concentration, as for instance, UV absorbance, or radioactivity β.[14]

The adsorbant can be easily conditioned by flowing large volumes of solvent through the column. The direct measurements on the column, with γ-labeled tracers, have already been published.[11,12] Thus we focus below on the recorded elution curves at the column exit.

Let C be the concentration of a solution whose solute molecules may adsorb on the column packed beads. At first the liquid phase in the column is

a pure solvent or buffer. Let us inject at time zero a solution of concentration C. The eluted solution at the column exit starts from zero during at least the time corresponding to the void volume, then increases to reach progressively the entrance concentration C. The slower is the increase, the higher is the adsorption in the column. No adsorption leads to the steepest increase. Recording of the exit concentration with (C') and without (C'') adsorption allows determination of the adsorbed amount, which is proportional to the area between the two curves (Fig. 1). Let J be the flow rate common to the two injections. The total adsorbed amount is given by summing over time the entity $[C''(t) - C'(t)]J$. From a general point of view, we measure

$$\int_0^\infty [C''(v) - C'(v)]dv$$

where v is the elution volume.

The foregoing principle leaves however a crucial question: How, in practice, does one obtain the curve without adsorption? There we take advantage of one particular property of proteins and macromolecules in general. They are in an adsorbed metastable state in the presence of pure solvent. Therefore the following procedure (Fig. 2) is used: (i) passage of the solution (adsorption curve), (ii) rinsing with solvent (washing curve), (iii) second passage of the solution (dilution curve). We show in Figure 3 a practical example where UV absorbance was measured at the column exit, with a polymer solution at pH 7 (C = 0.42 mg/mL). Let us note that the curve which is symmetrical to the washing curve about the horizontal axis at $C/2$ almost coincides with the dilution curve. We therefore have a good estimation of the adsorbance with one passage of solution followed by washing with solvent. We used typically a column of internal diameter 2 mm with 100 mg of silica (about 1 m^2) and a flow rate of 8.5 mL/h. A thermostated jacket allowed stabilization of the temperature at 20 ± 0.05°C.

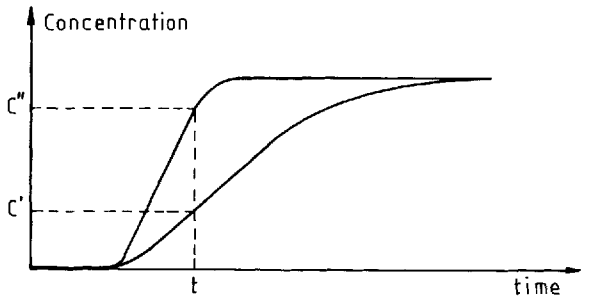


Figure 1. Schematic representation of the concentration of the eluted solution with (C') and without (C'') adsorption.

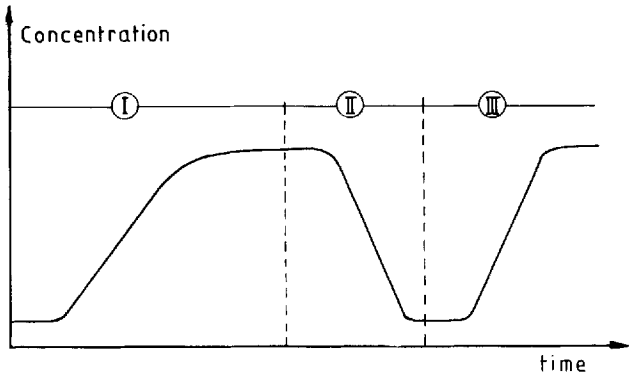


Figure 2. Schematic representation of the successive steps leading to the determination of the adsorbed amount: (I) First passage of the solution (adsorption), (II) rinsing with solvent, (III) second passage of the solution (no additional adsorption).

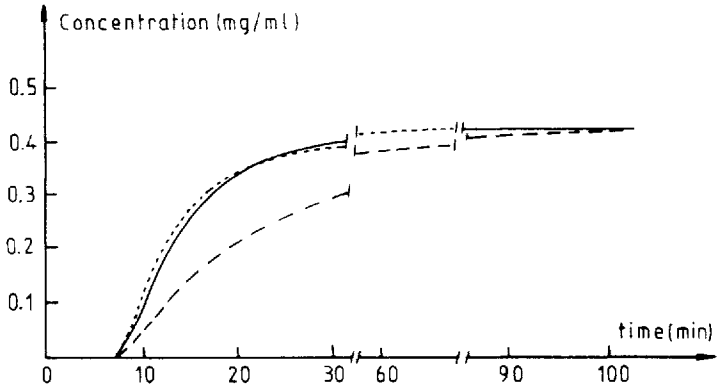


Figure 3. Illustration of the three superposed curves: (---) First passage of the solution, adsorption curve; (——) second passage of the solution, dilution curve; (---) symmetric of the washing curve with respect to the horizontal axis at $C/2$. C = 0.42 mg/mL, pH 6.5, H_2O, 20°C.

Measurement of platelet accumulation

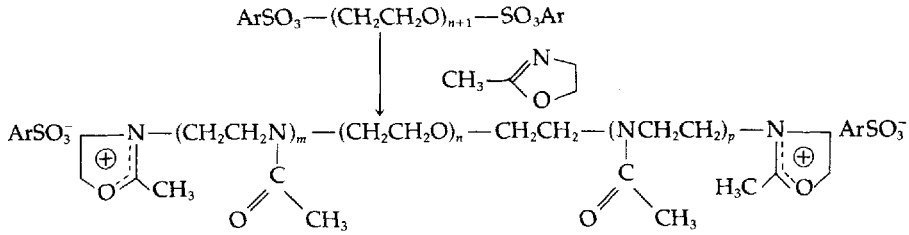
Platelet accumulation was measured as previously described[15] in glass capillary tubes of internal diameter 0.56 or 0.80 mm, uncoated or precoated with copolymer. Briefly, the capillaries were precoated by static adsorption for 1 h at 22°C from a polymer solution of concentration 4.0 mg/mL in phosphate buffer (pH 7.4). After rinsing with the same buffer, the capillaries were perfused at 37°C with a suspension of washed ^{111}Indium-labelled human platelets (180,000/mm^3) in Tyrode's-albumin buffer containing 40%

washed human red blood cells. Perfusion time was 5 min at flow rates corresponding to wall shear rates of 50 s^{-1} or 2,000 s^{-1}. Platelet deposition was determined from the ^{111}Indium radioactivity deposited on 1 cm tube segments (1282 Compugamma, LKB, Turku, Finland) and the specific activity of the platelet suspension.

RESULTS AND DISCUSSION

Synthesis, purification, and characterization of the block copolymer

The copolymer synthesis depends on the cationic ring-opening polymerization of 2-methyl-2-oxazoline[16] (MOXZ) initiated by the α,ω-aromatic disulfonate of polyoxyethyleneglycol (PEO), according to the following schema:



with Ar = phenyl, *p*-tolyl.

This method, previously developed in our laboratory[17] and then by Miyamoto et al.[18] for low-molecular-weight polyoxyethylenes ($\overline{M}_n < 7 \times 10^3$ daltons), was transposed to a precursor for significantly higher molecular weight, $\overline{M}_n = 2.43 \times 10^4$ daltons.

Functionalization of the PEO end-groups

The condensation of aromatic sulfonylchloride onto the PEO alcohol end groups was performed in two different ways:

a) directly under Schotten-Bauman conditions (pyridine, 0°C), according to an established procedure for low-molecular-weight PEO ($\overline{M}_n = 3 \times 10^3$ daltons).[8]

b) after previous activation of the alcohol functions to sodium alkoxide (tetrahydrofuran, room temperature).[7]

Neither process involves degradation and both lead with good yields (75 and 80% for a and b, respectively) to selective and quantitative functionalization, as derived from molecular weight measurements and UV spectroscopy ($\varepsilon = 582$ L · $mole^{-1}$ cm^{-1} at 261 nm in methanol solution for ethyl tosylate[8] chosen as a model compound for the functionalized PEO end groups).

$$\text{Average functionality: } f = \frac{[ArSO_3]\overline{M}_n^0}{1 - [ArSO_3][M(ArSO_2) - 1]} = 2.02 \pm 0.08$$

where $[ArSO_3]$ is the concentration in arylsulfonate function per gram of polymer, $\overline{M}_n^0$ the number average molecular weight of the PEO precursor, and $M(ArSO_2)$ the weight increment arising from the functionalization reaction.

In our hands, the separation of the NaCl by-product by centrifugation in method b was easier than that of the pyridinium hydrochloride in method a, which requires a series of tedious recrystallizations.[8]

Polymerization of 2-methyl-2-oxazoline (MOXZ) initiated by the α-ω-aromatic disulfonate of PEO

The experimental results of this polymerization performed either in homogeneous solution or in bulk are given in Table I. As expected the bulk process leads to higher conversion of the MOXZ monomer.

Fractionation of the crude copolymers is a necessary step in order to separate pure block copolymers. Because of the crystallinity of both homopolymers, PEO and PAEI, selective extraction was assumed to be more selective and was thus preferred to the usual successive precipitation. Such a process, involving benzene as a selective PEO solvent and dioxane as a swelling agent but nonsolvent of PAEI (see experimental part), allowed the separation of three main fractions, as shown in Table II for a representative fractionation:

a) A benzene soluble fraction, containing PEO-rich copolymer and eventual residual PEO initiator or diblock PEO-PAEI.

b) A benzene and dioxane insoluble fraction, containing PAEI-rich copolymer and eventual PAEI homopolymer (see further discussion).

c) A benzene insoluble but dioxane soluble fraction, containing essentially the pure block copolymer free of its parent homopolymers.

TABLE II
Fractionation and Characterization of a Representative Copolymer

Fraction	Brut	Soluble Benzene	Insoluble Dioxane	Soluble Dioxane
w% of fraction	100	21	68	11
w% PAEI	70	16	84	54
dn/dc (mL · g^{-1})[a]	—	0.158	0.201	0.183
$\overline{M}_w$ (Dalton)	—	43800	140000	55500
$\overline{M}_w$ (Dalton) calculated[b]	—	29800	156000	54400

[a]The experimental dn/dc values (40°C, MeOH, 632 nm) are in good agreement with the values calculated according to the additivity rule: $dn/dc = (dn/dc)_{PEO}(1 - w_{PAEI}) + (dn/dc)_{PAEI} w_{PAEI}$.

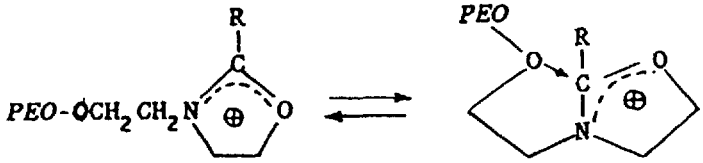
[b]Computed from the elementary analysis and the molecular weight of the PEO sequence.

The ^{1}H-NMR spectrum of this last fraction, very similar to that previously published for analogous low-molecular-weight copolymer,[18] clearly points out the presence of both PEO and PAEI blocks in the copolymer chain. The compositional data, expressed in weight percent of PAEI, derived from nitrogen elemental analysis, UV spectroscopy (II $\Rightarrow$ II* transition of the tertiary amide bond: ε = 5900 L · mol^{-1} · cm^{-1} at 197 nm in aqueous solution for PAEI homopolymer) and ^{1}H-NMR spectroscopy (analysis of the well resolved >N—CO—CH$_3$ singlet) are quite self-consistent: 54.0, 55.2, and 56.8, respectively.

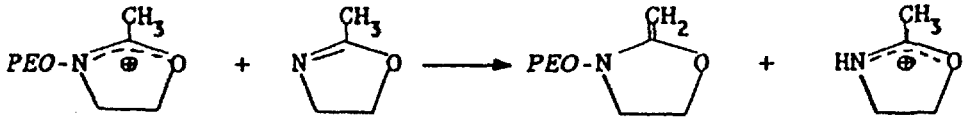
The purity of the block copolymer is demonstrated by its solubility properties and by the very good agreement between the calculated and experimental molecular weights (see Table II).

The analysis of the fractionation data suggests the following comments.

a) The relative high amount of benzene soluble and PEO rich fraction probably arises from a slow initiation (rate constant (k_i) with respect to the propagation step (rate constant k_p): the low values of the ratio k_i/k_p (<1) already observed for analogous systems[18,19] were ascribed to the stabilization of the first propagation center according to an equilibrium which would favor the less reactive bicyclic conformation.[19]



b) The PAEI-rich fraction is by far the major one and results from the low value of the k_i/k_p ratio and from a possible transfer reaction from the propagating species to the 2-methyl-2-oxazoline monomer,[20] leading to homopolymer PAEI:



c) The very low yield in isolated pure block copolymer (#11%) mainly arises from important losses in the two other fractions: here again the low k_i/k_p ratio implies a high compositional polydispersity in the copolymer chains, which display a wide range of solubility properties according to their composition.

To conclude, the cationic polymerization of 2-methyl-2-oxazoline initiated by the α, ω-ditosylate of polyoxyethylene ($\overline{M}_w$ = 2.43 × 10^4 daltons) leads to a relatively high molecular weight triblock copolymer ($\overline{M}_w$ = 5.5 × 10^4 daltons) but with a very low efficiency, which appears to be related to the polymerization mechanism itself. With respect to this important restriction, our

results are less optimistic than those of Miyamoto et al.,[18] who did not perform any fractionation of the crude copolymers and claimed quantitative yields of di- and triblock copolymers starting from low molecular weight PEO ($\overline{M}_w = 2.8 \times 10^3$ daltons). Our results are, however, in good agreement with the most recent and in-depth study of a similar system, PEO-tosylate and 2-isobutyloxazoline.[19]

Adsorption of the copolymer on silica

Preliminary experiments showed that the adsorbance of the polymer on silica passes through a maximum in the neutral range when varying the pH. Afterward we examined specifically the range around pH 7.5, close to the natural blood pH. We proceeded by an adsorption at pH 7.5 (C = 0.40 mg/mL), followed by two successive adsorptions on the same column at pH 6.5 and 8.5. We detected an increase of 25% in the amount adsorbed when passing from pH 7.5 to 6.5, while no variations were observed when passing from pH 6.5 to 8.5. Therefore pH 6.5 was chosen in most of the experiments.

Figure 4 shows the adsorption isotherm of the copolymer on silica at pH 6.5, obtained by the chromatographic technique described in Methods. The additional amount adsorbed becomes negligible above 0.20 mg/mL. The plateau value (90 ng/cm^2) corresponds to 100 nm^2 per macromolecule of molecular weight 55,000.

Joppien[21] measured, with Aerosil as adsorbant and a PEO of 22,000, an interfacial concentration of 40 to 50 ng/cm^2. The higher molecular weight in our case and the larger affinity of the *N*-acetylethyleneimine group may be responsible for the higher interfacial concentration. Let us note, however, that since specific areas are not known with accuracy, the orders of magnitude of the interfacial concentrations are similar.

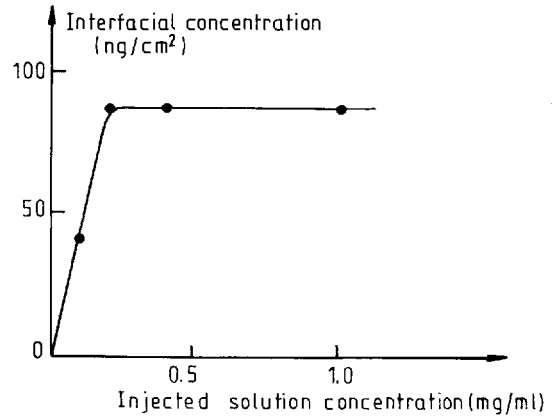


Figure 4. Adsorption isotherm of a copolymer PAEI–PEO–PAEI onto silica (Spherosil XOC 005) in water, pH 6.5, at 20°C.

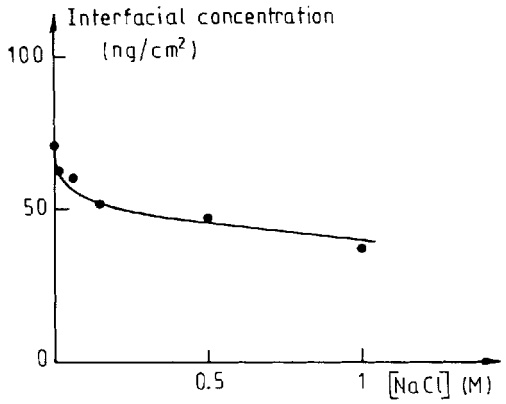


Figure 5. Influence of NaCl concentration on the interfacial concentration of a copolymer PAEI–PEO–PAEI on silica (Spherosil XOC 005) C = 0.40 mg/mL, pH 6.5, 20°C.

Interesting too is the effect of the saline concentration. Figure 5 illustrates the decrease of the interfacial concentration when increasing NaCl concentration in a solution of the copolymer at C = 0.40 mg/mL. All measurements were done on the same column, starting from [NaCl] = 1 mole/liter to zero through several steps. The additional amount adsorbed was measured at each step. The ions Na^+ might play a role by complexation with ether groups of PEO or by breaking hydrogen bonds between N-acetylethyleneimine groups and silica. Let us note that the final interfacial concentration (71 ng/cm^2) is smaller than when starting directly without salt (90 ng/cm^2). It could be that the interfacial conformation changes when passing from concentrated saline solutions to diluted ones are too slow to be entirely achieved with a flow rate of 8.5 mL/h.

Adsorption of fibrinogen and chromatography test

As a first step, a column of silica was precoated with copolymer, in the optimal conditions described above: 20°C, no salt, pH 6.5, C = 0.40 mg/mL. Interfacial concentration was determined to be 90 ng/cm^2.

In a second step, the column was washed with the buffer of the fibrinogen solution. We used a solution of Tris (hydroxymethyl) aminomethane 0.05M, NaCl 0.15M, NaN_3 $10^{-3}M$, adjusted to pH 7.35 with concentrated hydrochloric acid. Recording of the UV absorbance at the column exit revealed slight desorption of the copolymer, which may be explained by the presence of NaCl.

Afterwards a solution (0.40 mg/mL) of radiolabeled fibrinogen in Tris buffer was passed through the column. Recording of the γ activity of the column allows one to follow the replacement of the buffer by the solution of fibrinogen and the possible adsorption until a stable value which corresponds to the sum of the γ-activities of (i) the interstitial solution and (ii) the

adsorbed molecules. Replacement of the solution by the buffer alone leads to a fast signal decrease corresponding to the activity of the interstitial solution, the adsorbed molecules remaining in a metastable state. The activity recorded is then proportional to the amount adsorbed. Calibration according to the surface area and the specific activity of the fibrinogen solution allows one to deduce the interfacial concentration (1.4 ng/cm^2) which is 0.8% of the value observed on bare silica (180 ng/cm^2). This result illustrates the efficiency of the polymer precoating on silica with respect to inhibition of the adsorption of fibrinogen.

The influence of such a coating on the adsorption of other proteins was checked via calibration curves in size exclusion chromatography. The support (Nucleosil 300-10) was impregnated from a copolymer solution (50 mg/mL) in methanol. One gram of silica filled a 4–5-cm column of diameter 0.4 cm. Figures 6a and b show the calibration curves obtained at low and high ionic strength, respectively. Two observations are in order: (i) at low ionic strength, the three albumins are well separated while the cationic proteins (lysozyme and cytochrome) remain adsorbed; (ii) at high ionic strength, both albumins HSA and ovalbumin appear at a similar elution volume while lysozyme and cytochrome are eluted from the column. These results are relevant to the potentialities of copolymers with a PEO sequence in the field of biopolymer chromatography.

Platelet accumulation

Results for platelet accumulation in glass capillary tubes, uncoated or precoated with copolymer, are presented in Table III and compared with

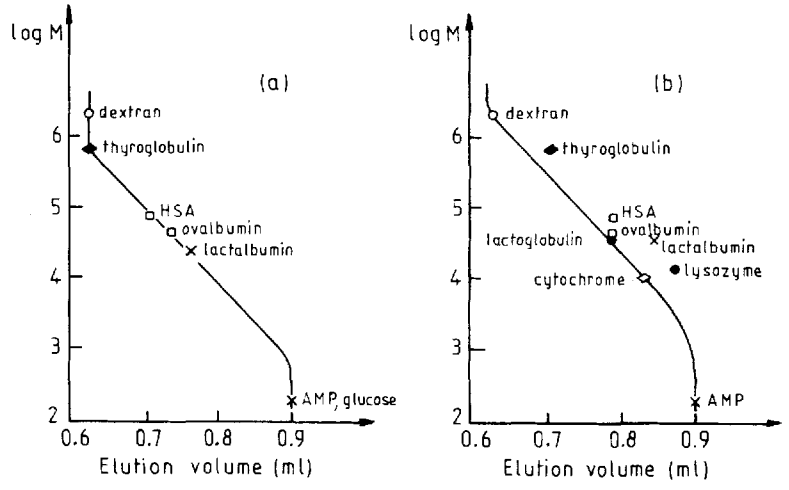


Figure 6. Chromatography of some proteins on Nucleosil 300-10 precoated with a copolymer PAEI–PEO–PAEI. pH 7.4, flow rate = 0.5 mL/min, phosphate buffer 10^{-2}M. a) ionic strength = 0.04, b) ionic strength = 1.0; $(NH_4)_2SO_4$ 0.32 M.

TABLE III
Platelet Accumulation on Glass Capillary Tubes, Uncoated or Precoated with PEO-PAEI, Perfused with Washed ^{111}Indium-Labeled Human Platelets

Wall Shear Rate (s^{-1})	Adherent Platelets (/mm^2)		
		Precoated with	
	Uncoated	Albumin	PEO-PAEI
50	9900	11300	1200
	100%	100%	12%
2000	24400	11000	4600
	100%	45%	19%

Note. Results for albumin-precoated capillaries taken from ref. 15. Perfusion time 5 min at 37°C, platelets 180,000/mm^3, hematocrit 40%.

platelet deposition under the same conditions in glass capillaries passivated by albumin preadsorption (3.5 g/L in phosphate buffer[15]). Precoating the glass surface with PEO-PAEI leads to a marked reduction in platelet accumulation. The surface concentration of adherent platelets falls to 12% and 19% of its value on bare glass at 50 s^{-1} and 2,000 s^{-1} respectively, a degree of passivation superior to that observed on albumin coated glass. The low platelet reactivity of the triblock PAEI-PEO-PAEI copolymer could be due to preferential orientation of the hydrophilic PEO groups towards the solid/solution interface.[5] Alternatively, the surface adsorbed polymer may form a microdomain structure similar to that exhibited by block copolymers of 2-hydroxyethyl methacrylate (HEMA) with ethylene oxide (EO), propylene oxide (PO), styrene (St) or dimethylsiloxane (DMS). Blood compatibility *in vitro* and *in vivo* of HEMA copolymers has been extensively investigated by Okano et al.[22,23] In particular, using polymer coated arteriovenous shunts (1.5 mm i.d., 20 cm length) implanted in rabbits, occlusion times were 12 days for poly(HEMA-DMS) and 20 days for poly(HEMA-St), as compared to 2–3 days for the homopolymers PHEMA, PDMS, and PSt.[23] The block copolymers also suppressed platelet shape change and aggregation in an *in vitro* column elution model. Since poly(HEMA-St) has been shown to exhibit preferential adsorption of albumin on hydrophilic microdomains and of fibrinogen and γ-globulin on hydrophobic microdomains,[24] these authors propose that the improved hemocompatibility of the hydrophilic-hydrophobic microphase structure could be related to an organized array of adsorbed plasma proteins. Such a conjecture, however, was not checked in our study. Let us note that the ways to coat the surface could be very important. We expect an adsorbed monolayer when processing by adsorption from dilute solutions, while dense (organized?) multilayers are probable when evaporating the solvent. Anyway the copolymer PEO-PAEI appears a highly promising new compound for the preparation of stable artificial surfaces of improved hemocompatibility.[2,5]

CONCLUSION

We report the synthesis and purification of a triblock copolymer PAEI–PEO–PAEI. The sequence PAEI was chosen as a potential good anchor on surfaces such as silica, through hydrogen bonding between silanols and the polymer tertiary amide functions. The aim of the PEO sequence was to provide a hydrophilic interface to improve compatibility with biological solutions or suspensions. Comparison between bare and precoated silicas with regard to the adsorption of fibrinogen and the chromatography of proteins shows the efficiency of such a precoating. Hemocompatibility with respect to platelet adhesion seems to be of good quality. Therefore this type of copolymer should be valuable for biological applications.

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