

X-ray photoelectron spectroscopy of ABA polypeptide–polybutadiene–polypeptide triblock copolymers

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The surface of various triblock ABA copolymers formed from polypeptide as the A component and polybutadiene as the B component was studied by X-ray photoelectron spectroscopy. The composition of the surface was shown to be the same as the overall bulk composition for copolymers with hydrophobic polypeptide blocks: poly(γ -benzyl-L-glutamate) or poly(N^{ϵ} -trifluoroacetyl-L-lysine). The surface was shown to be richer in polybutadiene for copolymers with hydrophilic polypeptide blocks: poly(N^5 -hydroxyethyl-L-glutamine) or polysarcosine. X-ray diffraction studies of the dry copolymers showed that they display lamellar structures. The experimental results suggest that these structures are perpendicular to the air–polymer interface.

(Keywords: block copolymers; polypeptides; mesophases; X-ray photoelectron spectroscopy; X-ray diffraction)

INTRODUCTION

Synthetic block copolymers with microphase separation and good mechanical properties are expected to be of interest in the biomedical field. The surfaces of such copolymers were pointed out to have good blood compatibility¹. The antithrombogenicity was shown to be improved with the presence of a polypeptide block in the copolymer². Past investigations on block copolymers have often shown that the composition at the surface can differ greatly from that in the bulk^{3–9}.

For all the preceding reasons, we have undertaken a study of the surface of block copolymers formed with at least one polypeptide block together with the study of their bulk structure with a view to clearing up the relationship between typical bulk and surface features.

The present paper reports on triblock ABA copolymers consisting of polybutadiene as the B component and different polypeptides as the A component. The polyvinyl chain is expected to improve the mechanical properties of the material. Polypeptides linked to the ends of the polybutadiene block are:

Poly(γ -benzyl-L-glutamate) (G)
Poly(N^5 -hydroxyethyl-L-glutamine) (EGn)
Poly(N^{ϵ} -trifluoroacetyl-L-lysine) (Kt)
Polysarcosine (Sar)

EXPERIMENTAL

Materials

Peptide monomers. γ -Benzyl-L-glutamate was obtained from L-glutamic acid and benzyl alcohol¹⁰. N^{ϵ} -

trifluoroacetyl-L-lysine was prepared from L-lysine and ethylthiotrifluoroacetate¹¹, which was synthesized from ethylmercaptan and trifluoroacetic anhydride¹². They were transformed into the corresponding *N*-carboxyanhydrides (NCA) by the action of phosgene in tetrahydrofuran (THF) suspension¹³.

Polybutadiene. The starting material is a commercial dicarboxyl-terminated polybutadiene, CTB 2000.156 Hycar from Goodrich (Cleveland, Ohio, USA)¹⁴. This dicarboxyl-terminated polybutadiene was transformed into diamine-terminated polybutadiene (ATB) by a coupling reaction, in THF solution, with a large excess (30 times) of diaminehexane (Fluka) in the presence of dicyclohexylcarbodiimide (Fluka) as coupling agent and *N*-hydroxysuccinimide (Fluka) as nucleophilic agent. The resulting product was purified by several precipitations in methanol. The complete elimination of diaminehexane was verified by thin-layer chromatography. There is no residual carboxylic group, as confirmed by titrating with sodium methanolate¹⁵ and by i.r. spectroscopy. The number-average molecular weight was obtained by titrating the amine functions with perchloric acid in chloroform/acetic acid solution¹⁵:

$$M_n = 6990 \text{ for ATB 15609}$$

$$M_n = 8100 \text{ for ATB 15610}$$

Synthesis of block copolymers

The block copolymers G–B–G, Kt–B–Kt and Sar–B–Sar were obtained in THF solution, at room temperature, by polymerization of the respective NCA initiated by the terminal amine functions of ATB. The block copolymers G–B–G and Kt–B–Kt were submitted to fractional

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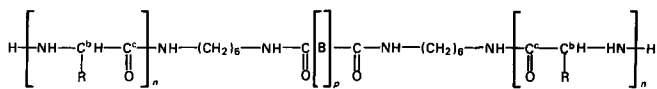
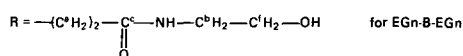
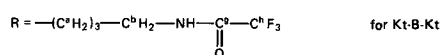
Table 1 Average molecular weights and bulk compositions for the ABA triblock copolymers

Sample	M_n		Bulk composition*	
	B	A	Wt% A	Mol% A
G-B-G ^a	901	8110	53.7	22.7
	902	6990	73.9	41.8
	904	6990	81.5	52.8
	1011	8100	69.4	36.4
	1013	8100	81.3	52.2
EGn-B-EGn ^b	6990	19195	73.3	52.8
Kt-B-Kt ^c	8100	28718	78.0	46.8
Sar-B-Sar ^d	8100	8988	52.6	46.3

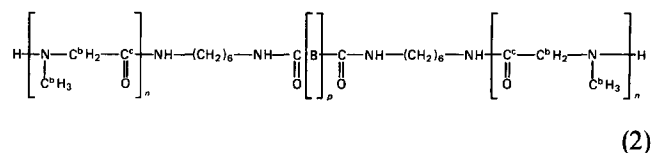
* Determination of the bulk compositions:

^a By u.v. absorption of the benzylic groups at 258 nm^b From the bulk composition of the G-B-G parent copolymer^c By the chemical analysis of the C, H, N and F elements^d By ¹H n.m.r. at 300 MHz

precipitation by water. The block copolymer Sar-B-Sar was precipitated by diethyl ether and eventual homopolysarcosine was removed by extraction in methanol. The copolymer EGN-B-EGN was obtained from G-B-G copolymer by aminolysis of the benzylic ester by a large excess of ethanolamine at 50°C in THF solution¹⁶. The copolymer was precipitated by cold diethyl ether. The complete removal of the benzylic groups was confirmed by the disappearance of u.v. absorption at 258 nm. The triblock copolymers G-B-G, Kt-B-Kt and EGN-B-EGN have the general formula:

with B = $-(\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2)-$ 

The triblock copolymer Sar-B-Sar has the chemical formula:



In Table 1 are listed the compositions and the number-average molecular weights of the copolymers studied.

Sample preparation and techniques

The sample preparation and the techniques used (X-ray diffraction and X-ray photoelectron spectroscopy) are described elsewhere⁹.

RESULTS AND DISCUSSION

Bulk structures of dry copolymers

Block copolymers G-B-G, Kt-B-Kt and EGN-B-

EGn studied by x.r.d. and i.r. spectroscopy were shown to exhibit a periodic lamellar structure in the dry state after evaporation of THF for both the copolymers G-B-G and Kt-B-Kt and after evaporation of a system THF/H₂O for the copolymer EGN-B-EGn. This structure consists of plane, parallel, equidistant sheets. Each elementary sheet of thickness d results from the superposition of two layers: one of thickness d_B contains the B blocks in a disordered configuration, and the other of thickness d_A contains the polypeptide chains in an α -helix type conformation assembled in a hexagonal array^{17,18}.

The block copolymer Sar-B-Sar studied by x.r.d. exhibits a lamellar structure in the dry state after evaporation of chloroform. This lamellar structure resembles that described above except that the Sar chains are in a disordered conformation.

The lattice parameters of the lamellar structures were directly obtained from the X-ray patterns or calculated by formulae based on simple geometrical considerations¹⁹. Structural parameters are listed in Table 2.

Surface composition

X.p.s. analysis provides two types of information about the top 50 Å of the sample surface:

(a) The atoms present at the surface and their chemical environment are identified by the binding energies of the ejected photoelectrons²⁰⁻²²;

(b) The stoichiometry of the sample is determined from the ratios of the areas of the photoionization peaks^{23,24}.

The x.p.s. spectra for all the triblock copolymers studied display three signals associated with the direct photoionization of the C_{1s}, N_{1s} and O_{1s} levels (Figure 1). The x.p.s. spectra for the Kt-B-Kt copolymer displays an additional signal due to the photoionization of the F_{1s} level (Figure 1c). The assignment of the component peaks is obtained from the chemical formulae (1) and (2) of the copolymers, taking into account the previous experimental and theoretical analysis of the electron binding energies in polymers and model compounds.

The N_{1s} and F_{1s} signals fit a single peak centred respectively at 400.4 and 688.8 eV.

The O_{1s} signal consists also of a single peak for the copolymers Kt-B-Kt and Sar-B-Sar (Figures 1c and 1d). This peak is centred at 532.3 eV (Table 3) and corresponds to the carbonyl oxygen. For the copolymers G-B-G and EGN-B-EGN, the O_{1s} signal arises from two components (Figures 1a and 1b) with a separation of respectively 1.6 and 1.3 eV (Table 3). The component at lower binding energy was attributed to the carbonyl oxygen, and the component at higher binding energy was attributed to the alcohol oxygen. Their positions are in reasonably good agreement with the literature data^{20-22,25,26}.

For each copolymer studied, the lineshape analysis of

Table 2 Parameters (in Å) of the lamellar structures displayed by the dry copolymers

Copolymer	d	d_A	d_B
G-B-G	901	130	58
	902	123	86
	904	146	110
	1011	208	127
	1013	154	114
EGn-B-EGn	180	123	57
Kt-B-Kt	195	134	61
Sar-B-Sar	225	96	129

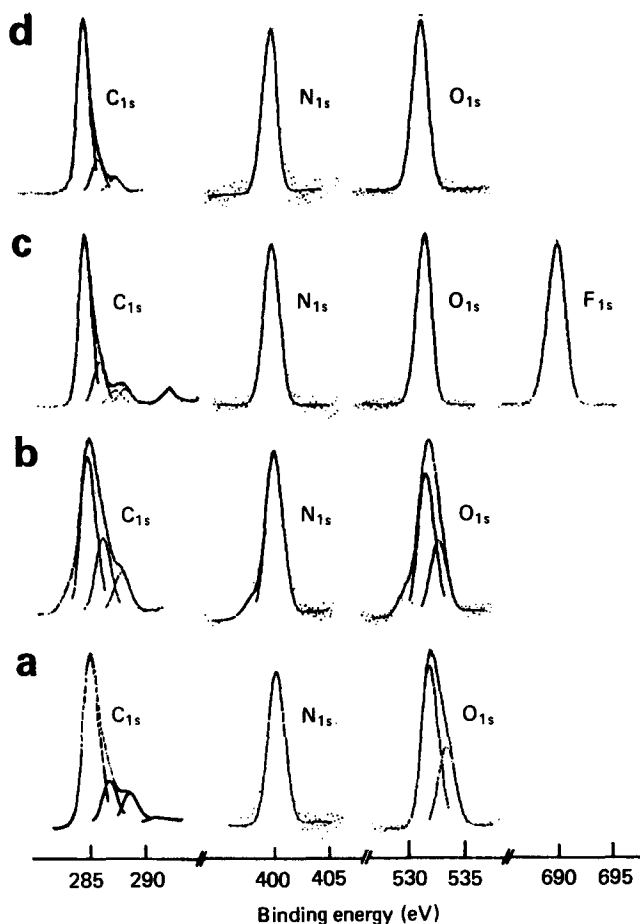


Figure 1 Examples of the x.p.s. spectra for the ABA copolymers: (a) copolymer G-B-G; (b) copolymer EGN-B-EGN; (c) copolymer Kt-B-Kt; (d) copolymer Sar-B-Sar. The experimental data recorded are shown as dots, along with the calculated curves for the various components

Table 3 Experimentally determined binding energies (in eV) for the C_{1s} and O_{1s} signals in the ABA copolymers

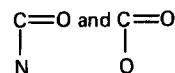
Copolymer	C_{1s}					O_{1s}		
	C^a	C^b, C^f	C^c	C^d	C^g	C^h		
	C-C	C-N C-O	C=O N	C-O O	C=O N	CF ₃	C=O	C-O
G-B-G	285	286.7		288.8			532.3	533.9
EGN-B-EGN	285	286.4	288.2				532.9	534.2
Kt-B-Kt	285	286.4	288.0		289.0	293.3	532.3	
Sar-B-Sar	285	286.4	288.1				532.3	

the C_{1s} spectra reveals the presence of three components at approximately the same binding energy which arise from carbon atoms in the same electronic environment (Table 3). The lower binding energy component at 285 eV is due to carbons bound only to carbon and hydrogen (C^a). As the binding energy increases, a second component shifted by 1.4 eV arises from carbons bound to nitrogen (C^b). Then the third component is attributed to carbons bound both to nitrogen and to oxygen (C^c). For the copolymer Kt-B-Kt, two additional peaks appear at higher binding energies. The component at 289 eV arises from the carbonyl carbon pertaining to the side chain of the polypeptide (C^d). It is indicative of the

secondary substituent effect of the nearby fluorine⁹. The component at 293.3 eV arises from the carbon bound to three fluorines (C^h).

Binding energies of carbons singly bound to one oxygen (C^f) or bound to nitrogen are closely similar. Consequently, the relevant carbons in copolymers G-B-G and EGN-B-EGN are expected to contribute to the same peak.

Copolymers G-B-G display carbon atoms in both



functionalities. Binding energies for such carbons are expected to be respectively of 288 and 289 eV^{25,26}. As can be seen from Figure 1a and Table 3, the C_{1s} spectra display one component at 288.8 eV. This peak was attributed to both types of carbons noted above. The stoichiometry of the G homopolymer calculated with this peak assignment is in good agreement with the theoretically expected stoichiometry.

All the copolymers studied are unsaturated in the B blocks, and their C_{1s} signals are expected to display a shake-up satellite. Only the C_{1s} spectra of the G-B-G copolymer exhibits a shake-up satellite at 291.4 eV (Figure 1a). It was assumed to arise from the $\pi^* \leftarrow \pi$ transitions accompanying the core ionization in the benzyl groups of the A blocks³. The satellite is very weak and consequently was not taken into account in this study.

The chemical formulae (1) and (2) of the copolymers show that:

(a) the block of polybutadiene contributes only to the C_{1s}^a signal;

(b) the A blocks contribute to this signal except for the copolymer Sar-B-Sar, which gives rise to all the other peaks.

Therefore, setting the C_{1s}^a signal aside, the ratios between the area of the signals arising from each copolymer was checked to be in good agreement with the stoichiometry of the A blocks.

Let us assume that in a sample studied $N(C_B^a)$ is the number of carbons of type C^a pertaining to the B blocks and $N(A)$ is the number of peptide monomers. The ratio $N(C_B^a)/N(A)$ was determined in two independent ways: first by comparing the ratios between the area of the C_{1s}^a and the area of the other C_{1s} peak components for the copolymer and the A homopolymer; secondly by comparing the peak area ratios C_{1s}/O_{1s} , C_{1s}/N_{1s} and possibly C_{1s}/F_{1s} for the copolymer and for the A homopolymer. The B content at the surface of the copolymers (mol% B) is then given by the relation:

$$100/\text{mol \% B} = 1 + 4N(A)/N(C_B^a) \quad (3)$$

Examples of peak area ratios measured and the value of the B content of the surface deduced from these ratios are gathered in Table 4. It can be seen that (i) for the copolymers G-B-G and Kt-B-Kt the B content at the surface is approximately the same as the B content in the bulk, and (ii) for the copolymers EGN-B-EGN and Sar-B-Sar the B content at the surface is higher than the B content in the bulk. Therefore a trend for the B component to aggregate at the surface is shown for copolymers with more polar polypeptide blocks. It may

Table 4 Experimentally determined peak area ratios and surface composition for the ABA copolymers

Copolymer	Mol% B in bulk	$\frac{C_{1s}^a}{C_{1s}^b}$	$\frac{C_{1s}}{O_{1s}}$	$\frac{C_{1s}}{N_{1s}}$	Mol% B at surf.
G-B-G 901	77.3	7.40	2.67	13.0	73.0
902	58.2	4.65	2.02	9.90	57.0
904	47.2	3.70	1.80	8.78	46.0
1011	63.6	5.50	2.25	10.90	64.0
1013	47.8	3.85	4.83	8.95	48.0
EGn-B-EGn	47.0	4.40	1.85	5.20	73.6
Kt-B-Kt	53.2	4.00	2.26	3.68	55.0
Sar-B-Sar	53.7	5.20	4.80	7.80	72.8

be that the rearrangement of the B chains allows a decrease in the surface free energy^{27,28}.

Surface morphology

All the copolymers studied exhibit a lamellar structure. The knowledge of the surface composition gives information on the structure at the surface. For all the copolymers studied, x.p.x. experiments sampling the top 50 Å of the surface reveal the presence of both polybutadiene and polypeptide components.

The layers of the lamellar structure may be assumed to be parallel to the air-polymer interface or tilted with respect to this interface. In a lamellar structure parallel to the surface the overlayer may be expected to contain the A blocks or the B blocks if the chains of polybutadiene are refolded in two superposed layers. In most copolymers studied, the values of half the thickness of the polypeptide chains are close to the depth probed by x.p.s. (Table 2). In the case of an overlayer of A blocks, a great excess of polypeptide chains would be measured by x.p.s. Any excess of the polypeptide blocks was evidenced at the surface of the copolymers (Table 4). Therefore the model with an overlayer of A blocks was set aside.

Copolymers G-B-G and Kt-B-Kt. In the copolymers G-B-G and Kt-B-Kt, the thickness of the polybutadiene chains refolded in two superposed lamellae varies from about 20 to 40 Å (Table 2). In the case of an overlayer of B blocks a great excess of the B chains would be measured by x.p.s. The composition at the surface was found to be the same as in the bulk (Table 4). Therefore the model of a lamellar structure parallel to the surface does not fit the experimental results. The latter results can be explained by assuming the layers of the lamellar structure to be arranged perpendicular to the surface. This model has been proposed for various block copolymers^{3,7,9}.

Copolymers Egn-B-EGn and Sar-B-Sar. In the copolymers Egn-B-EGn and Sar-B-Sar the values of the layer thicknesses (Table 2) show that the excess in the B content of the surface (Table 4) is consistent with both an overlayer of the B blocks and with layers of the lamellar structure tilted to the air-polymer interface. The presence of a B block overlayer would lead to a fast disappearance of the X.p.s. signals arising from the A blocks with the lowering of the sampling depth. A depth profile analysis was performed by recording x.p.s. spectra for take-off angles θ ranging from 0 to 75°. The sampling depth is then varied from approximately 50 to 12 Å^{31,32}.

In the copolymer Egn-B-EGn, the B content at the surface was shown to increase gradually as the air-

polymer interface is approached (Figure 2). Both the A and B blocks are present at each depth probed. These results are then incompatible with an overlayer of the B blocks. The model of a lamellar structure perpendicular to the air-polymer interface was retained. In this model the continuous increase of the B content as the sampling depth decreases may be explained if the B regions are assumed to be raised above the polypeptide regions. The pre-eminence of the B chains gives rise to a 'shadowing effect' where the atoms of the polypeptide chains are screened by the atoms of the B chains leading to an increase of the B signal as the outermost surface is approached. This model has been developed previously from x.p.s. studies on a number of copolymers^{3,4,9}. It is consistent with the morphological observations on replication electron micrographs made from various block copolymers^{8,32,33}.

In the copolymer Sar-B-Sar the B content does not vary in a significant way as the sampling depth decreases from 50 to ~12 Å (Figure 2). Consequently, for the copolymer Sar-B-Sar, the layers of the lamellar structure were assumed to be perpendicular to the air-polymer interface.

CONCLUSIONS

X.p.s. studies were used to determine the surface composition and topography of a series of triblock ABA copolymers where B is formed from polybutadiene and A from various polypeptides.

The composition at the surface was found to be the same as in the bulk for the copolymers G-B-G and Kt-B-Kt formed from two hydrophobic blocks. The surface composition was found to be richer in butadiene than the bulk for the copolymers Egn-B-EGn and Sar-B-Sar where the polypeptide component is hydrophilic. For the copolymer Egn-B-EGn, the B content of the surface was shown to increase as the air-polymer interface is approached.

X-ray diffraction studies of the block copolymers in the dry state have shown that they exhibit lamellar structures formed by the superposition of layers containing alternatively B blocks and A blocks. For all the copolymers studied, the experimental results suggest a surface model where the microdomains formed from the B and A blocks are arranged perpendicular to the surface. For the copolymer Egn-B-EGn, the B component was assumed to be slightly raised above the polypeptide chains.

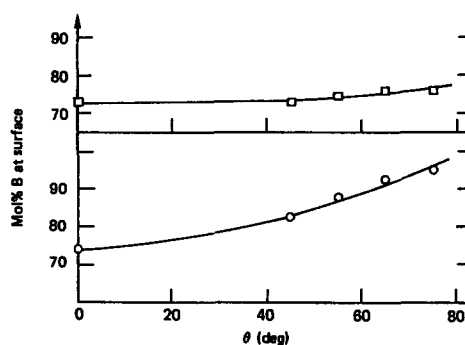


Figure 2 Variation of the B content at the surface versus the take-off angle θ : (○) copolymer Egn-B-EGn; (□) copolymer Sar-B-Sar

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