Anticoagulative Surfaces: XPS Study of Polyamidoamine Derivatives Bonded to Glass Surface and of Their Complexes with Heparin

R. BARBUCCI

Istituto di Chimica Generale, Università di Siena, Siena, Italy

C. BISI CASTELLANI

Istituto di Chimica Generale, Università di Pavia, Pavia, Italy

M. DELFINI*

Istituto di Chimica e Tecnologia dei Radioelementi del C.N.R., Area della Ricerca di Padova, Padua, Italy

and P. FERRUTI

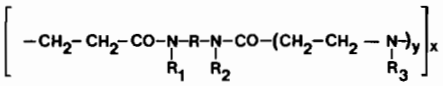
Facoltà di Ingegneria, Università di Brescia, Brescia, Italy

Received June 18, 1984

One of the most relevant difficulties concerning the use of non-physiological materials for biomedical purposes is that of their thrombogenic interaction with the blood. This is for instance the case for glass used for blood and plasma storage. This problem has been partially resolved by the addition to the stored blood of anticoagulative substances. The presence of such substances prevents fast coagulation but they can lead to additional risks, especially if the transfusions are frequent. Furthermore, the stability of the blood or plasma stored in the presence of such anticoagulatives is short-lived.

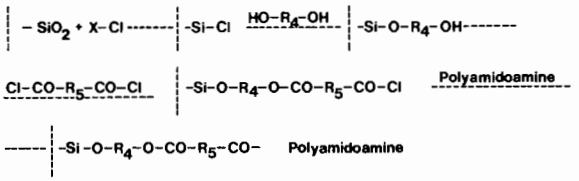
With the aim of preventing blood coagulation or at least enabling the reduction of the quantity of anticoagulative substances added for storage, we studied the possibility of obtaining an anticoagulative surface by the use of chemical substances covalently bonded to the storage glass surface.

Polymeric substances, covalently bonded to the glass surface and able to form complexes with heparin, seem to be promising in this respect. The polymers used are various polyamidoamines of general formula:



where R, R_1, R_2 and R_3 are linear, branched or aromatic hydrocarbon chains [1].

The process of covalent binding of these polymers to the glass surface can be summarized as:



Heparin can subsequently be bonded to the modified surface. In order to follow and verify the different steps of this process XPS experiments have been carried out. This spectroscopy is in fact a powerful tool for analysis at the molecular level of the external layers of solids a few Å deep [2, 3].

Experimental

Materials and Methods

The glass samples (1 × 5 × 10 mm) were prepared as previously described [4].

For chlorination reactions, thionyl chloride was used. The polyamidoamine used was a copolymer obtained by polymerization of 1,4-bisacryloyl-piperazine and N,N′-dimethylendiamine [5]. The glass samples treated and untreated were washed with $CHCl_3$, H_2O, EtOH and then dried under vacuum.

The reaction with heparin was carried out in a solution of H_2O:EtOH:acetic acid in the ratio 40:60:1. The samples obtained were washed with EtOH and dried under vacuum.

The X-ray photoelectron spectra were recorded using an AEI-200 B spectrometer equipped with a $Mg_{K\alpha}$ source. The reported binding energies were measured at halfwidth of the band maximum using carbon 1s as internal standard.

Results and Discussion

The reference glass samples show the characteristic XPS peaks of silicon (153,101 eV), oxygen (531 eV), calcium (348 eV) and sodium (1071 eV). After the chlorination process a new peak, centered at 198 eV, is connected to the chlorine 2p ionizations. A less intense peak at 167 eV is also present in the samples treated with thionyl chloride and indicates the presence of small amounts of sulphur.

The further reaction with polyamidoamine of the samples gives rise to the appearance in the XP spectrum of a signal at 398 eV, assigned to the nitrogen 1s, together with a marked lowering of the silicon

*Author to whom correspondence should be addressed.

0020-1693/84/$3.00

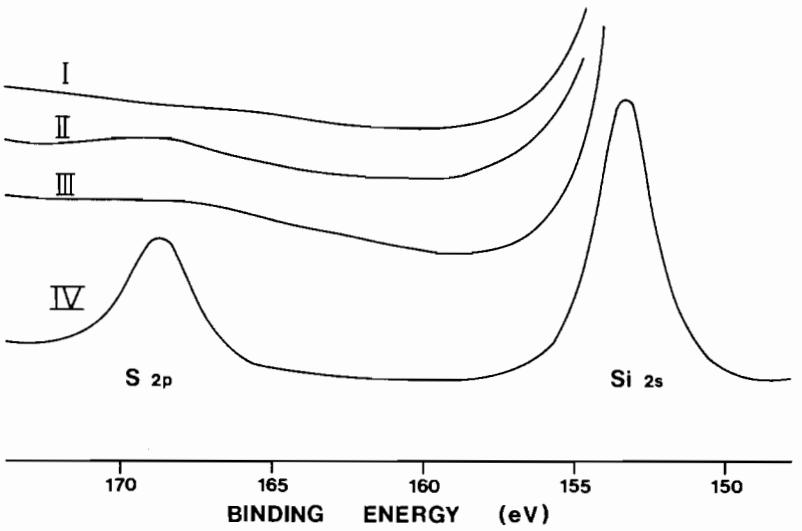


Fig. 1. XPS spectrum of the S 2p region of a glass surface. Curve I: untreated glass. Curve II: glass after chlorination. Curve III: glass treated with polyamidoamine. Curve IV: the glass surface of curve II after treatment with heparin.

signals. Sulphur traces are also detectable. Finally, the heparin treated samples show XP spectra with very intense and broad peaks in the regions of nitrogen and sulphur binding energies.

The XP spectra in the silicon and sulphur binding energies region are reported in Fig. 1. With respect to such experimental features the following conclusions can be reached: the efficiency of the chlorination process is shown by the presence of the chlorine signals as well as that of the polyamidoamine process by the nitrogen signals. The dramatic decrease of the silicon signal after these treatments strongly supports a large layer coating of the surface. Moreover, after the treatment with heparin, the photoelectron spectrum is consistent with a large quantity of such a substance bonded to the polyamidoamine treated surfaces. Work is still in progress to obtain a quantitative knowledge of the processes and of the reaction rates.

Some experiments have been carried out with bottles having glass surfaces treated as summarized before and filled with human blood. The stability of the stored blood towards coagulation showed a very significant improvement, confirming the high ability of surface supported polyamidoamines to react with heparin to form anticoagulant complexes.

References

1 M. A. Marchisio, T. Longo and P. Ferruti, *Eur. Surg. Res., 4*, 312 (1972).
2 T. A. Carlson, 'Photoelectron and Auger Spectroscopy', Plenum, New York, 1975.
3 D. Briggs, (ed.), 'Handbook of X-ray and Ultraviolet Photoelectron Spectroscopy', Heyden, London, 1977.
4 *U.S. Pat. 3 865 723* (1975) to M. A. Marchisio, T. Longo, P. Ferruti and F. Danusso.
5 M. A. Marchisio, T. Longo and P. Ferruti, *Experientia, 29*, 93 (1973).