HYBRID SILOXANE-POLYAMINOAMIDES FOR THE ABSORPTION OF HEPARIN FROM BLOOD

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Abstract. Heparin is an anticoagulant which is widely used during blood-dialysis. To now, there is not a commercial device able to "filter" heparin from blood after or during the medical treatment. Heparin can give, on life-long treatments, several health problems. We prepared siloxane-modified hybrid poly(aminoamides) which can be obtained from water solution. They shown good heparin absorption properties.

Keywords: Sol-gel, heparin, polymers.

1. Introduction

The use of an extracorporeal apparatus for the medical treatment of blood, implies the presence of anticoagulant substances such as heparin, in order to avoid the formation of blood-clots inside the artificial circuit. Haemodialysis is widely used to remove waste products form blood and often the patient undergoes life-long cares with a frequency of two-three times a week. Generally, heparin is used as an injectable preparation.

Heparin is a mucopolysaccharide acting on the antithrombin mechanism which inhibits the serine-proteases involved in the coagulation processes.

Heparin is a polymer with variable chain lengths having a molecular weight ranging from 3,500 to 35,000 Da. It is formed by different di-saccharide units and is acidic, due to the presence of a high content of sulphate ($HOSO_3$) groups. The most common disaccharide found in heparin is formed by 2-O-sulfated iduronic acid and 6-O-sulfated, N-sulfated glucosamine.

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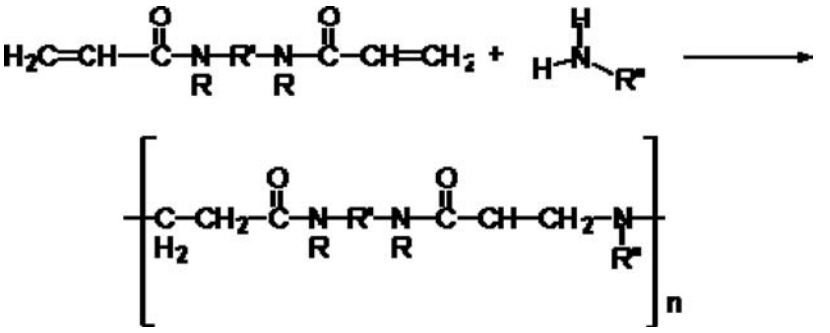
At the physiological pH, heparin is found in its poly-anionic form; the cations present in the medical preparations can be changed following the treatment needs; sodium and calcium are the most used. Heparin binds to the enzyme inhibitor antithrombin III (AT-III) causing a conformational change which results in its active site being exposed. The activated AT-III then inactivates thrombin and other proteases involved in blood clotting. The rate of inactivation of these proteases by AT-III increases 1,000-fold due to the binding of heparin .[1]

Heparin is normally absorbed and degraded in the human body with a half-life of about one hour, depending on its molecular weight. Sometimes heparin, mostly on life-long treatments, can give several problems like hemorrhage, hypersensitivity, bone decalcification. As far as we know, the only practically applied method of de-heparinization is a plasmapheresis system that uses polylysine cationic polymers.[2] Another approach that seems effective, is the use of poly (amido-amine)s (PAAs), polymers obtained by the reaction of bis-acrylamides and primary or bis-secondary amines. They were widely studied by the group of Ferruti.[3] It appears that hydrolysis and solubility of these polymers, even when organically crosslinked, is preventing them to be practically applied. PAAs can be easily prepared in water or alcohol as solvent and without the need of a catalyst through a Michael addition:

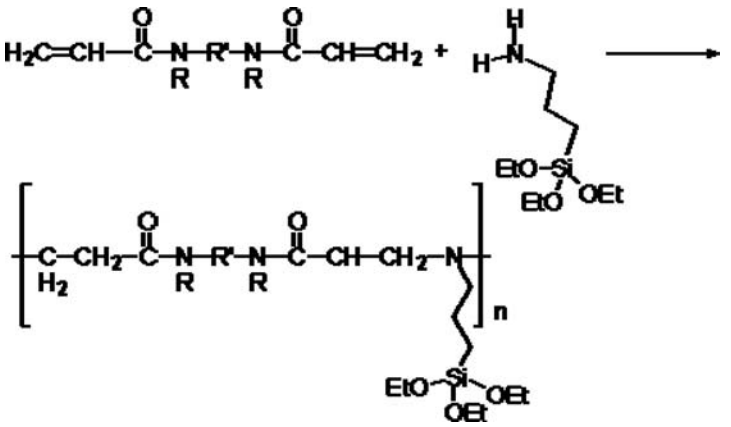


Every aminic hydrogen is a point of polymerization by reacting with an acrylamidic group. Primary amines and secondary diamines will yield linear polymers. Primary diamines, e.g. ethylenediamine, will produce branched polymers.

2. Results

Organic PAAs can be modified in order to be used for the preparation of hybrid materials by the sol-gel method.

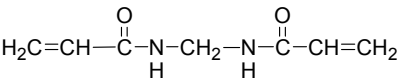
In fact, the use of 3-aminopropyl-triethoxysilane (APTES), allows for the introduction of the $(EtO)_3Si$- group covalently bonded to the polymer chain.[4]



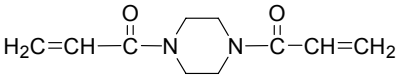
Different formulations have been tested, by changing the reaction components. The aim of the research was to find a formula able to yield a flexible polymer, to be used as a thick coating on the surface of biomedical PVC objects. The better composition was found to be a mixture of a bis-acrylamide, APTES or APMDES (3-aminopropyl-methyldiethoxysilane) and 1,6-dimethylaminohexane, a secondary diamine. The polymers obtained from water only are transparent, flexible and insoluble in water or any other solvent. They possess, as expected (Figure 1), an intermediate character with respect to the brittle and opaque TEOS xerogel (on the left in the figure) and the sticky an water soluble PAA polymer (on the right in the figure).

Figure 1. TEOS xerogel, hybrid siloxane-PAA, organic PAA.

Two bis-acrylamides were used, methylenebis-acrylamide (MBA) and bis-acryloylpiperazine (BAP).



MBA



BAP

The choice was dictated by their low cost required in the case of real production. 1,6-dimethyl-diaminohexane was found to infer elastic properties to the polymer, together with a higher capacity to bind heparin.

Typical infrared and X-Ray powder diffraction are reported in Figure 2:

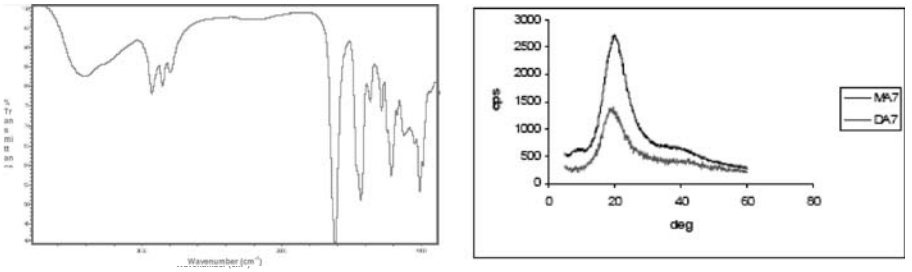


Figure 2. Infrared spectrum and XRD spectra of typical hybrid PAAs.

Absorption tests *in vitro* and on human blood

Heparin absorption *in-vitro* tests were performed on these materials using com-mercial heparin solutions (Sodium heparin, Liquemin Roche). Following the published literature, we decided that spectroscopic determination would be the quickest way to analyze many samples. Other methods, based on biological activity, could be used but were to complex for a quick screening of the best formulation. We then applied an indirect method,[5] which consists in measuring the lowering of the absorption bands of the dye O-Toluidine blue, due to the formation of a heparin-toluidine complex. Suitable PVC medical devices were coated using a water solution of the forming polymers. In order to produce more robust coatings, a PVC-SiO2 blend was also tested (Figure 3).

Surface heparin absorption values ranges from 250 to 750 mg/m^2, which are more than enough to absorb the heparin injected in one treatment. The highest values were found for polymers obtained from MBA with a high content of secondary diamine, and probably depends on the swelling ability of the coating.

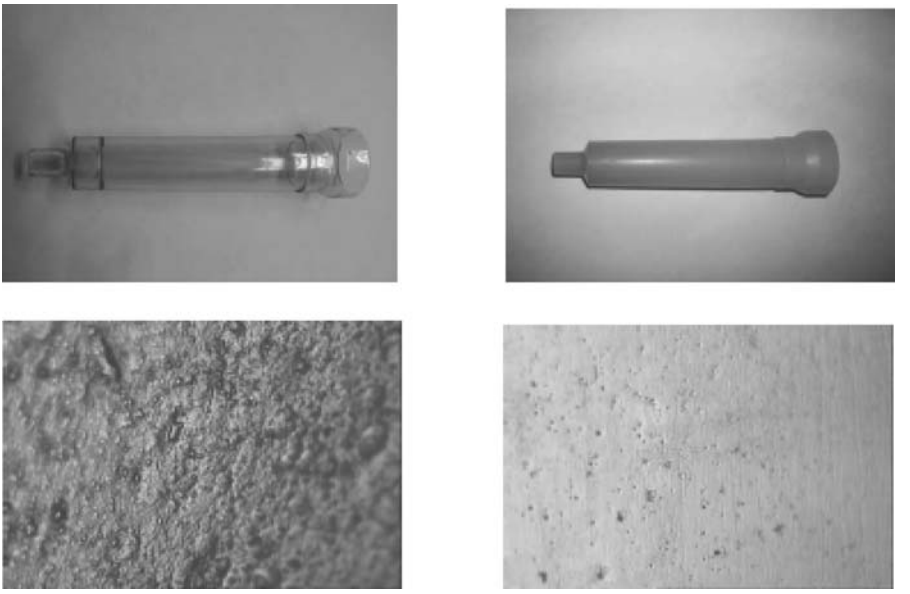


Figure 3. On the upper left, a PVC blood dropper, upper right a PVC/SiO_2 blood dropper; down left: the surface, washed with ethanol to remove the plastifier, of a PVC/SiO_2 dropper. down right; the same surface after coating with a hybrid polymer.

Tests performed on human blood were also very promising. Human blood contains a very high quantity of albumin which is in competition with heparin in the absorption process; polymers obtained from MBA resulted more selective towards heparin when in competition with albumin.

Acknowledgments

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