Fractionation of Human Plasma Proteins Based on Heparin Affinity and Magnetic Separation

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INTRODUCTION: Heparin affinity has been be used in the process of purification and identification of several proteins and separation using magnetic composites has the great advantage of being a rapid process as well as allows reuse. This process is based on the formation of reversible and specific complexes between the highly charged heparin and the proteins to be purified. **OBJECTIVES:** This work aimed to immobilize heparin onto magnetite particles coated with polyaniline and to use for fractionation and isolation of proteins from human plasma. **MATERIAL AND METHODS**: Magnetite particles were synthesized by co-precipitation method with salts of iron II and III. Then they were coated with polyaniline by oxidation of aniline with $KMnO_4$ (MAG-PANI). The obtained material was characterized by transmission electronic microscopy, magnetization measurements and powder X-ray diffraction. Heparin solution (3 mg/mL activated with EDAC/NHS) was incubated with 30 mg of MAG-PANI for 72h at 25°C yielding MAG-PANI-HEP. This composite was incubated with 1.0 mL of human plasma (1:5) for 1 h at 4°C and recovered with a magnet. The proteins complexed to MAG-PANI-HEP were eluted with increasing concentrations of NaCl (0.25 to 2.0 M) and subjected to electrophoresis. **RESULTS AND DISCUSSION**: MAG-PANI analysis showed a size of 14.4 ± 0.9 nm and XRD indicated the presence of polyaniline and magnetite phases. MAG-PANI exhibited superparamagnetism and magnetic saturation equal to about 66 emu/g to 293, 300 and 313 K. Heparin was covalently immobilized: 26.4 ± 0.09 µg per mg of support. The first fractions indicated the presence of proteins of 23.9 to 151.3 kDa and at the 9th fraction showed the presence of antithrombin (58 kDa). **CONCLUSIONS**: This method can be biotechnologically applied as either depletion of plasma proteins as well as to purify plasma proteins such as human antithrombin.

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