Research project objectives/ Research hypothesis

The need to lower the excessive blood coagulation down to the normal level has resulted in the advent of a large group of pharmaceuticals called anticoagulants, among which heparin is a notorious example. Chronic therapeutic dose anticoagulation is indicated, e.g., in prevention of stroke in patients with atrial fibrillation, deep vein thrombosis and pulmonary embolism. Anticoagulants are also very often used episodically, e.g., during cardiac surgeries when temporary lowering of blood coagulation below normal level is usually indispensable. The application of anticoagulants, on the other hand, created the need for the development of their efficacious and safe antidotes, which in emergency such as hemorrhage from an injury, would allow quick recovery of normal blood coagulation. Protamine sulfate is the only approved antidote of unfractionated heparin (UFH), yet may produce life threatening side effects such as systemic hypotension, catastrophic pulmonary vasoconstriction or allergic reactions. Unfortunately, it only partially reverses low molecular weight heparins (LMWH), e.g., enoxaparin, and it is ineffective against fondaparinux, a synthetic anticoagulant. Within the planned studies synthetic block polyelectrolytes will be synthesized and studied which will perform two opposite tasks. The first group of the polymers will inhibit heparinoid anticoagulants (i.e. UFH, LMWH and fondaparinux). The polyelectrolytes of the second group will show expected anticoagulatory activity. The hemostasis control system: synthetic polymeric anticoagulant – synthetic polymeric inhibitor, will thus be obtained. The research is planned based on the hypothesis that: 1) one can obtain well defined, synthetic polymeric inhibitors of heparin-like anticoagulants, which would be more effective, safer and have a wide spectrum of action than currently used protamine sulfate, 2) one can obtain well defined, safe, synthetic polymeric heparin-like anticoagulant, and 3) one can obtain an anticoagulant-antidote system for hemostasis control based on block polyelectrolytes. In the research planned we will use our knowledge and experience gathered in physicochemical studies, in *in vitro* and *in vivo* experiments on the interaction of modified natural polymers with heparin, as well as on interaction of polycations with biomolecules. The research will be carried out by chemical and biomedical/pharmacological research teams within the scientific consortium.

Research project methodology

The polyelectrolytes, which will be synthesized to play a role of the inhibitors of heparins and heparinoid anticoagulants, will be block copolymers containing a cationic block of poly(methacrylamidepropyltrimethylammonium chloride) (PMAPTAC). The second block will be poly(ethylene glycol) (PEG) or poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), polymers known for their biocompatibility and many biomedical applications. The thorough chemical and physicochemical characterization of the obtained macromolecules will be performed. Their ability to complex anticoagulants including those obtained within the project will be determined using chemical tests. Those cationic polymers which will show this property will undergo *in vitro* and *in vivo* tests in order to determine their ability to restore normal blood coagulation changed by anticoagulants.

The polymers of the second group, showing expected anticoagulant activity, will be anionic block polymers. They will be obtained using the monomers containing sulfate or sulfonate groups in their structure, i.e., sodium 2-acrylamido-2-propyl-1-sulfonate (AMPS) and sodium styrene sulfonate (SSS). The literature reports indicate that these polymers show anticoagulatory action. They will be thoroughly characterized both chemically and physicochemically. Their anticoagulatory action and the possibility of its inhibition using the synthetic polymeric inhibitors obtained will be studied at *in vitro* conditions and in rodents.

The polymers belonging to both groups will be synthesized using the controlled radical polymerization (CRP) technique allowing preparation of well-defined polymers, having intended molecular weight and low dispersity of the sizes of the macromolecules. The efficacy, safety and pharmacokinetic properties of the anticoagulation system consisting of a new block copolymer containing sulfonate groups with its complementary cationic block inhibitor will be developed for potential use in humans. Polymers of both group will undergo biological tests determining basic parameters of their safety (toxicity, elimination, immunogenicity, etc.).

Expected impact of the research project on the development of science, civilization and society

It is expected that the obtained polymers belonging to the first group will show the ability to neutralize the anticoagulant action of heparins and heparinoid compounds. The development of such substances is of great importance, since some of the anticoagulants belonging to this group still do not have any antidote, while in the case of UFH the antidote is protamine sulfate, a protein substance, which shows a number of severe adverse effects. Therefore, it is very desirable to develop an alternative antidote for UFH with superior parameters of pharmacological safety. Successful development of the whole anticoagulation system could replace not only protamine, but also heparin, which is diverse mixture of glycosaminoglycans (GAGs) isolated from animal mucosa in nonefficient and tedious process, with unpredictable dosing and showing some severe adverse effects.