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THE APPLICATION OF THE ELECTRON-BEAM PLASMA FOR THE PRODUCTION OF NOVEL EFFECTIVE PLATELET AGGREGATION INHIBITORS

T. VASILIEVA[1], D. CHUHCHIN[2]

[1] *Moscow Institute of Physics and Technology, Institutsky per., 9, 141700 Dolgoprudny, Moscow region, Russia*
E–mail: protein7@rambler.ru

[2] *Arkhangelsk State Technical University, nab. Severnoy Dviny, 17, 163002 Arkhangelsk, Russia*
E–mail: dimatsch@mail.ru

Abstract. The modification of the artificially synthesized derivative of the natural amino acid alanine with pirozolidine cycle in its structure, the blood protein fibrin-monomer, and heterocyclic compounds (6-R-1,3,4-thiadiazine-2- amines) by the electron-beam plasma (EBP) was studied experimentally. All studied substances were effectively and controllably modified due to non- equilibrium plasmachemical processes in the EBP. The technique involved is likely to be useful to produce compounds with new pharmacological activities (for instance, to produce the effective platelet aggregation antagonists).

1. INTRODUCTION

Low-temperature strongly non-equilibrium plasma generated by means of the electron beam (EB) was used for the materials modification to engineer active agents for new drugs. The study objective was to produce agents inhibiting the platelet aggregation activity using EBP-assisted processes of the biomolecules modification. The modification of the artificially synthesized derivative of the natural amino acid alanine with pirozolidine cycle in its structure, the blood protein fibrin-monomer (FM), and heterocyclic compounds (6-R-1,3,4- thiadiazine-2-amines) by the EBP was studied experimentally. The substances were treated in the EBP of helium (He-EBP) or water vapor (H_2O-EBP). Our previous studies showed the combination of plasma-chemical processes, the fast electrons bombardment, and X-ray irradiation to be responsible for the modification of the original biomaterials but the effects appearing due to the plasmachemical modification predominate. The modification usually appears as a formation of new macromolecules, the structure and molecular mass of these macromolecules differing from those of the original ones (Vasilieva 2007). As a result the products can exhibit absolutely new and unique bio-properties. In our study the conventional techniques of the IR-spectroscopy, NMR-spectroscopy, ion-exchange chromatography, exclusion chromatography were used to characterize macromolecules

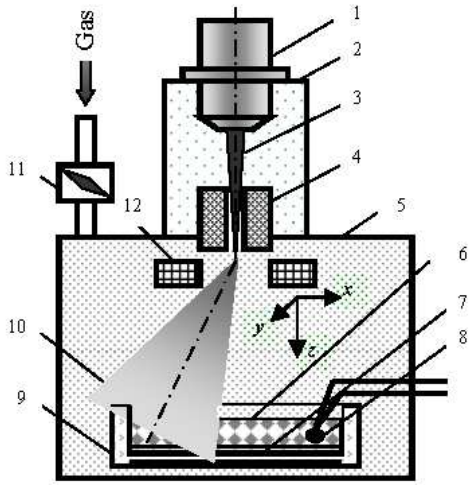

Figure 1: Scheme of the Electron-Beam Plasmachemical Reactor

modification, and the human platelet aggregation *in vitro* was measured to demonstrate the bioactivity of the products obtained, adenosine diphosphoric acid ADP (final concentration 1×10^{-5} M) being used as an aggregation agent. All effects were studied as functions of the plasma treatment conditions.

2. TREATMENT PROCEDURE

Fig. 1 illustrates a way of the substances powder treatment. The focused electron beam (EB) 3 generated by the electron-beam gun 1 which is located in the high vacuum chamber 2 is injected into the working chamber 5 filled with the plasma-generating gas (helium or water vapor) through the injection window (IW) 4. In passing through the gas the EB is scattered in elastic collisions and the energy of fast electrons gradually diminishes during various inelastic interactions with the medium (ionization, excitation, dissociation). As a result the plasma cloud 10 is generated. In general, all plasma parameters are functions of x, y, and z coordinates (z is the axis of the EB injection). Special electromagnetic scanning system 12 is placed inside the working chamber near the IW. The system is able to deflect the EB in x and y directions: being fed with sinusoidal or saw-tooth voltages the system controls the spatial distribution of the plasma particles over the plasma bulk. The working chamber is preliminary evacuated to pressure $\sim10^{-3}$ kPa and then filled with the plasma-generating gas through the feeder 11. The powder of the substance to be treated partially fills the glass container 9. Thin plate 7 made of piezoelectric ceramics is placed on the container bottom. Being fed with alternative current voltage the plate vibrates, throws up the powder particles and forms the mixing layer 6 of the treated material. The miniature thermo-sensor 8 is inserted into the container to monitor the material temperature T_s during the treatment.

3. THE PRODUCTION OF PLATELET AGGREGATION INHIBITORS BY THE EBP-TREATMENT

The treated alanine derivative became partially water-soluble even at room temperature whereas the untreated compound was not dissolvable in distilled water either cold or heated up to 90 °C. The water-soluble products of the treated alanine derivative reduced the aggregation degree down to $\approx$ 30 % and suppressed the platelet aggregation by $\approx$ 45 % more effectively than the untreated substances. The effect of the treatment duration on their anti-aggregation activity increased as the treatment prolonged, the anti-aggregation activity rising sharply at $90 < \tau < 180$ s (Table 1).

Table 1: The effect of the plasma modification in the EBP of water vapor on the anti-aggregation activity of the alanine derivative (*in vitro*): the aggregation degree as a function of the treatment duration τ and temperature of the substance T_s under the treatment procedure

ADP	ADP + untreated amino acid	ADP + treated amino acid				
		$\tau = 45$s, $T_s = 38$ °C	$\tau = 90$s, $T_s = 38$ °C	$\tau = 180$s, $T_s = 38$ °C	$\tau = 180$s, $T_s = 55$ °C	$\tau = 300$s, $T_s = 55$ °C
56±2%	46±2%	41±3%	41±3%	34±3%	32±3%	31±3%

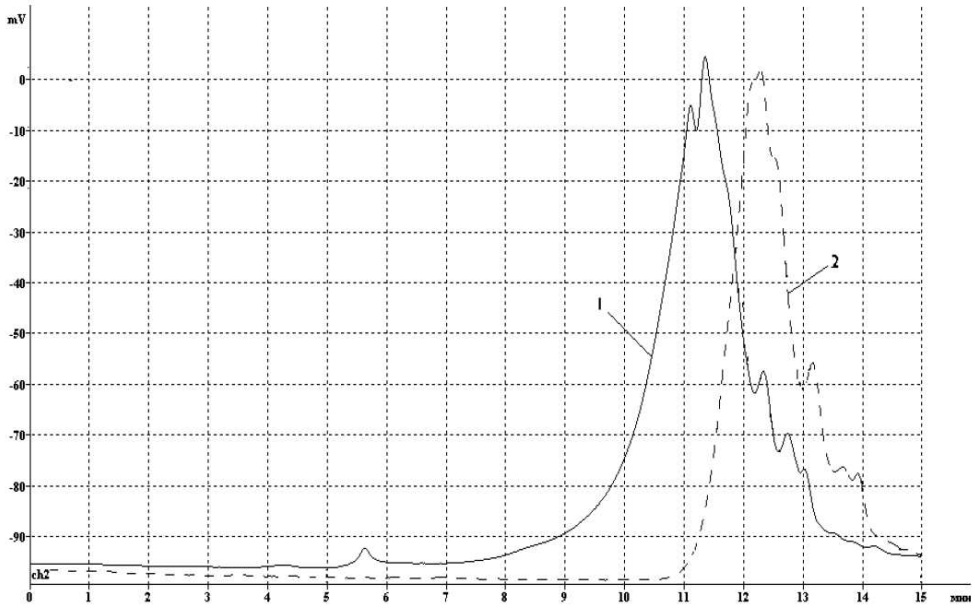

Figure 2: The chromatograms of the FM treated in the EBP of helium (1), water vapor (2).

The EBR-treatment was found to cause the partial destruction of peptide –CO-NH-bounds in the primary FM structure and the oxidation of disulfide bounds stabilizing tertiary peptides structure. All changes were more significant after the treatment in the H_2O-EBP in comparison with the treatment in the He-EBP. The EBP-treatment reduced the amount of some amino acids forming the primary protein structure. The percentage of lysine, threonine, cystine, tyrosine and phenylalanine was found to reduce significantly (down to 2 times with respect to the native FM). The reduction of other amino acids was not so sharp (only 1.3-1.5 times with respect to the native FM. The EBP-modified products did not exhibit the specific antigenic properties of original FM and did not react with specific antibodies, while the native substance gave specific precipitation line. The water-soluble products of the FM modification decreased the platelet aggregation up to $\approx$ 33-35 % *in vitro* at concentrations 1×10^{-5}-1 mg/ml, treatment in the plasma of water vapor being more effective than that in the plasma of helium. The peak corresponding to the elution time 12.3 min was observed at the exclusion chromatograms of the FM modified both in the H_2O- and He-EBP. It is this peptide that is likely to inhibit the platelet aggregation (Fig. 2).

The modification of 6-R-1,3,4-thiadiazine-2-amines in the H_2O-EPB resulted in their solubility increase as well. The plasma treatment increases the antiaggregating activity of the substance involved up to twofold with respect to that of the untreated substance. The NMR-spectroscopic analysis showed the sample of 6-R-1,3,4-thiadiazine-2-amines after the EBP-treatment to contain new products in $\approx$ 6% concentration.

Thus, fine powders of some natural substances dispersed in the EBP cloud can be effectively and controllably modified due to non-equilibrium plasmachemical processes in the EBP. The technique involved is likely to be useful to produce compounds with new pharmacological activities (for instance, to produce the effective platelet aggregation antagonists). The electron-beam plasmachemical reactors with the aerosol reaction volume seem to be competitive with technologies conventionally used in pharmaceutical industry.

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