Electron-beam plasma in the production of bioactive agents and drugs

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Abstract. The modification of some biopolymers and amino-acids by the Electron-Beam Plasma was studied experimentally. The plasma was generated by injecting the continuous electron beam in gaseous or vapor media. The powders of the substances under consideration were found to change their physical-chemical and biological properties due to the treatment. The aggregation degree of human blood platelets in vitro was chosen as the quantitative characteristics of the biological effect. The untreated compounds were not dissolvable in distilled water at room temperature and did not inhibit the human platelet aggregation. The modified by the Electron-Beam Plasma synthetic derivative of 2-aminopropanoic acid (alanine) was proved to acquire the anti-aggregation activity for platelets. Products of the plasma modified fibrin-monomer were found to be soluble in water at room temperature and reduced the aggregation degree up to ≈ 33-35 % in vitro, treatment in the water EBP being more effective than the treatment in helium.

1. Introduction

The Electron-Beam Plasma (EBP) is generated by injecting an electron beam (EB) into a gaseous medium. Under typical conditions of the EBP generation (medium pressure \( P_m < 10 \text{ kPa} \) and moderate EB power \( N_b < 1 \text{ kW} \)) the plasma is strongly non-equilibrium and cold. Being injected into the gas the EB ionizes the gas, excites the gas molecules and is able to cause molecule dissociation. As a result, the EBP composition is complicated and there are a lot of chemically active particles that do not exist under equilibrium conditions. With respect to non-equilibrium plasmas generated in conventional ways (for instance, the plasma of gas discharges) the EBP has the following advantages:

- the EB can be injected into any gases, vapors and gas-vapor mixtures; the EBP bulk does not contract even at very high gas pressures (\( P_m \sim 10 \text{ kPa} \) and higher).
- the solid powders and liquid droplets injected into the gas do not prevent the EBP generation; large-size bodies can be inserted into the plasma bulk.

The EBP was proved to effectively modify properties of materials thanks to the plasma chemical activity, complicated organic and polymer molecules being the most modifiable even at low temperature.

2. The Electron-Beam Plasmachemical reactor and the treatment procedure

Figure 1 illustrates a typical way of the EBP generation. The focused EB 3 generated by the electron-beam gun 1 located in the high vacuum chamber 2 is injected into the working chamber 5 filled with the molecular gas 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). Eventually the EB power...
transforms into heat increasing the temperature of the medium and heating the unit elements. It may also cause phase transformations or is radiated. As a result the plasma cloud 6 is generated, all plasma parameters being functions of $z$- and $r$-coordinates.

**Figure 1.** The Principle of the EBP generation and the powder treatment procedure; the comments are given in the text.

**Figure 2.** The reaction chamber of the rotating plasmachemical reactor; the comments are given in the text.

The working chamber is preliminary evacuated to pressure $\sim 10^{-3}$ kPa and then filled with the plasma generating gas through the feeder 8. The pressure $P_m$ varied within the range 1-10 kPa depending on the gas composition and treatment conditions required. The following gases were used to generate the EBP: air, nitrogen, oxygen, noble gases (He and Ar), gaseous hydrocarbons, CO$_2$, water vapor and vapors of some organic liquids. Gaseous and vapor-gas mixtures were used as well. A specially designed double-stage gas-dynamic window is used to transport the EB of energy $E_b = 20$-40 keV from high vacuum $\sim 1$ Pa (that is a typical pressure required for the operation of electron guns with thermoionic cathodes) into the working chamber filled with the gas.

The powder of the substance to be treated is filled in the glass container 11. A thin plate 10 made of piezoelectric ceramics is placed on the container bottom. Being fed with AC voltage the plate vibrates, throws up the powder particles and forms the mixing layer 9 of the treated material. The miniature thermo-sensor 7 is inserted into the container to monitor the material temperature $T_s$ during the treatment. Adjusting the EB power the temperature $T_s$ could be varied within the range 300-450 K, no additional heaters or coolers being used to maintain the constant $T_s$-value. Note that the power is heated by the EB more effectively than the gas and usually $T_s > T_m$, where $T_m$ is the plasma generating medium temperature.

Another version of the reactor developed to treat larger amounts of biomaterials is illustrated by figure 2. The general axis of this reactor (and the axis of the EB injection) is horizontal, the reaction chamber being a cylindrical or barrel-shape vessel 1 equipped with internal ribs 3. The EBP cloud is formed in the vessel hollow by the EB 2 injected through the open end of the chamber. The powder 4 of material to be treated is mixed by means of the vessel rotation; as a result the aerosol reaction zone 5 is formed inside the chamber. Note that the reaction chamber 1 is placed inside the working chamber (that is not shown in figure 2) filled with the plasma generating gas at the required pressure $P_m$. 
3. The biopolymers treatment in the Electron-Beam Plasma

The plasmachemical modifications of cellulose materials (pure cellulose, wooden sawdust and crushed paper), peat and chitosan in EBP of O₂, He, H₂O, NH₃, CO₂, SF₆ are studied in detail. The yield (S, %) and the average molecular mass (M, amu) of water-soluble products extracted from the solution were taken to be the quantitative criteria for the modification. Note that typical content of water-soluble substances in untreated cellulose materials does not exceed 1-2%. The molecular mass of the cellulose may reach several million.

The water-soluble products yield depends upon the processing conditions and has a maximum value Sₘₐₓ for each plasma-generating gas: for O₂ plasma Sₘₐₓ ≈ 45 %, for H₂O-plasma Sₘₐₓ = 56 %, for (H₂O + NH₃) plasma Sₘₐₓ = 70 % which are reached during certain time periods of the treatment τ₀. At first the dependence S(τ) increases smoothly, then – steeply close to τ₀ after which the yield of the water-soluble products does not change whatever the time of the treatment could be (figure 3). The relation between S and the pressure of O₂ - plasma is shown in figure 4. The NMR- and IR-spectroscopy analyses showed the final water-soluble products of the cellulose modification to be β-(C₁→C₄)-tetrasaccharide, the molecule structure and chemical bonds of the products being identified [1]. The average molecular mass of the products was M = 400-800.

The products of the EBP modification of all substances mentioned above turned out to be bioactive. For instance, modified cellulose and peat are effective substrates for microorganisms and fungi: the yield of the yeastrel on the plasmachemically treated peat attained 0,14 kg per 1 kg of the peat, whereas productivity of the untreated substrate was only 0,053 kg. The yield of water-soluble products from plasmachemically modified chitosan achieves 75-80% whereas the efficiency of conventional technologies does not exceed 35-40%. Preliminary tests showed the water-soluble products of the chitosan modification to be promising for skin therapy.

![Figure 3](image1.png) **Figure 3.** The yield of water-soluble products from the cellulose treated by the H₂O-EBP as a function of the treatment duration.

![Figure 4](image2.png) **Figure 4.** The yield of water-soluble products from the cellulose treated by the O₂-EBP as a function of the gas pressure.

4. The amino-acids treatment in the Electron-Beam Plasma

Plasmachemically modified organic substances were supposed to be active agents for drug production. Some natural amino acids with artificially inserted pirozolidine cycles into their structures were used as original substances and the products of their modification in the EBP of helium and water vapor were tested as inhibitors of the human platelet aggregation. Preliminary analysis showed the substances of this class to be promising as active agents for medical therapy of acute coronary events, and cardiovascular diseases that remain the leading cause of mortality. Their advantages are due to selectivity of the pharmacology action and limited side effects.

Biological effect appearing due to plasmachemical modification can be detected by means of the standard techniques in vitro, the aggregation degree being chosen as the quantitative characteristics of...
the biological effect. The platelet aggregation $A$ (%) were measured by the turbidimetric method and $A$ was defined as the ratio of the light transparency of the platelet suspension after ceasing the aggregation process to the initial value of the light transparency [2]. The aggregation was monitored by the aggregometer Biola (Russia), ADP (final concentration $1 \times 10^{-5}$ M; Boehringer Mannheim, Germany) being used as an aggregation agent.

The experimental data were statistically analyzed by Student’s test, P-values smaller than 0.05 were considered as reliable. Table 1 presents the results of the statistical analysis.

The powder samples (≈ 50 mg in mass) of the original substance were treated in the EBP of water vapor at pressure $P_m = 2$ kPa for variable time duration $\tau = 45$-300 s. The monolayer of the powder to be treated was filled on the glass substrate and placed into the reaction chamber of the electron beam plasmachemical reactor. The typical EB power was $N_b = 0.1$ kW, the sample temperature $T_s$ under the treatment could be varied within the range 30-110 °C.

Table 1. The effect of the plasma modification in the EBP of water vapor on the anti-aggregation activity of the tested 2-aminopropanoic acid derivative (in vitro): the aggregation degree $A$ as a function of the treatment duration $\tau$ and the substance temperature $T_s$ under the treatment procedure.

| 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% | 34±3% |
| 32±3% | 31±3% |

The treated substance became partially water-soluble at room temperature and the solution at maximum concentration was added to the platelet suspension to measure the aggregation degree. The main results are:

- The untreated derivative of 2-aminopropanoic acid decreased human platelet aggregation in vitro from 55.82±2.53% (control experiments) to 45.97±3.51%. The untreated compound was not dissolvable in distilled water at room temperature and the water heating up to 90 °C followed by cooling to 25 °C was required to carry out the control experiments.
- The water-soluble products of plasma treatment reduced the aggregation degree up to ≈ 30%, i.e. being treated by the EBP for 5 min the studied substance reduced the platelet aggregation activity by approximately 45%.
- 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. Our previous experiments with cellulose, starch, chitosane show that the yield of the plasmachemically modified products began to rise abruptly at some duration $\tau_0$. At shorter durations $\tau < \tau_0$ the plasma did not modify the original substance and the longer treatment $\tau > \tau_0$ resulted in insignificant additional effect (see figure 3 and [1]).
- Moderate sample heating amplified the treatment effect slightly, i.e. plasmachemical processes are responsible for the modification. Cellulose and chitosane modification was found to occur due to plasmachemical processes, whereas the substance heating was a minor factor only.

5. Fibrin-monomer treatment in the Electron-Beam Plasma
Fibrin-monomer is the natural protein which is contained in the blood of mammalians. The powder of fibrin-monomer industrially made of the human blood (Company Technology-Standart, Barnaul, Russia) was treated in the Electron Beam Plasmachemical Reactor in the EBP of water vapor and helium, then the modification products were tested as aggregation inhibitors. Original substance was
not water-soluble and its influence on the platelet aggregation could not be studied both \textit{in vitro} and \textit{ex vivo}. Products of the plasma modification were found to be soluble in water at room temperature without bunching. The anti-aggregation activity of the treated fibrin-monomer was studied as described above (see section 4). Products of plasmachemically treated fibrin-monomer were found to reduce the aggregation degree up to $\approx 33-35\%$ \textit{in vitro} (figure 5), treatment in the water EBP being more effective than the treatment in helium. Figure 6 presents the UV-spectra of absorption of original and treated fibrin-monomer. The spectral curves of the modified products radically differ from the curve of the original substance. This supports the hypothesis of the changes in the physical-chemical properties due to the EPB action.

![Graph](image)

**Figure 5.** The effect of the plasma modification on the anti-aggregation activity of the fibrin-monomer ($A_{\text{max}},\%$); the treatment in the H$_2$O-EBP.

![Graph](image)

**Figure 6.** UV-spectra of the light absorption of fibrin-monomer solutions.

6. Mechanisms of the plasmachemical modification of biomaterials

The properties modification of biomaterials and, in particular, the higher anti-aggregation activity of 2-aminopropanoic acid derivative after EBP-treatment is assumed to be due to the following reasons:

- Plasma treatment increases the solubility of the studied substance. Regarding the anti-aggregation activity, it means that the concentration of the active agents in platelet suspension during the aggregation degree measurements was inherently higher in comparison with the non-soluble original substance.
- Possible chemical transformations of substances are also supported by the observed changes of the original color due to the plasma treatment. The color transformations in the powder of studied amino acid derivative support the suggestion regarding the modification due to the treatment in both helium and water vapor plasmas.
- Changes of the compound conformation could occur without alteration of its structure. This could improve the affinity of the substance under consideration to the platelet GPIIb/IIIa-receptors.
- Some new chemical groups might be included into the molecule structure during the treatment in the EBP. For instance, the EBP of the water vapor is enriched with H, OH and H$_2$O$_2$ radicals that can react with the amino acid molecules.
These explanations seem to be reasonable and qualitatively correspond to the data of our previous experiments on biopolymers modification by the EBP. Undoubtedly, the higher effect of the plasma modification can be attained by optimizing the treatment regimes and adjusting the design of the plasmachemical reactor.

7. Conclusions

- The plasmachemical treatment of natural organic materials and substances is very promising for bioactive agents production and electron beam plasmachemical reactors are able to controllably change the product properties. For instance, the technique involved is likely to be useful for the platelet aggregation antagonists development since inhibitors that can reduce the aggregation activity by fifty percent are conventionally considered to be effective.
- The modification of the natural amino acid derivatives in the EBP of the water vapor can be explained by the same plasmachemical mechanisms that were found and confirmed in the experiments with cellulose, peat and chitosane.

References

[1] Aleksandrov I V, Bychkov V L and Vasiliev M N 1997 Chem. of High Energies (in Russian). 31 82-8
[2] Born G 1962 Nature 194 927-9