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Metrological Approaches to Drug Development. Fast Screening Using Universal Biosensors at the Stage of Drug Development

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Abstract—We expect that in the near future drug development will become the most interesting and demanded application of the suggested approach. Taking a known drug as the reference point and performing its chemical modification, one can sharply reduce the time and financial expenses for bioactivity testing of new substances and experimentally predict its general toxicity on a biological model the most adequate to human body.

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Development and commercialization of a new drug is a long-term and expensive process. It takes 10–20 for a biologically active substance to pass way from laboratory synthesis or isolation to application as a drug [1]. If 20 years ago this process costed slightly more than $300 mln, then now the cost may reach $1 bln. Therewith, only 3 of 5 newly commercialized drugs make a profit greater or equal to expenses on its development.

One of the approaches to the problem of reduction of terms and costs of drug development involves screening of biologically active substances and drugs on their basis at the development stage. Such screening can be performed by means of biosensors.

The end of XX century was marked by the advent of a new field of science—biosensing. Unfortunately, already in a few years the term "biosensors" started to be related to electronic devices, and attention started to be focused exclusively on the development of high-selectivity specific biosensors. However, with time, researchers returned to nonspecific universal bio-sensors on the basis of live systems. Biosensing as one of the components of the methodology of drug development allows fast selection of physiologically active substances as potential candidates for medical applications.

Existing Methodologies of Drug Development

The methodical aspects of drug development was be presented as three principal problem blocks which describe the drug development process in cases, when one or several preselected active substances are available, as well as production of generics, i.e. copies of a brand-name drug, whose share among drugs registered in the Russian Federation is high and continues to increase.

These blocks include the aim, tasks, expected results, and limitations of pharmaceutical developments.

Block 1

Aim: prediction of the demand for a drug to be developed at the pharmaceutical market and forming the commercial series of drugs with a similar action.

Tasks: study of existing and being tested approaches to treatment of diseases belonging to leading nosologic groups (by medical and patent literature data). Study of the marketing dynamics of commercial series of drugs for a concrete nosological group of diseases.

Expected results: choice of an object for research (chemical class for targeted drug search), choice of a

drug development strategy, and defining expected characteristics of the drug to be developed.

Limitations: economical; marketing research is required.

Block 2

Aim: Drug development with account for preset pharmaceutical and commercial characteristics.

Tasks: Study of the composition of and production technology of the drugs to be developed, feasibility assessment, synthesis and biopharmaceutical research, stability assessment, standardization of the developed drugs, and development of regulatory documents.

Expected results: development of a drug dosage form meeting genenal regulatory requirements and comparing with analogs in biological availability; submission of regulatory documents for approval.

Limitations: chemical limitations associated with drug development and testing.

Block 3

Aim: complex technical and economic assessment of the developed drug, competitiveness assessment.

Tasks: Bioequivalence assessment of the developed drug, economic assessment, complex assessment of desired characteriastics of the drug, competitiveness assessment.

Reached results: developed drug meeting requirements of practical medicine, production feasibility, and price affordability.

Limitations: engineering, competitiveness assessment is required.

When new biologically active compounds are searched for to be used as active substances of a drug to be developed, Block 2 comes first. Note, by the way, that over the past years the field of science focusing on search for new biologically active substances has been more and more frequently referred to as medicinal chemistry. This appears to be associated with the fact that the IUPAC defines medicinal chemistry as a brach of chemical science dealing with search for and development, identification, and mechanisms of action of biologically active substances at the molecular level. Most attention is therewith given to drugs, and the interests of medicinal chemistry also extend to study and synthesis of drug metabolites and related compounds.

Block 3 is in many cases closely associated with Block 1, since in is just economic limitations that dictate one or another drug dosage form to be produced. Apart from already mentioned limitations, the entrance of a drug to the pharmaceutical market can be affected by several other reasons. For example, the retail chain poorly sells preparations with shelf lives less than 2 years for distribution and logistic management reasons.

The way from a biologically active substance to a drug can be outlined in terms of a series of successive stages [2]: (1) synthesis and physicochemical study of a substance; (2) study (prediction) of its biological activity; (3) toxicity testing (first phase of preclinical testing); (4) specific biological activity testing (second phase of preclinical testing); and (5) clinical testing (phases 3.1 and 3.2).

The first stage is meant to include a complex of chemical or biotechnological approaches. The chemical approaches suggest targeted synthesis of regulators and products of the energetic and plastic metabolism, phytotherapeutic drugs, compounds belonging to known drug series, compounds with programmable properties, modifications of polymorphous drugs, as well as stereoselective synthesis of eutomers and the most active tautomers of drugs.

The biotechnological approaches to new biologically active substances are realized in two ways: selection of the most active drug sources and genetic engineering of active drug sources.

The practice shows that the number of substances-to-cost ratio changes an order of magnitude in going from one to next stage. The number of substances decreases by an order of magnitude in going from the first to fifth stage, and, accordingly, the cost of each stage increases by an order of magnitude.

Thus, the most costly stages in drug development are the stages 2 to 4. According to [3], before marketing 68 randomly selected pharmaceuticals, American pharmaceutical companies spent $802 mln for research on pharmaceuticals rejected at the stage 2, 3, or 4.

Biosensor Screening at the Stage of Drug Development

Over the past decades, considerable progress has been reached in biochemistry, molecular and combinatorial chemistry, and computer techniques, which prompted pharmaceutical companies to turn to screening approaches for decreasing the number of

candidates for clinical testing and, as a result, decreasing the cost of introduction of drugs to the pharmaceutical market [4–6].

Beginning in 1990s the scale of screening research has risen several times. The most actively developing screening techniques are high--throughput screening (HTS) (computer simulated molecular targeting based on biochemical pathway), new chemical entities (NCE) (a new therapeutic molecule or compound that has not been tested on humans), and (3DMD) (3D molecular docking), specifically 3D modeling of interacting molecules with a known structure, based on their spacial arrangement [7, 8]. However, ever though research in this has made a great progress, at present certain crisis is observed in the methodology of search for new drugs, since computer modeling has failed to justify expectations and investments, and this situation forces pharmaceutical companies to search for new approaches to investing into pharmaceutical industry [9].

At present NCE is considered as the most promising screening technique. High--throughput screening gives no way for predicting the therapeutical properties of new compounds but is quite useful for predicting new application fields for already known compounds. With accumulation of experimental data on the 3D structures of protein molecules, 3DMD is coming to the fore in computer screening. The commercial success of this technique is associated with the fact that, when pharmaceutical companies initiate production of generics, they make use of the clinical characteristics of brand-name medicines and certify and patent generics as a new application of the brand-name medicines. Over the past time combined approaches have received wide acceptance [10–12].

Fast screening of biologically active substances, including drugs, is made possible by the biosensor technology [13].

According to expanded definitions, biosensors are detectors whose action is based on the sensitivity of cells and molecules to one or another effect, in particular, to exposure to chemical substances. When the test substance binds with the biological component of the biosensor, the transducer generates an electrical or optic signal with a power proportional to the concentration of the substance. The possible uses of biosensors in domestic science and industry include measurement of nutrition value, freshness, and safety of food and alcohol [14]; express analysis of blood; identification and measurement of environmental pollution [15]; and detection and quantitation of explosives, toxins, and biological warfare agents [16, 17].

Search for new drugs is not mentioned among priority uses of biosensors in the Russian scientific and technical literature. However, we can expect that this direction will gain in significance in the next few years with the harmonization of the legislation and pharmaceutical markets in Russia and foreign countries.

At present biosensing has received the widest application in the regulation of environmental pollution and environmental quality assessment of natural ecosystems. The Russian environmental protection bodies recommended a number of approved biological test systems for assessing the environmental hazard of substances and preparations on their basis, as well as of environmental matrices exposed to such substances and preparations. The biological test systems are certified at the state level and included in the Federal register of procedures and so-called register of environmental regulators. Certified laboratories are required to have in their disposal at least two test systems including tests organisms belonging to different taxonomic groups [18]. As a rule, laboratories analyzing environmental matrices, raw materials, and wastes use at least 2–4 procedures for both acute and chronic toxicity testing. Thus, for example, at the Lomonosov Moscow State University four test systems are used for acute and chronic toxicity biotesting: *Paramecium caudatum* infusoria*, Daphnia magna* and *Ceriodaphnia affinis* entomostracans, as well as *Scenedesmus quadricauda* green protococcus algae [19]. Toxicity is measured in terms of LD_{50} values for test species [20]. The recommended procedures also suggest measurement of safe dilution factors for water samples or for aqueous extracts from solid samples. The test systems used as environmental biosensors are listed in the table in [21]. At the same time, the search for a universal biological model on the basis of protozoa for chemical toxicity assessment can be easily adapted for searching for new drugs. As the most suitable candidates among protozoan biosensors we would like to mention *Tetrachymena pyriformis* used in ecotoxicology [22, 23] *Paramecium caudatum* infusoria used an *in vitro* model in biological activity testing [24, 25].

As to the preferred uses of protozoan biosensors in foreign countries, they include search for new biologically active substances. In this context, qualitatively new biosensing technologies are being

Biotesting methods and environmental toxicity parameters

Method	Measured parameter
Toxicity assessment of wastes, soils, sediments, and waste, surface, ground and drinking waters using *Paramecium caudatum* holotrichs (FR 1.1.39.2003-00-923)	Acute toxicity, $LM_{50\text{-}1}$ Safe dilution multiplicity, $SM_{10\text{-}1}$
Toxicity assessment of water and aqueous extracts from soils, sediments, waster waters, and wastes by changes in the death and birth rates of *Daphnia magna* (FR 1.1.39.2001-00-283)	Acute toxicity, $LM_{50\text{-}96}$ Safe dilution multiplicity, $SM_{10\text{-}96}$ Chronic toxicity, $LM_{50\text{-}672}$
Toxicity assessment of water and aqueous extracts from soils, sediments, waster waters, and wastes by changes in the chlorophyll fluorescence and cell number of *Scenedesmus quadricauda* algae (FR 1.1.39.2001-00-284)	Acute toxicity, $LM_{50\text{-}96}$ Safe dilution multiplicity, $SM_{20\text{-}96}$ Chronic toxicity, $LM_{50\text{-}672}$
Toxicity assessment of water and aqueous extracts from soils, sediments, waster waters, and wastes by changes in the death and birth rates of *Ceriodaphnia affinis* (FR 1.1.39.2001-00-282)	Acute toxicity, $LM_{50\text{-}48}$ Safe dilution multiplicity, $SM_{10\text{-}48}$ Chronic toxicity, $LM_{20\text{-}7}$

developed for biological activity testing at all stages of the pharmaceutical technology: from the stage of synthesis and isolation of a substance to production of a new pharmaceutical preparation [26–30]. These biosensors can be used in pharmaceutics for functional screening at the stage of drug design and discovery [31] and for predictive screening at the stage of planning the targeted synthesis and determination of the mechanism of action of the biologically active substance [32], and for toxicity predictions over a wide concentration range without animal experiments [33, 34].

Experts in analytical control consider that complex mixtures are best analyzed by means of nonspecific biosensors which allow identification of different chemical effects by measuring different parameters of a single biomaterial [35].

An alternative technology on the basis of cellular biosensing is molecular genomics which makes it possible to create cells (primarily yiest cells) with molecular chains responsible for biosensing built into cellular membranes [36]. Admitting that this approach holds some promise, we would like to note that this technology is quite costly both in terms of preparation [37] and in terms of use of biosensors [38]. Results of research in this field are published almost exclusively in the patent literature [39].

The measuring unit in biosensors transforms an input signal $x(t)$ into an output signal $y(t)$:

$$y(t) = F[x(t)], \quad (1)$$

where $x(t)$ and $y(t)$ are vector values and $F(x)$ is the required transformation function.

Equation (1) can be considered as a mathematical model of a biosensor, where an input information is transformed into an output information. In a more general representation, a biosensor fulfills the operation of transforming a set of input signals $x \in X$ into a set of output signals $y \in Y$, and, therewith, the transformation can be single-values for one and the same stimulus (biologically active substances). In real nonspecific sensors, the transformation function depends not only on the signal $x(t)$ (stimulus concentration), but also on the perturbation $\xi(t)$ produced by the signal $x(t)$ (state of the biosensor in a concrete time moment), on the interference $\upsilon(t)$ (concentration of the interfering compound) affecting instrument parameters $q(t)$, on imperfections in the instrument manufacturing $\eta(t)$ (scatter of parameters of the live system), on the interference $\nu(t)$ (changes of physicochemical and other factors affecting the live system), i.e.

$$y(t) = F[x,\xi,q(\eta, \upsilon), \nu], \quad (2)$$

where ξ,q,η,υ,ν are vectors.

Fugure 1 shows the functional scheme refelecting Eq. (2).

The signal from a test object is detected as a change of some physicochemical parameter. Such a detected signal can be exemplified as a change of fluorescence intensity under the action of genetically modified *Saccharomyces cerevisiae* yeasts [40]. However, the

final signal of a biosensor is almost ways is an electric signal which relates to the intensity of the measured physical characteristic [41].

In should be noted that electric signals are not the only possible measured physical characteristics. Thus, for example, by measuring the variability of the nature of formation of two *Saccharomyces cerevisae* strains of different ploidities under the action of the meadium one can control the state of the environment. The toxic effect of a test sample is measured by the lethality parameter, by the increase of the rate of mutant colonies, and by the rate of morphoses and changes of the size of colonies to assess heritable cell lethality and predict long-term pathology [42]. One of *Saccharomyces cerevisiae* strains is also used for biological activity assessment of adaptogens. In this case, activity is measured by the increase of cell number in the presence and in the absence of an adaptogen in the cell culture grown in a special nutrient medium decelerating yeast cell growth [43]. The same approaches allow indentification of biologically active substances by tracing changes in yeast cell cultures [44].

Dynamics of Blood Sedimentation Rate as a Biosensor

According to the general theory of biosensing, we suggested that human cells would be the best object for prognostic NCE research, and blood cells would be the most promising biosensors, since all diagnostics of human pathologies is based on comparative analysis of blood in norm and in pathology. Further evidence in favor of blood cells as candidates for universal pharmaceutical biosensor applications comes from the fact that in vitro blood experiments are presently a standard and widely used procedure. Thus, if it would be shown that some single-type action on blood (blood cells) in vitro induces a predictable (reproducible) and quantitatively mesurable response, then one could develop a high-sensitivity biosensor. Both whole blood and separate cell polulations could be used as biosensors. This suggestion has found experimental confirmation [45]. Banerjee et al. [46] developed biosensors for quantitative determination of toxic substances in solutions on the basis of immobilized lymphocytes.

As one of the first realizations of this approach we can mention the antigen leukocyte cellular antibody test (ALCAT) which allows individual selection of pharmaceuticals and food products by following changes in the quantity, shape and size distribution of

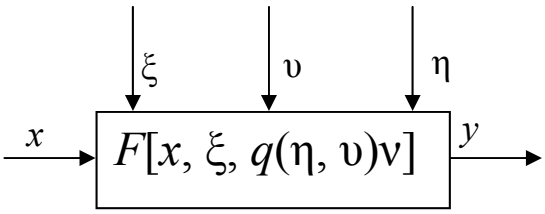


Fig. 1. Functional scheme of a biosensing instrument.

immune-competent protein blood cells in response to an applied stimulus (chemical substance or protein) [47]. An analogous approach is also used in Europe, but it is based on only one fraction of leukocyte, i.e. neutrophiles [48]. With the progress in electronics, analogous approaches have started to be applied for measuring erythrocyte sedimentation rates (ESR) [49]. Note that these approaches are only applied for individual diagnostics, rather than for searching for new drugs.

In our research on the assessment of the effect one or another stimulus induces in blood cells we suggested ESR dynamics as a cell biosensor. This suggestion was motivated, in particular, by the reported use of the classical ESR parameter as a measure of an individual toxicity of substances *in vivo* [50]. By following dynamics of blood sedimentation parameters one can gain a more reliable information compared with that provided by a static ESR test [51–53].

The classic ESR test is performed in the following way: an anticoagulated blood sample is withdrawn into an upright tube to a standard height (usually 10 cm), and after an hour the height of the plasma column over the layer of sedimented erythrocytes is measured. This value is just the ESR (in mm h^{-1}). In medicine, high ESR values are treated as evidence of inflammation processes and tissue necrosis, even though in cases where tissue necrosis is evident (active tuberculosis, terminal stages of cancer, intact acute appendicitis, etc.) the ESR values fit the normal range. Chizhevskii was the first to note that analysis of blood sedimentation dynamics favors a more efficient diagnostics. He obtained experimental evidence (in 1930s) that the ESR parameter in its dynamic version can be used for in vitro registration of blood cell responses.

Detailed study of blood sedimentation dynamics (BSD) or an immediate sedimentation rate allowed us to suggest a number of new parameters of the process, which, along with traditional ESR, sharply enhance the information content of the test and its diagnostic value.

The classical ESR test, while being helpful in diagnosing inflammatory diseases, is useless when a concrete nosology is to be identified.

More recent works reported that the immediate blood sedimentation rate, i.e. a derivative of the sedimentation curve (*dh/dt*) which was later defined as BSD, is a more useful diagnostic tool that an integral 1-h ESR value [54].

The most successful use of the BSD parameter for extended diagnostics was presented in [55]. It was found that from the curve of immediate blood sedimentation rate one can reveal sedimentation features which are not explicitly evident in the integral sedimentation curve. Fourier transform of BSD curves reveals differentiated and multiply repeated elements (characteristic frequencies) typical of different nosologies, which "can be useful in diagnostics" [55].

Thus, the available evidence gives grounds to conclude that measuring the ESR dynamics under standardized conditions can be used both for specific individual diagnostics (direct task) and for in vitro assessment of the effect of biologically active substances, irrespective of indivudual features of blood samples, i.e. as a biosensor (reverse task). Solution of the reverse task is based on the suggestion that if the effect a test substance has on rheological blood parameters is analogous to that of a known (reference) substance, irrespective of individual blood features, then the test substance will be analogous to the reference sampe in the biologic effects on all processes that occur in sedimented blood cells. Consequently, knowing, for eample, toxicity (or activity) of the reference substance one can predict or assess toxicity (or activity) of the test substance. The above regularity makes it possible to develop fast screening procedures for solving a great variety of pharmaceutical tasks. This is assessment of authenticity, biopharmaceutical efficiency, and stability of drugs, comparison of original and generic drugs, express toxicity testing of biologically active substances in drug development, assessment of the biopharmaceutical efficiency of excipients in pharmaceutical industry, etc. Thus, we are speaking here about the emergence of a new scientific field in pharmacy, namely application of nonspecific universal biosensors in drug research and development.

Only a few examples are known for the use of reference compounds for tuning biosensors, even though this methodical approach holds great promise. A procedure for biological activity assessment was described, involving the following steps: preparation of a test object from a live organism, introduction into the cell suspension of the reference and test substances, incubation, preparation of the reference and test solutions, and measurement of their optical densities. It should be noted that this work was published well after our research, and it provides evidence for our working hypothesis.

The methodical substantiation of our suggested approach to a universal biosensor for express pharmaceutical screening is based on the postulate that the introduction of a biologically active substance in blood affects the BSD parameter. This postulate could be accepted, provided the mechanism of action of a substance on the sedimenting system. However, regardless of the wide practical use of ESR, no uniform commonly accepted mechanism of this process has still been developed.

As known, many biological processes are oscillatory in nature. The spectral and correlation analyses of the dynamics of biological processes give information essential for understading of their mechanisms [56]. Kuo et al. [57] reported evidence for a nonmonotonic character of the blood sedimentation process.

The existing theories of blood sedimentation can arbitrarily be divided into calculational and phenomenological.

The simplest model is based on the Stoke's law which describes sedimentation of a spherical particle in an infinite volume of liquid. However, the Stoke's law can only arbitrarily be applied for the blood sedimentation process, since this law is valid for single free particles moving in a viscous medium and for colloid low-concentrated systems. The more advanced sedimentation model considers blood sedimentation as a process involving sedimentation of conglomerates.

The nonlinear dynamics of blood sedimentation was also explained in terms of electrostatic phenomena. As known, erythrocytes and their aggregates in solutions bear a negative charge, and, therefore, they should repulse from each other. There were different estimates for the strength of this repulsion, but no experimental evidence for mutual repulsion of erythrocytes has still been obtained.

Another phenomenological explanation of the mechanism of erythrocyte aggregation is provided by the fibrinogenic hypothesis [58, 59]. According to this

hypothesis, aggregates are formed in three stages: initially erythrocytes approach to each other by a distance of about 0.01 μm under the action of hydrodynamic forces, then fibrinogen molecules form bridges between erythrocytes, and, finally, slow mutual motion of erythrocytes (under the action of surface forces) establishes the final configuration.

Many researchers [60] consider blood sedimentation as a cooperative process of settling of the 3D erythrocyte network, the experimental visual observation of which was described in 1920s [61].

Of the mathematical models of erythrocyte network settling, the most interesting is the fractal model [62] which describes the process by an S-shaped sedimentation curve. A distinguishing feature of this theory is its fractality. The fact that the model operates by fractal dimentionality suggests that the system involves internal periodic processes responsible for external ordering. Consequently, having studied factors controlling formation and rearrangement in the process of sedimentation of the erythrocyte network, one can transform the sedimenting blood column into a sensitive biosensor.

Our literature analysis showed that until now no mathematical model including biochemical processes occurring in whole blood during its sedimentation has been developed. We suggested a base model of an active colloid system in which the properties of sedimenting particles are changing during sedimentation [63]. The suggested model was tested to describe the process of sedimentation of form blood elements, including intercellular interactions (erythrocyte aggregation−deaggregation and interaction of the resulting structures with leukocytes).

The developed model which describes the behavior of blood as an active colloid system, including erythrocyte aggregation−deaggregation processes and interactions of the resulting structures with leukocytes, allows quantitative explanation of the changes in the ESR parameters, observed in clinical practice, and provides evidence for the applicability of this parameter for pharmaceutical screening, when tests substances affect either cellular membranes or intercellular interactions.

Our developed biosensor on the basis of blood cell sedimentation was used for express screening of new platunim complexes for antitumor activity.

Platinum complexes, specifically cisplatin, carboplatin (carboplatam), and oxaliplatin, as well as their modifications or combinations with other pharmaceutical preparations, are widely used in the moderm clinical practice in the therapy of malignant tumors and their metastases [64, 65]. An essential drawback of these drugs is their high toxicity (LD_{50} 12.5 mg kg^{-1} for cisplatin and 36 mg kg^{-1} for carboplatin), narrow range of therapeutic doses, and quite low selectivity [66].

A series of Pt(IV) complexes containing a pyridine ring with various substituents has been studied.[1] The complexes taken for screening were not labeled, and experimenters had no information on their chemical composition.

Before screening solutions of complexes were normalized by platinum contents [67]. Based on the average therapeutic dose of 5 mg kg^{-1} and taking into account that a human body 70 kg in weight contains 5 l of blood, and the blood sample in the capillary contains 0.1 ml of blood, we prepared a stock solution with the initial platinum concentration of 7 μg ml^{-1} and then prepared test solutions by its successive dilutions.

Screening was performed by our earlier modified procedure of ESR measurement using reference compounds ($K_2[PtCl_6]$ and cisplatin). The donor blood stabilized with an anticoagulant (sodium citrate) was used no later than 6 h after sampling. A test solution (10 μl) of a metal complex or a reference compound was placed into a well of an immunological plate, after which 90 μl of donor blood was added in one portion, and the plate was incubated for 20 min at 28°C. The coltrol series was blood diluted with a solvent. Each compound was tested on no less than 30 different samples of donor blood.

The ESR parameter was generally recorded 30, 60, 90, and 120 min after the well content was withdrawn into a capillary, but calculations were performed with data after 60 min, when the height of the plasma column over the layer of sedimented bood was 10−20 mm.

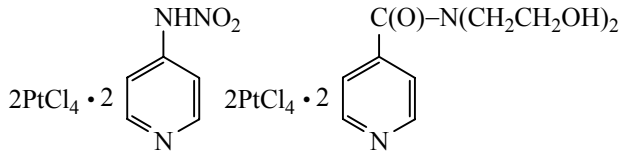
For comparable results, for each blood sample were used centered data, i.e. we calculated the deviation of measurement results from control: $dA_i(x) = A_i(x) - A_i(0)$. The rms deviation δ was calculated from the data for blood without additions (is spanned the range

[1] The authors are highly grateful to B.S. Fedorov and co-workers for the synthesis of tested compounds and providing them for the screening research.

1.03−1.57 mm). For each test and reference sample we found the number of results falling into the following ranges: $dA_i(x) \le \delta$ (series 3: no response), $\delta \le dA_i(x) \le 2\delta$ (series 2: weak response), and $dA_i(x) \ge 2\delta$ (series 1: strong response).

The resulting data allowed us to suggest that the strength of response, estimated by the deviation from control, correlates with the general toxic effect on blood. Consequently, of the greatest interest are compounds which cause the weakest response at a high concentration of platinum.

By the results of screening we selected two Pt(IV) complexes: bis(4-nitraminopyridine-*N*)tetrachloroplatinum(IV) (**I**) and bis(diethanolisonicotinamide-*N*) tetrachloroplatinum(IV) (**II**).



I **II**

The structure of complex **I** was proved by elemental analysis and IR and NMR spectroscopy. The solutions of the complex in DMSO remained unchanged within 25 days. The complex is scarcely soluble in water and Tween-80, which makes difficult its use with biological samples.

Complex **II** was identified by elemental analysis and NMR spectroscopy. Its solutions in DMSO and water undergo no changes within 30 days.

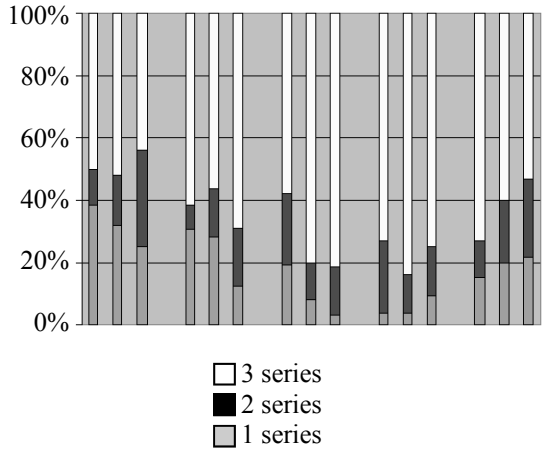


Fig. 2. Results of screening research of $K_2[PtCl_6]$, bis(4-nitraminopyridine-*N*)tetrachloroplatinum(IV) and bis(diethanolisonicotinamide-*N*)tetrachloroplatinum(IV) on a pooled blood with ESR > 15 mm h^{-1}.

To gain more information on the toxicity of complexes **I** and **II** and choose one of them for preclinical testing, we performed additional screening. In these experiments we used the donor blood with ESR values higher than 15 mm h^{-1} (blood from apparently diseased, according to the medical classification). As reference we used platinum hexachloroplatinate. Test solutions were normalized by platinum cincentration. The resulting data are shown in Fig. 2. The response columns for five different concentrations are shown in the following order: potassium hexachloroplatinate, complex **I**, and complex **II**. The series are defined above in the text.

Assuming that the effect of a compound on the cell sedimentation process correlates with its toxicity, for planning subsequent animal experiments we summarized the data on expressed responses (strong and medium) over all concentrations of each compound. After normalization to the number of reactions for the reference compound it was established that complex **I** causes a stronger response compared by complex **II** (by 14±6 %) and reference (by 15%), which implied its lower toxicity. As a result, we decided to perform preclinical tests on complex **I**.

The results of preclinical tests on laboratory animals provided evidence for the results of screening with a nonspecific biosensor based on blood cell sedimentation, which showed that complex **I** is less toxic than cisplatin. Tests for antitumor activity of this complex on tumor models gave the following results.

The complex was found to exhibit no antitumor activity with respect to a continuous line of mouse L1210 lymphoma cells (five injections); the test doses varied from 62.5 to 2.0 mg kg^{-1} (toxic dose 125 mg kg^{-1}).

No antitumor activity was also revealed on the model of Ca-755 mammary adenocarcinoma after five injections (dose range 31.3−1.0 mg kg^{-1}, toxic dose 62.5 mg kg^{-1}). After ten injections (at 48-h intervals), certain antitumor activity was revealed at a dose of 6.0 mg kg^{-1} (higher doses were not studied): The lifespan of mice with Ca755 increased by 27%.

It was reliably established that complex **I** exhibits antitumor activity with respect to continuous Ca-755 mammary adenocarcinoma and antitumor and antometastatic activity on metastasizing continuous B-16 melanotic cancer [68]. This complex is less toxic than the reference compound cisplatin-LENS (Verofarm).

CONCLUSIONS

In the presented work we made an attempt to convert blood into a biosensor, by studying response of one of its parameters on a stimulus. The suggested line of research forms a theoretical and methodological basis for a new field in pharmacy, specifically individual or targeted pharmacy.

Further research in this direction will allow a qualitative breakthrough in different fields of knowledge. For example, this methodology is used to analyze protein carbon extracts from food products during development of an individual diet aimed at restoring nonspecific immunity [69, 70]. The fact that the suggested approach allows testing preparations with a single active substance opens up the way to developing an express and virtually noninstrumental test for bioequivalence of drugs and identification of adulterates [71, 72]. Other medical applications are possible, for example, by slightly changing the methodical approach to study blood response of one patient on a series of single-type pharceutical preparations, one can develop a methodology for selecting drugs in terms of a minimum individual toxicity [73].

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