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THE ROLE OF MICROCALORIMETRIC RESEARCH IN MEDICINE AND PHARMACY

The article is devoted to an overview of some practical applications of microcalorimetric research methods for the needs of medical and pharmaceutical science and practice. The laws of thermodynamics are the main regulator of chemical processes, i.e. the processes of metabolism in biological systems. Biological thermodynamics deals with the quantitative study of the energy transformations occurring in living organisms, structures and cells, or the nature and function of the chemical processes underlying these transformations. Microcalorimetry is an indispensable tool for determining the thermodynamic parameters of a system, which is necessary both in the study of the structure of the biological system and the processes occurring in the system. The effects of drugs on the biological system and the processes of creating new drugs are also characterized by changes in thermodynamic parameters. Bibl. 43.
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Introduction

Microcalorimeters are devices for measuring the small amount of heat that occurs in closed volumes, called reaction chambers. Microcalorimetric studies are important in medicine and pharmacy, since most physico-chemical and biological processes are accompanied by thermal effects, yielding fundamental information on the nature of energy conversion in the system. Microcalorimeters are used to study the phase transitions in solid and liquid states, the formation of complexes, equilibrium constants, to study the interactions between solids and gases and liquids, to measure the heat of hydration, dissolution, adsorption, enthalpy, heat, and thermal conductivity. Also, important is the ability to study the thermogenesis of microorganisms, metabolic processes at different levels of living system organization, etc. [1].

Microcalorimeters are classified by thermal measurement conditions and by the interaction of the reaction chamber with the environment [2].

Main part

Thermodynamics of biological systems

The laws of thermodynamics have been developed for many years as fundamental rules that are satisfied when energy is exchanged in a thermodynamic system. The implications of the laws of thermodynamics are relevant to almost every aspect of scientific research.

Such concepts as thermal energy, temperature, heat transfer, thermodynamic process are handled to understand the laws of thermodynamics [3]. The laws of thermodynamics do not take into account the specific nature of heat transfer at the atomic or molecular level, but characterize the total energy and thermal transitions in the system [4].

A living organism is an energy system where the same laws of thermodynamics apply as in inanimate nature. There are various energy processes in the biosystems: breathing, photosynthesis, muscle contraction, transport of substances, etc. Processes that take place in biosystems are irreversible (non-equilibrium), that is, when the system goes from one state to another, it is impossible to return to the initial state without additional energy flow from the outside. The study of the biological process presents the following three tasks: the transfer of energy, which depends solely on the initial and final state of the system; the mechanism of reactions involved; the speed of these reactions.

According to the first law of thermodynamics, different types of energy can pass from one kind to another, but with these transformations energy does not disappear and does not appear from nothing. The implementation of the first law of thermodynamics for biological systems was proved in 1780 by Antoine Lavoisier and Pierre Laplace. They measured the amount of heat (at the rate of snow melting) and carbon dioxide released by the guinea pig during its life, and compared these values with the thermal effect of combustion reactions on CO_2 products. The results showed that there was no difference between the internal energy of the consumption products and the heat that was emitted. This proves that living organisms are not independent suppliers of energy, but merely transform one type of energy into another. The application of the first law of thermodynamics to living systems is that the energy supplied to living organisms by food is distributed in the process of consumption into two parts:

- released into the environment in the form of heat and energy contained in life products;
- deposited in cellular material.

The sum of these two parts is equal to the internal energy of the food supplied to the body [5].

The first law of thermodynamics of biological systems indicates that an arbitrary biological system (cell, human body, etc.) is an open thermodynamic system. This law establishes quantitative relationships between the amount of heat, the work, and the change in the internal energy of a thermodynamic system, but does not determine the direction of thermodynamic processes.

The energy balance of the body is studied by direct and indirect calorimetry. In the former case, a person is placed in an isolated chamber, in which the amount of heat radiated by a living organism during various processes of normal physiological activity is determined. Indirect calorimetry is based on calculation methods using respiratory coefficients (the ratio between the amount of carbon dioxide that is released and the amount of oxygen that is absorbed, for carbohydrates it is 1.0, for proteins - 0.8, for fats - 0.7) and the caloric equivalent of oxygen (the amount of heat released at a flow rate of 1 liter of oxygen, for carbohydrates it is 21.2 kJ, for proteins - 20.09 kJ, for fats - 19.6 kJ).

Enthalpy is often used to describe thermal effects in biological systems (showing the total heat content of the system), since all processes in cells occur at constant pressure:

$$\Delta H = \Delta U + p \cdot \Delta V,$$

here, $p\Delta V$ is a "non-mechanical work.

Enthalpy reflects the ability of systems to perform non-mechanical work and release heat, as well as the number and types of chemical bonds in reagents and reaction products. Enthalpy is a function of state, so it is impossible to determine the absolute value of enthalpy, but the enthalpy change associated with the thermodynamic process can be measured accurately. In the isochoric process, all the heat received by the system goes to a change in internal energy, that is, $Q_V=\Delta U$. Therefore, the change in internal energy in a certain process can be measured when it flows in a calorimeter at a constant volume. In an isobaric process, the system spends heat to perform work, therefore $Q_P = \Delta H$.

Sufficient accuracy in measuring the energy balance is achieved if the body does not perform mechanical work and does not accumulate biomass. In this case, the energy changes ΔH in the biological system can be accurately recorded by a calorimeter when measuring the heat released. If $H > 0$, the heat is absorbed and the reaction is called endothermic. If $\Delta H <0$, then the system releases heat, and the reaction is called exothermic. Most metabolic reactions are exothermic. .

All the energy entering the body turns into heat. During the formation of ATP, only part of the energy is stored, most of it is dissipated in the form of heat. If ATP energy is used by the functional systems of the body, then most of this energy is also converted into heat. Part of the energy left in the cells is spent on the functions they perform, but it still turns into heat. For example, the energy used by muscle cells is spent on overcoming the forces of viscous resistance of muscles and other tissues. Viscous movement causes friction, which causes the formation of heat, for example, the expenditure of energy transmitted by the heart to the blood flow. During the movement of blood through the vessels, all energy is converted into heat due to friction between the layers of blood and between the blood and the walls of the vessels. So, in fact, all the energy that has been expended by the body ultimately turns into heat, with the exception of the case when the muscles perform work with external bodies [6].

For a long time, it was believed that only processes that are accompanied by a decrease in the energy of the system (exothermic) proceed spontaneously. However, many unauthorized endothermic processes (for example, dissolution of some salts, decomposition of carbonic acid, etc.) are known in which heat is absorbed. At low temperatures, mainly exothermic reactions occur.

In chemical reactions, under the action of the same principle, atoms tend to merge into such molecules, the formation of which leads to the release of energy (coupling reaction). But more likely are those reactions that result in an increase in the number of particles (decomposition reactions). Thus, unauthorized processes that occur without a change in energy state occur only in the direction in which disorder in the system increases and it passes to a more probable state.

Among the thermodynamic functions characterizing the energy state of a biological object, entropy plays an important role. Entropy characterizes energy costs usually in the form of heat during irreversible processes. Thus, entropy reflects that part of the energy of the system that has been dissipated, degraded in thermal form, and cannot be used to carry out work at a constant temperature. In reverse processes the entropy change is zero ($\Delta S = 0$), and in the case of irreversible it is positive ($\Delta S> 0$). Therefore, the smaller the energy gradients in a system and the more heat dissipated in the form of degraded energy, the greater its entropy.

Energy is spent on ordering the system, so an ordered system has a certain amount of energy and can do the work. This energy supply will inevitably be wasted due to insufficient isolation of the system from the environment.

According to the Boltzmann-Planck formula, the entropy of a system is related to the probability:

$$S = k \cdot \ln W,$$

where S is entropy, k is the Boltzmann constant, W is thermodynamic probability.

If we artificially create a closed isolated system with a very unlikely structure and leave it on its own, then it will evolve into a more probable structure. Therefore, we conclude that probability has a natural tendency to increase, as well as entropy. Entropy can be interpreted as a measure of disorder in a physical system or as a measure of a lack of information about the structure of a system. Since information can only be obtained as a result of energy expenditure, any experience that provides information about the physical system or arbitrary physical measurements of system parameters can be made as a result of increasing the entropy of the system or its environment. Moreover, the average entropy increase according to the second law of thermodynamics is always greater than the information obtained [7].

Entropy has positive values associated with increasing disorder and vice versa. In particular, the release of structured water molecules surrounding the binding surfaces is generally considered to be a source of positive entropy due to increased system disorder. Conversely, an increase in system ordering due to, for example, the introduction of conformational constraints in the binding complex is reflected by the negative entropy values [8].

The practical absence of reversible processes in biological systems causes the fact that all the processes that occur in them are accompanied by an increase in entropy. So, in biosystems, not all the free energy consumed by a particular process passes into useful work. Part of it dissipates in the form of heat. The ratio of the amount of work done to the amount of free energy expended on it is called the efficiency of the biological process.

Thus, muscle contraction is carried out with an efficiency of ~ 30%, glycolysis ~ 36%. However, there are also processes that are close to the reverse, that is, the efficiency of which is high. For example, the luminescence of some tropical insects has an efficiency of 98-99%, the discharge of electric fish - 98%. The reason for this highly efficient use of free energy is not yet fully understood. Thus, the more significant is the increase in entropy in a given process, the more probable is the irreversibility of this process.

Living organisms retain low levels of entropy over time because they receive energy from the environment in the form of food. This energy is released during the oxidation of the substance, which is accompanied by the consumption of oxygen and the release of carbon dioxide.

The second law of thermodynamics implies the principle that total entropy must increase steadily. Although thermodynamics does not independently describe processes as a function of time, the second law of thermodynamics determines the direction in which the value of total entropy increases.

The question is can the arbitrary process be reversed? The second law of thermodynamics answers that this is possible by creating an equivalent or even greater disorder elsewhere. A clear example is photosynthesis. Carbon dioxide, water, and other nutrients are absorbed by plants, and complex molecules of carbohydrates are synthesized at their expense. This process is accompanied by a decrease in entropy. Photosynthesis is impossible without sunlight. Therefore, the decrease in entropy in the synthesis of carbohydrates in plants is offset by the increase in entropy in the Sun. Many other important biochemical processes are also carried out with the reduction of entropy - the formation of biopolymers (proteins, nucleic acids, etc.), the active transport of ions through cell membranes, etc. But a living organism is an open system, and in it entropy can grow, remain unchanged or decrease depending on the amount of entropy generated inside the system, its inflow

from the outside or outflow into the environment. Our Universe is also not an isolated system, and therefore it does not face “thermal death” - a state of maximum entropy.

Entropy is a driving force for the unfolding of proteins. The natural state of a protein is characterized by a low value of entropy, since its conformation is very limited. On the other hand, the expanded form can exist in many different conformations, even if each amino acid takes only three positions, the polypeptide chain of 100 amino acids can take 3^{100} or 10^{47} different conformations. Since the result of the unfolding reaction of a protein can be in a large number of equivalent states in comparison with the natural state, entropy increases during the unfolding reaction. During protein folding, entropy loss must be balanced by the contribution of enthalpy to the free energy that promotes folding. Significant non-covalent forces of hydrogen bonds and other physical interactions compensate for small entropy. Changes in the entropy of the solvent - water - play an important role in compensating for the loss of conformational entropy. In the natural state, many non-polar amino acid residues, which are packed in protein, are sequestered (separated) away from water. In the unfolded form, these residues are treated with water molecules that are formed around nonpolar residues into cellular structures. The hydrogen bond network is reorganized so that the number of hydrogen bonds remains. This ordering of water molecules reduces its entropy. When the protein folds, these water molecules are released and the non-polar residues are separated from the water. The resulting recovery of entropy through water is a dominant force in protein folding, and this effect is generally known as the hydrophobic effect. Therefore, the solvent has a significant effect on biological reactions.

Entropy plays an important role in enzymatic catalysis. Usually, the reactions in solution are slow due to the entropic cost of bringing the reagents and catalyst together. Two or more molecules combined together into one entails significant entropy loss. On the other hand, when enzymes bind to the substrate, the released bond energy is used to compensate for the entropy losses caused by the formation of low-probability enzyme-substrate complexes as the enzyme-catalytic groups are very precisely oriented. This is due to an increase in the dissociation constant of enzyme-substrate complexes. Small losses of entropy occur during the stages of the chemical reaction, since the catalytic groups are already properly oriented in the enzyme – substrate complexes, and therefore their effective concentration is very high compared to the corresponding biochemical reactions that occur freely in solution [9].

According to the second law of thermodynamics, spontaneous chemical reactions, which in biological processes typically occur at constant temperature and pressure, are always accompanied by a decrease in free energy. Free energy is the energy spent to perform useful work. All reactions occur in the direction of the equilibrium state (where the free energy decreases no longer).

The stability of any isolated system is determined by the ratio of enthalpy and entropy factors. The former characterizes the system’s desire for ordering, since this process is accompanied by a decrease in internal energy, the second - shows a tendency to disorder, since this situation is most likely. So, if in the process $\Delta S = 0$ - the degree of disorder does not change, then the process goes in the direction of decreasing enthalpy, that is, $\Delta H < 0$. If in the process no energy changes occur ($\Delta H = 0$), then the process goes towards increasing entropy, that is, $\Delta S > 0$.

As a criterion of arbitrariness in non-isolated systems, a new state function was introduced that takes into account both of these factors. This state function for isobaric processes is called Gibbs energy or isobaric isothermal potential G:

$$\Delta G = \Delta H - T\Delta S.$$

For isochoric processes, a similar Helmholtz energy or isochoric isothermal potential F is introduced:

$$\Delta F = \Delta U - T\Delta S.$$

At a constant temperature and pressure, only those processes can occur arbitrarily for which the change in the Gibbs (or Helmholtz) energy is negative [8].

Lost free energy appears as heat (enthalpy) or an increase in entropy. Thus, spontaneous chemical reactions on which life depends can occur with heat consumption, but only at the cost of an increase in free energy. For example, protein unfolding consumes a large amount of heat and is an example of a reaction caused by an increase in entropy. From the definition of entropy, when considering a biological process at constant temperature, the entropy change is equal to the heat given by the heat divided by the temperature. Since the heat of the protein unfolding is positive because it is consumed, the entropy changes are always positive.

Pharmaceutical materials, crystalline and amorphous, absorb water from the atmosphere, which affects critical drug development factors, such as the isolation of the crystalline form of the drug, compatibility with excipients, dosage form selection, packaging and shelf life. In order to improve knowledge of pharmaceutical materials and to bypass potential problems by studying the thermodynamics of the interaction of solids with sorbed water, a study was conducted [10].

In the study of increasing the bioavailability of an effective therapeutic and prophylactic agent of lovastatin, which blocks the initial stages of cholesterol synthesis, the thermodynamic parameters (ΔG, ΔH, ΔS) characterizing the process of solubility of lovastatin in aqueous solution were systematically determined along with other characteristics. The data obtained will ultimately enable the development of the desired highly soluble, effective and safer lovastatin preparations [11].

The review [12] discusses the role of thermodynamics in allosteric mechanisms, that is, when behavior in one part of a molecule changes due to a change that has occurred in another part of it. Conformational changes are accompanied by a significant increase in entropy. The effect of point mutations on the thermodynamic parameters of binding and function may reveal the energy pairing of neighboring (and distant) amino acid residues upon activation.

The determination of whether the interaction of a particular ligand-receptor in equilibrium is enthalpy or entropically stabilized can be achieved by thermodynamic analysis. Studies show that enthalpy stabilization is usually associated with the formation of new bonds (e.g, hydrogen bonds and van der Waals interactions) in the ligand-receptor-membrane matrix, whereas entropy stabilization is typically characterized by displacement of ordered water molecules associated with the formation of new hydrophobic interactions [13].

The energy exchange in living systems is organized in such a way that reactions that are possible from a thermodynamic point of view (for example, the breakdown of carbohydrates to water and carbon dioxide) and impossible (biosynthesis of complex molecules, active transport through cell membranes, etc.) are simultaneously running in it. This is achieved through energy coupling, the transition of the process into a multi-stage mode and the functioning of multi-enzyme systems. An energy coupling mechanism occurs when, in terms of the entropy criterion, the reaction is combined with the reaction, thermodynamically impossible, and gives it energy. The free energy of the former must exceed the energy consumed by the latter. Conjugating reactions should have a common component, a coupling factor, which is usually a phosphate ion. The conversion of the biochemical process into a multistage mode allows the living organism to easily regulate the synthesis of certain substances in the required quantities. This is because the difference in the free energies of the initial and final states for each of the individual stages is usually small, and therefore the probability of equilibrium is greater for it than for the process as a whole. Multistage passage of chemical transformations in living systems is ensured by the functioning of multi-enzyme systems operating on

the principle of molecular conveyor - the product of one enzymatic reaction serves as a substrate for subsequent transformation.

In living organisms, the most common conjugated process is active transport, that is, the transfer of a substance from a region of less concentration to a region of greater concentration. Such a process cannot arbitrarily occur because it is accompanied by an increase in the orderliness of the system, i.e. a decrease in entropy. Therefore, active transport occurs only in the case of coupling with another process - the source of energy. In the process of conjugation, some of the energy is converted into heat. The energy conversion efficiency in conjugated processes for biological systems is 0.8-0.9.

In [14], a methodology of linear irreversible thermodynamics is applied to study general systems in nonequilibrium states, which take into account both internal and external entropy contributions to analyze the efficiency of two conjugated processes. The results show that there are optimization criteria that can be used specifically for biological systems, where the optimal design of biological parameters created by nature at maximum effective power results in more efficient tools than those created at maximum power or in the best environmental conditions.

The study of the state of a living organism, as an open thermodynamic system, underlies the method of calorimetry (Latin calor - heat + Greek metreo - measure) - the measurement of the amount of heat released during various physical, chemical or biological processes. Calorimetry of biological and biochemical processes (biocalorimetry) allows quantifying the energy and thermal effects of individual biochemical reactions, the activity of cellular organelles and cells, tissues and organs, the body as a whole. Calorimetric studies measure the values of heat fluxes from a living object into the environment and calculate the amount of heat produced and the heat capacity of the organism; the measurement of heat capacity is based on the mass, heat capacity and temperature change of the object.

The direct detection of small thermal changes accompanying biological reactions provides for a universal method for the detection of molecular interactions and offers a significant advantage over biochemical analyses requiring specific development and optimization for each investigated target. Another advantage of calorimetry is that it is carried out in the liquid state of a substance and does not necessitate chemical modification, marking or immobilization.

Microcalorimetric methods in medicine, biology and pharmacy

The principle of calorimetry is based on the statement that in all chemical reactions there are changes in energy, which are usually accompanied by the release of heat (exothermic) or its absorption (endothermic). Microcalorimetry is a highly sensitive method that detects even the smallest temperature changes in small volume samples, which makes it possible to use it when developing biomaterials.

Microcalorimetry is used to study reactions involving biomolecules, including molecule interactions and conformational changes, such as protein folding. Areas of application range from confirmation of target binding in the development of low molecular weight drugs to the development of stable biotherapeutic drugs.

Consider microcalorimetry methods that have been widely used in biomedical and pharmaceutical research.

Isothermal microcalorimetry

Isothermal microcalorimetry (IMC) is a laboratory method of real-time monitoring and dynamic analysis of chemical, physical and biological processes. Within hours or days, the IMC determines the

onset, rate, degree, and energy of these processes for samples in small ampoules (e.g, 3-20 ml) at a constant set temperature (in the range of 15 ° C to 150 ° C).

IMC carries out this dynamic analysis by measuring and recording, compared with the previous time, the heat flow rate ($\mu J/s = \mu W$) to the ampoule with or from the sample, as well as the total amount of heat consumed or produced.

IMC is a powerful and versatile analytical tool for the following closely related reasons:

- all chemical and physical processes are either exothermic or endothermic - produce or consume heat;
- heat flow rate is proportional to the speed of the process;
- IMC is sensitive for detecting and monitoring very slow processes in a few grams of material or processes that generate a small amount of heat (for example, the metabolism of several thousand living cells);
- IMC instruments typically have a wide dynamic range - heat fluxes of approximately 1 μW up to 50,000 μW can be measured with the same instrument.

The IMC method for studying the speed of processes is widely used in practice, provides constant data in real time, and is sensitive. Measurements are quite simple, do not require supervision and fluorescent or radioactive markers.

However, there are cautions that should be kept in mind when using IMC:

- if the ampoules prepared from the outside are used, approximately 40 minutes are required to slowly insert the ampoule into the instrument without significantly disturbing the set temperature in the measuring module. However, arbitrary processes occurring during this time are not controlled;
- IMC records the total net heat flow produced or consumed by all processes occurring in the ampoule. Therefore, to be sure which process or processes produce the measured heat flux, one must be very careful, both in the experimental design and in the initial use of appropriate chemical, physical and biological analyzes.

It is believed that the possible use of IMC is limited only by the imagination of the person who chooses to use it as an analytical tool and the physical limitations of the method. In addition to the basic warnings described above, the limitations also include sample size and ampoules, as well as the temperatures at which measurements can be taken. IMC is generally best suited for evaluating processes that occur over the course of hours or days. Next, we will focus on the use of IMC in medicine, biology, and pharmacy, highlighted in recent publications.

The term metabolism is used to describe studies of the quantitative measurement of the rate at which heat is produced or consumed by whole small organisms, tissue specimens, or cells (including microbial) in culture. Metabolism can be useful as a diagnostic tool, especially for identifying the nature of a sample by its heat flux under specified conditions, or for determining metabolic processes.

To determine the metabolism using IMC, there should be enough cells, tissues or organisms that were initially present (or added later if replication occurs during the measurement of IMC) to generate a heat flux signal above a given detection limit of the device.

[15] describes the relationship of metabolic rate to the mass of an object and how it scales throughout the range from molecules and mitochondria to cells. The authors note that although the rate of metabolism of this type of mammalian cells in vivo is significantly reduced with increasing size (mass) of animals, the size of the donor animal does not affect the rate of cell metabolism when cultured in vitro. Extending theoretical and empirical analyzes of scaling to sub-organism levels can potentially be important for the cellular structure and its functions, as well as for the metabolic basis of aging.

Mammalian cells in culture have a metabolic rate of approximately 30 × 10−12 W/cell. IMC instruments have a sensitivity of at least 1×10^{-6} W (ie 1 μW). Therefore, the metabolic heat of approximately 33.000 cells is practically detected. Based on this sensitivity, the IMC was used to conduct a large number of pioneering studies of the metabolism of cultured mammalian cells in the 1970s and 1980s in Sweden. There are known IMC studies of heat flux from cultured human red blood cells, platelets, lymphocytes, lymphoma cells, granulocytes, adipocytes, etc., skeletal muscles and myocardial tissues. Studies were conducted to determine the possibility of using IMC as a method of clinical diagnosis and establishing metabolic differences between the cells of healthy people and people with various diseases or health problems [16].

IMC was used to evaluate antigen-induced proliferation of lymphocytes [17] and found aspects of proliferation that were not observed with the conventional method of analysis of a continuous radioactive marker. IMCs have also been used in the field of tissue engineering. Studies [18] showed that IMC can be used to measure the growth rate (i.e. proliferation) in a culture of human chondrocytes harvested for tissue engineering.

IMC is used in toxicology to monitor the metabolism of cultured cells in real time and to quantify the rate of metabolic decline as a function of the concentration of a possibly toxic agent. In the study of implant materials [19], both fast-growing fungal cultures and human chondrocyte cultures were exposed to calcium hydroxyapatite particles (<50 μm in diameter) and bioactive silicate glass particles. Glass particles slowed or reduced the growth of fungi as a function of increasing the concentration of particles. Hydroxyapatite particles had a much smaller effect and never completely reduced fungal growth at the same concentrations. The effect of both types of particles on chondrocyte growth was minimal when using the same concentration. The authors concluded that the cytotoxicity of solid particles such as bioactive glass and hydroxyapatite particles can be estimated using the microcalorimetry method. This is a modern method of studying in vitro biocompatibility and cytotoxicity of biomaterials, which can be used along with conventional analyses.

In the 1980s, publications appeared indicating the use of IMC in microbiology. Although some microbiological studies of IMC were aimed at viruses [20] and fungi [21], the entire research concerned bacteria. In [22], methods of using IMC in medical and environmental microbiology are considered. The article reports how accurate are the data on heat flow and fluctuations in the metabolic activity of microorganisms and the rate of replication in this environment.

In [23], the use of the microcalorimetric method for the evaluation of E. coli and S. aureus metabolism is highlighted. Measurements were taken in sealed 24-ml glass ampoules in the temperature range from 5 ° C to 90 ° C. The temperature measurement error was ± 0.02 °C. The detection limit was estimated to be 2 μW and the baseline stability was 2 × 106 μW for 24 hours. By registering heat transfer in real time, the metabolic activity of bacteria was evaluated and the effect of the extracts was investigated. Using kinetic and thermodynamic information from the microcalorimetric method, a number of important kinetic parameters were obtained: the growth rate constant, when reaching a maximum, the inhibition coefficient and the drag coefficient. Escherichia coli growth rate constant showed slight changes with increasing concentration of Aconitum alkaloids. However, the growth rate constant of S. aureus increased and then decreased as the concentration of Aconitum alkaloids increased. This indicated that treatment with Aconitum alkaloid slowed the growth and metabolism of S. aureus. Based on the study of the effect of various concentrations of Aconitum alkaloids on the growth of E. coli and S. aureus, it was concluded that Aconitum alkaloids do not affect the growth of E. coli, but potentially inhibit the action of S. aureus.

Modern isothermal microcalorimeters (IMC) are able to detect the metabolic heat of bacteria with the accuracy sufficient to recognize even the smallest bacterial infection of water, food and medical samples. IMC methods are superior to conventional detection methods in terms of detection time, reliability and technical efforts. In [24], a linear relationship was observed between the calorimetric detection time and the initial concentration of bacteria. This can be used to quantify bacterial infection. A study of the relationship between the level of filling (in mm) of the calorimetric capacity and the specific maximum heat flux (in mW.g-1) illustrated two completely different results for liquid and solid media. The time to detect the presence of bacteria by IMK depends on the initial amount of bacteria present, the sensitivity of the instrument and the level of heat flux above the initial level, which is chosen as an indicator of bacterial growth. In general, bacteria are about 1/10 the size of mammalian cells and possibly produce only 1/10 of the metabolic heat of the cell. However, some bacteria grow faster than mammalian cells, often their number increases within minutes. Therefore, a small initial bacterial count in a culture that does not initially detect IBC quickly yields a detectable amount. For example, 100 bacteria that double every 20 minutes in less than 4 hours will give more heat than 330,000 bacteria, and heat flow will be determined by IMC. Therefore, IMC can be used for easy, rapid detection of bacteria, in particular for the detection of tuberculosis mycobacteria [25]. The metabolic evolution of mycobacterial heat during cell proliferation is measured by the IMC and is considered as a possible alternative to conventional diagnostic agents.

Staphylococcus aureus biofilm plays a major role in implant-associated infections. The sensitivity of S. aureus biofilm to daptomycin, phosphomycin, vancomycin, trimethoprim/ sulfamethoxazole, linezolid and rifampicin has been investigated by isothermal microcalorimetry [26]. In addition, the permanent status of cells isolated from S. aureus biofilm after vancomycin treatment was also analyzed. S. aureus biofilm was resistant to all tested antibiotics except daptomycin. Step-by-step treatment with vancomycin to destroy all metabolically active cells and with daptomycin to destroy persistent cells has destroyed the entire bacterial population. These results support the use in clinical practice of a therapeutic regimen based on the use of two antibiotics to kill persistent cells and eradicate S. aureus biofilm. IMC is an appropriate technique for characterizing real-time reversion from resistant to metabolically active cells.

Candida auris has emerged worldwide as a multi-resistant fungal pathogen. Using IMC, the heat production profiles of C. auris and other Candida spp. strains were compared and evaluated their antifungal susceptibility [27]. C. auris showed a peculiar heat production profile that distinguished it from other species. The thermogenic parameters of C. auris offer slower growth rates compared to C. lusitaniae and another clear thermal profile compared to complex strains of C. haemulonii species. Amphotericin B-based treatment has been identified as a potential therapeutic option for C. auris infection.

The IMC for thermally viable microorganisms in pure cultures and stable formulations was investigated in [28]. Quantifying viable microorganisms is an important step in microbiological research as well as in the formation of microbial products for the development of biological control products or probiotics. Thermal viability methods are new and effective methods for rapidly quantifying different species of bacteria and fungi and for increasing the speed, sensitivity and accuracy of routine viability estimates for pure cultures and controlled microbiomes such as plant seed coatings.

Despite significant advances in diagnostic and therapeutic approaches, fungal infections caused by C. albicans continue to be a serious problem in intensive care units worldwide. The economic cost of fungal blood infections and associated mortality, especially in debilitated patients, remains high. C. albicans is a highly adapted microorganism capable of developing resistance after prolonged exposure

to antifungal agents. The formation of a biofilm that reduces the availability of an antifungal drug, the release of spontaneous mutations that increase expression or reduce the susceptibility of targets, altered chromosomal abnormalities, overexpression of waste from several drugs, and the ability to avoid the body's immune defenses are some of the factors that can contribute to antifungal tolerance and resistance. Knowledge of the mechanisms of antifungal resistance may allow the development of alternative therapeutic options to modulate or restore resistance. The review [29] focuses on the factors involved in antifungal resistance and tolerance in patients with C. albicans blood infections.

In a study [30], the effects of fluconazole, caspofungin, anidulafungin, and amphotericin B are investigated against Candida species in planktonic form and biofilms using an IMC that measures the growth of heat production. An isothermal microcalorimeter equipped with 48 calorimeters and a sensitivity threshold of 0.2 μW was used. The heat flow was recorded for 48 hours. The study demonstrated the potential of microcalorimetry to test real-time antifungal sensitivity and evaluate antifungal activity against planktonic film and Candida biofilm.

Antibiotic abuse has led to increased bacterial resistance, which significantly limits the use of antibiotics for the treatment of bacterial infections. Therefore, it became necessary to develop new antibacterial drugs. Work [31] gives an idea of the effect of traditional Chinese medicine on drug-resistant bacteria. Dracotomelon tao is a traditional medicinal material derived from Anacardiaceae with a long history of treating various infectious diseases such as decubitus and skin ulcers. Recent studies have shown that various extracts from D. tao leaves containing flavonoids and phenolic acids exhibit potent antibacterial activity. In this study, the combination of bacteria-resistant action of these active ingredients was studied. Microcalorimetric measurements and principal component analysis were performed on samples from D. tao leaves in vitro. The results showed that all six samples had significant antibacterial activity, so the drug from the leaves of D. tao can be used as a potential antimicrobial resource in the treatment of infectious diseases.

With the addition of an IMC, which made it particularly valuable for biomedical and pharmaceutical applications, P. mirabilis was found to increase in the Luria broth environment between 2 and 9 h. research. The culture emitted 2.1 J with a maximum thermal output of 76 μW. The growth rate, calculated using calorimetric and spectrophotometric data, was 0.60 and 0.57 h – 1, respectively. Additional information gathered on the protease activity of P. mirabilis, which corresponds to the last peak in heat production. Tumor microtissues growth was also monitored, which exhibit a maximum thermal power of 2.1 μW, which corresponds to an increase in microtissue diameter from about 100 to 428 μm. This opened up new areas of research in oncology, diagnosis and the development of new antitumor drugs. For parasitic worms, the technique allows one to evaluate the survival of parasites using motor and metabolic activity of even one individual [32].

Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) is one of the most powerful methods for obtaining accurate information about the energy of biomolecules, which are associated with other biological macromolecules. ITC is a thermodynamic technique that directly measures the release or absorption of heat in intermolecular interactions such as ligand-protein, protein-protein [8]. An ITC experiment consists of calorimetric titration of a specific volume of one of the reagents, usually a macromolecule, with a controlled amount of another reagent, usually a ligand, at a constant temperature and pressure. Thus, the measured heat during titration corresponds to the enthalpy of such interactions [10]. This relatively simple experiment allows for a complete and accurate thermodynamic characterization of

the binding event (binding constant, enthalpy change, reaction stoichiometry, process heat change) that are important for understanding and optimizing molecular interactions [33].

Most biological phenomena affect intermolecular recognition and interaction. It is the main tool for the development and study of drugs and the regulation of protein interactions.

Over the last thirty years, ITC has become a powerful tool for studying a wide variety of molecular interactions. This technique is able to provide a complete thermodynamic profile of the interaction process in one experiment, with several advantages over other comparable methods, such as a smaller sample size or no chemical modification or labeling. Therefore, it is not surprising that ITC is used to study various types of interactions of natural products to gain new insights into the key molecular factors involved in the complexation of this type of compounds. A review article [34] describes the methodology of ITC and discusses some applications of ITC for studying protein-ligand interactions, protein-protein interactions, self-association, and drug development processes. The use of ITC to determine the kinetic parameters of enzyme catalyzed reactions as well as thermodynamic parameters is discussed. The review [35] confirms the use of ITC as a powerful tool for investigating the interaction of natural products with proteins, nucleic acids, oligosaccharides, and other types of receptors.

The enthalpy and entropy of binding in the creation of complexes that provide the release of therapeutic substances was investigated in [36] by the method of ITC. The information obtained is very important for drug developers, as it warns against taking into account the particularities of drug behavior in different environments.

Calorimetry of isothermal titration is a tool capable of determining thermodynamic as well as kinetic parameters associated with protein-ligand recognition and plays an important role in drug design. Further efforts to investigate the protein-ligand binding characteristics of ITC with a large amount of thermodynamic and kinetic data will lead to new discoveries that will extend our ability to understand the full range of protein-ligand recognition and treatment and to use these results appropriately for medical applications [37].

Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and a reference is measured as a function of temperature. Both the samples and the reference are maintained at almost the same temperature throughout the experiment. As a rule, the temperature program for DSC analysis is designed in such a way that the temperature of the sample holder increases linearly as a function of time. The control sample should have a well-defined heat capacity in the temperature range to be scanned.

The DSC is based on the principle that during the physical transformation of the sample such as phase transitions, it will receive more or less heat than the reference, to maintain both samples at the same temperature. More or less heat will be supplied to the sample depends on whether the process is exothermic or endothermic. For example, if a solid sample is melted to a liquid state, it needs more heat entering the sample in order to increase its temperature at the same speed as the reference. This is due to the absorption of heat by the sample, since it experiences an endothermic phase transition from solid to liquid. If the sample is subjected to exothermic processes (such as crystallization), less heat is required to increase the sample temperature. By controlling the heat flux difference between the sample and the standard, the DSC is able to measure the amount of heat absorbed or released during such transitions. DSC can also be used to observe subtle physical changes such as glass transition.

The result of the DSC experiment is a heat flux curve depending on temperature or time. This curve can be used to calculate the enthalpy of transitions:

$$\Delta H = K S,$$

where ΔH is the enthalpy of the transition, K is the calorimetric constant, and S is the area under the curve. The calorimetric constant varies depending on the device, so it is determined using samples with known transition enthalpies.

DSC can be used for thermodynamic analysis of proteins, namely to reveal important information about the global structure of proteins and the interaction between proteins and ligands. In particular, mutations reduce protein stability, whereas ligand binding usually increases protein stability [38]. With DSC, stability can be measured by obtaining the Gibbs free energy value at any given temperature. This allows researchers to compare the free unfolding energy between a protein without a ligand and a protein-ligand complex, or between wild-type proteins and mutants. DSC can also be used to study the interaction between proteins and lipids, nucleotides, between drugs and lipids [39].

DSC provides a complete thermodynamic profile for unfolding of system energy. The determination of binding energy is then determined by considering the unfolding of energy of the biomolecules in the presence and absence of the binding component. The DSC method is widely used in the pharmaceutical industry to determine drug treatment parameters. Therefore, if the drug is to be delivered in an amorphous form, it is recommended to treat the drug at a temperature below crystallization temperature.

The solubility of gemfibrozil, a drug that lowers cholesterol and is poorly soluble in water, was studied by DSC. The solvents used in the pharmacy were tested: water, methanol, ethanol, isopropanol, 1-butanol, 2-butanol, ethylene glycol, propylene glycol, polyethylene glycol-400, ethyl acetate, dimethyl sulfoxide and transcutol in the temperature range from 298.2 K to 318.2 K at atmospheric pressure P = 0.1 MPa. It was found that the maximum solubility characteristic of transcutol is minimal for water. Thermodynamic analysis on experimental solutions showed endothermic and entropy dissolution of gemfibrozil in each pharmaceutically used solvent [40].

Micro DSC is capable of both isothermal and non-isothermal calorimetric studies. Due to the increase in cell size and sensitivity requirements, the scanning speed is usually low (up to about 1 °C/min). Temperature ranges from approximately - 40 °C to 100-200 °C. The device allows you to control a very slow scan speed, such as 0.001 °C/min.

Micro DSC in combination with x-ray diffraction is also used to study phase transitions in biological samples, degradation of medical preparations, study of antiviral drugs, to determine their thermodynamically stable forms [41 – 43].

Conclusions

An analysis of the practical application of isothermal calorimetry, isothermal titration calorimetry and differential scanning calorimetry in biology, medicine and pharmacy shows that these methods have been widely used to determine the thermodynamic parameters of the system, in particular, Gibbs free energy, enthalpy, entropy, which are important for understanding and optimizing molecular interactions, phase transitions in solid and liquid states, studying solubility and crystallization processes, which is important for better use of pharmaceuticals. Microcalorimetry methods are used to study the processes of metabolism, in tissue engineering and toxicology and for the practical needs of microbiology: the study of viruses, bacteria, fungi. The main purpose is to eliminate their resistance to

the action of drugs. The results of microcalorimetric studies are used to create new drugs, including anticancer therapies.

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РОЛЬ МІКРОКАЛОРИМЕТРИЧНИХ ДОСЛІДЖЕНЬ У МЕДИЦИНІ І ФАРМАЦІЇ

Стаття присвячена огляду деяких практичних застосувань мікрокалориметричних методів дослідження для потреб медичної і фармацевтичної науки та практики. Основним регулятором хімічних процесів – процесів обміну речовин у біологічних системах - є закони

термодинаміки. Кількісним вивченням енергетичних перетворень, що відбуваються в живих організмах, структурах і клітинах, чи природи та функції хімічних процесів, що лежать в основі цих перетворень, займається біологічна термодинаміка. Мікрокалориметрія є незамінним інструментом для визначення термодинамічних параметрів системи, що необхідно як при дослідженні структури біологічної системи, так і процесів, які відбуваються в системі. Дія ліків на біологічну систему та процеси створення нових лікарських засобів також характеризуються зміною термодинамічних показників. Бібл. 43.

Ключові слова: мікрокалориметрія, термодинаміка, фазові переходи, медицина, фармація

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РОЛЬ МИКРОКАЛОРИМЕТРИЧЕСКИХ ИССЛЕДОВАНИЙ В МЕДИЦИНЕ И ФАРМАЦИИ

Статья посвящена обзору некоторых практических применений микрокалориметрических методов исследования для целей медицинской и фармацевтической науки и практики. Основным регулятором химических процессов – процессов обмена веществ в биологических системах - являются законы термодинамики. Количественным изучением энергетических преобразований, происходящих в живых организмах, структурах и клетках, или природы и функции химических процессов, лежащих в основе этих преобразований, занимается биологическая термодинамика. Микрокалориметрия является незаменимым инструментом для определения термодинамических параметров системы, что необходимо как при исследовании структуры биологической системы, так и процессов, происходящих в ней. Действие лекарств на биологическую систему и процессы создания новых лекарственных средств также характеризуются изменением термодинамических показателей. Библ. 43.

Ключевые слова: микрокалориметрия, термодинамика, фазовые переходы, медицина, фармация

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