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Methods for determining the residual amount of antibiotics in food

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ABSTRACT

In the modern world, it is worth seriously thinking about the quality of food consumed. Eating livestock products, we often do not think about such things as the presence of antibiotics in them. However, this can have serious consequences for the body. That is why the sanitary services of all countries pay such attention to the definition of antibiotics. Somewhere very strict standards are set on them. This article is an overview of such antibiotics as sulfaguanidine, benzylpenicillin and chloramphenicol, we also discussed the methods for the determination of antibiotics, methods of fluorescence analysis and quantum chemistry, which we will use in future studies.

Keywords: antibiotics, chloramphenicol, benzylpenicillin, sulfaguanidine (sulgin), fluorescence, fluorescent probes, quantum chemistry methods

1. INTRODUCTION

Antibiotics are chemical compounds of natural, semi-synthetic or synthetic origin. They kill bacteria and microorganisms. But in addition to pathogenic bacteria, they also kill beneficial microflora in the body.

Now antibiotics are often used not only to treat humans and animals, but also to stimulate animal growth, packaging and preservation of food (meat, fish, milk, honey, etc.) [1]. Some medicinal substances persist for a long time in livestock products and can enter the human body with these products. At the same time, antibiotics can cause various allergic reactions, suppress the activity of enzymes, change the microflora of the body, promote the spread of resistant types of microflora, and cause dysbiosis. The high content of antibiotics in food products is due to their widespread use in industrial animal husbandry, poultry farming and fishing.

Antibiotics stimulate some biochemical processes in the body of animals, thanks to antibiotics in animals, reproductiveness increases, defense reactions are activated, growth is accelerated and the general condition improves. Therefore, they are often used not only for treatment. Also, antibiotics are added when canning meat, poultry, vegetables, fruits, and even animal feed. Antibiotics are given to animals with drinking water immediately before slaughter, or by injection. This procedure increases the shelf life of fresh meat by 2 to 3 days, and also improves its smell, appearance and color. Treatment of meat carcasses with antibiotic solutions is also effective. The same method is used to increase the shelf life of fish - fresh fish is dipped in ice with an antibiotic, or placed in an antibiotic solution. Antibiotics negatively affect the microbiological processes of fermented milk production, as a result of which the manufacture of dangerous products is possible.

The time required for the elimination of the antibiotic from the animal's body is not met. The residual amount of antibiotics with food enters the human body. In addition to the fact that antibiotics kill beneficial microflora, their constant presence in the human body can lead to antibiotic resistance. Which is a very dangerous phenomenon both for an individual person and regarding the issue of national security. Now one of the pressing issues in the sanitary service is the problem of determining antibiotics in livestock products. Also, their constant presence in the body of animals can lead to antibiotic resistance and become the strongest allergen. In addition to the above, their accumulation can provoke the development of various diseases. We were faced with the task of finding a sensitive and accurate method for the determination of antibiotics in livestock products, which would exclude long-term sample preparation. A spectral method based on the phenomenon of fluorescence could be a solution to this problem.

2. OBJECTS OF RESEARCH

Antibiotics such as the tetracyclines group are often used to stimulate the growth of animals [2]. Antibiotics of this group are important drugs in the treatment of humans, but in case of an overdose, like other antibiotics, they literally cripple the entire body [3].

Chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) is a broad-spectrum antibacterial agent. Often used in animal husbandry, although banned in many countries. Even a small amount of chloramphenicol in meat can cause serious illness in humans, the most common of which is aplastic anemia [4].

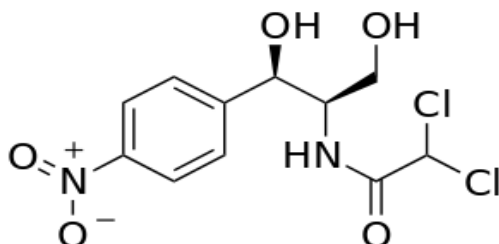


Figure 1. Structural formula of chloramphenicol.

The molecule of this substance includes two asymmetric carbon atoms, therefore, the existence of four spatial isomers is possible: D-threo, L-threo, D-erythro, L-erythro, Treo- and erythro-isomers differ in the spatial arrangement of functional groups in the molecule (Figure 2)

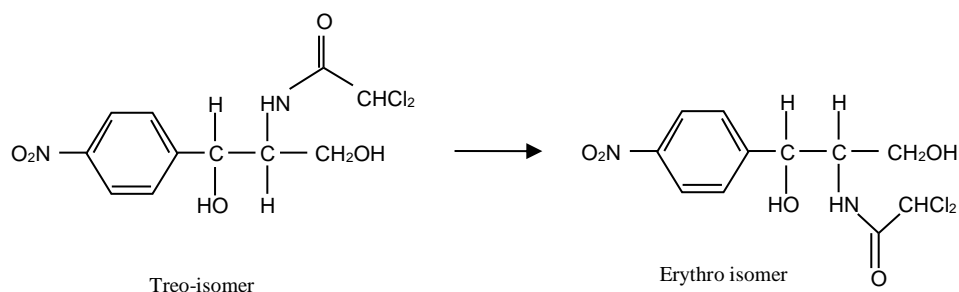


Figure 2. Spatial isomers of chloramphenicol.

The optical activity of the above structures depends on the configuration of all asymmetric carbon atoms; therefore, both the D-series and the L-series can contain levorotatory and dextrorotatory isomers.

Chloramphenicol is a white or white, odorless crystalline powder with a faint yellowish-green shade. Melting point 149-153°C. Specific rotation from +18 to +210 (5% solution in ethanol). Levomycetin is slightly soluble: in water, ether, chloroform, soluble in ethyl acetate, readily soluble in ethanol.

The UV spectrum of a 0.002% aqueous solution of chloramphenicol in the region of 220-400 nm has an absorption maximum at 278 nm and a minimum at 237 nm.

Penicillin is a beta-lactam antibiotic commonly used to treat lactational mastitis in animals. Although penicillins have long been considered safe, there is evidence of antibiotic transfer into milk. Residual levels of penicillin in milk are analyzed by liquid chromatography and mass spectrometry. Benzylpenicillin ($C_{16}H_{18}N_2O_4S$) is the first antibiotic used for the treatment of diseases, but in excessive concentrations it causes allergies. Since it is the most well-known antibiotic, it has been used in animal husbandry almost from the date of its discovery, including to stimulate animal growth [5]. Penicillin G-sodium is a penicillin derivative commonly used in the form of its sodium or potassium salts in the treatment of various infections. It is effective against most gram-positive bacteria and gram-negative cocci. Also used as an experimental convulsant for its effect on gamma-aminobutyric acid-mediated synaptic transmission.

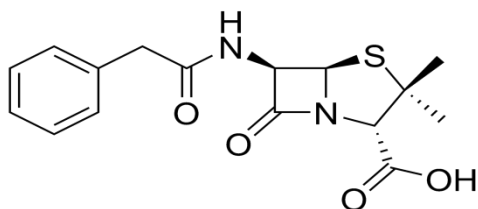


Figure 3. Structural formula of benzylpenicillin.

White fine crystalline powder of bitter taste, slightly hygroscopic. It is easily destroyed by the action of acids, alkalis and oxidants, by heating in aqueous solutions, as well as by the action of penicillinase. Degrades slowly when stored in solutions at room temperature. The melting point of benzylpenicillin is 209-212°C.

The sodium salt of benzylpenicillin ($C_{16}H_{17}N_2O_4SNa$) is readily soluble in water, ether, acetone, alcohol, and poorly in benzene, carbon tetrachloride, dioxane, pyridine. Has a melting point of 215°C.

Sulfaguanidine ($C_7H_{10}N_4O_2S \cdot H_2O$) is an antibiotic from the group of sulfonamides. Sulfonamides are antibiotics that are often used to treat animals. Substances of this class accumulate very quickly in the blood. In case of an overdose, sulfonamides hit almost the entire body: allergies, diseases of the nervous, cardiovascular systems, gastrointestinal tract organs, etc. [6] and therefore prohibited in many countries. In the year of its discovery, there was a terrible incident in America, in which more than a hundred people died because of these antibiotics [7].

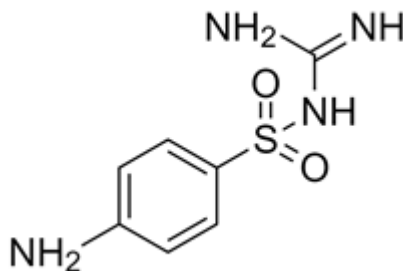


Figure 4. Structural formula of sulfaguanidine.

White or almost white fine crystalline powder.

We will very little dissolve in water and in 96% alcohol, we will slightly dissolve in acetone, practically insoluble in methylene chloride. Dissolves in diluted solutions of mineral acids. Has an absorption maximum at 470 nm.

3. METHODS FOR THE DETERMINATION OF ANTIBIOTICS

Today, three methods are used to determine the residual amount of antibiotics:

- Physicochemical. Such methods are quite sensitive, but require a complex sample preparation procedure and expensive equipment;
- Microbiological. These methods are imprecise and time consuming;
- Express methods of analysis. The advantages of these methods are the speed of determination and the relative cheapness. But often such methods are not highly accurate [8].

It is now important to find a sensitive and accurate method that would eliminate time-consuming sample preparation.

Among the physicochemical methods of analysis, fluorescence spectroscopy stands out. It has high sensitivity and accuracy. There are four directions of fluorescence analysis:

- Use of own antibiotic fluorescence;
 - Formation of fluorescent products as a result of the interaction of antibiotics with other compounds;
 - Application of the effect of quenching fluorescence;
 - Use of fluorescent markers.
- (there is no markers at all, but I added because it makes sense)

Some antibiotics have very little or no intrinsic fluorescence. The quenching effect occurs with a small amount of antibiotics, as does the formation of fluorescent products [9].

A method involving the use of fluorescent markers may be the most optimal solution to the problem. It will not be possible to exclude sample preparation, but the very study of products with the introduction of the marker will be reduced. This method is based on the phenomenon of fluorescence, which implies sensitivity to very low concentrations of a substance, therefore it is quite accurate. Finally, this method has a relatively low cost compared to other methods.

In analytical chemistry, there is a qualitative and quantitative definition of a substance. With a qualitative determination, only the presence of one or another component and only its approximate amount are recorded. The second method helps to determine a more accurate concentration of one or another component in the test sample. In this case, it is necessary to establish the sensitivity of the method, that is, to determine the minimum concentration of the substance in the sample.

4. METHOD OF FLUORESCENCE ANALYSIS

Fluorescence analysis methods are highly sensitive, inexpensive, easy to use, and the substances used in these methods are not toxic. Therefore, research methods related to fluorescence have been developing rather rapidly in recent decades. If a substance has its own fluorescence, its spectrum can tell about the molecular composition of the substance without resorting to additional tools. But most of the substances that need to be investigated have very little or no intrinsic fluorescence. For such cases, special fluorescent substances have been synthesized. All such substances can be divided into two groups: fluorescent labels and fluorescent probes. Markers work like beacons, they show where the investigated substance is located. Probes, however, respond to their environment [10].

In order to understand the advantages of this method, let's delve into the nature of the phenomenon of fluorescence.

An atom consists of a nucleus and electrons located at discrete levels with specific energies. In order for an electron to move from a lower level to a higher one, you need to give it energy. When passing from a high to a low level, the electron itself gives up energy. Fluorescence is a radiative transition from the lowest singlet vibrational level S_1 to the ground state S_2 [11]. But a process called fluorescence quenching can occur - a process that causes a decrease in the fluorescence intensity of a given substance. Quenching can be triggered by a variety of processes, including excited reactions, energy transfer, complex formation, and collisional quenching. This happens because the transition from S_1 to S_2 is not always accompanied by the emission of a quantum of light. Depending on the solution in which the fluorophore is located, the surrounding molecules can take up this energy. Naturally, each fluorophore reacts differently to different solutions. This is the basis of the method associated with fluorescent probes [12].

The fluorescent probe binds to the molecule under investigation, and after exciting radiation transmits information about the environment in which it is located. Since the intensity of the fluorophore emission, the decay time of the fluorescence and the maximum of the spectrum are very strongly influenced by the solution in which this fluorophore is located, the researcher can understand the composition of the solution by changing these parameters. Depending on which probe was used in the study, data on solution viscosity, temperature and polarity can be obtained.

5. METHODS OF QUANTUM CHEMISTRY

The development of molecular modeling techniques has opened a new avenue for a more detailed picture of information at the molecular level, molecular modeling is a rapidly growing discipline that has undoubtedly benefited from advances in computation [13].

Molecular modeling (mechanics and molecular dynamics) is a tool for calculating the energy of a molecular structure, it encompasses all theoretical methods and computational methods that are used to solve molecular structure problems

Quantum mechanics explains the energy of a molecule in terms of the interactions between electrons and nuclei, as far as the Schrödinger equation is concerned. To simulate molecular properties, electronic structure methods are used, such as the Hartree-Fock self-consistent field method and density functional theory (DFT) [14].

The theory is based on time-independent solutions of the Schrödinger equation, each of which at the output gives a new electronic state of the molecule; it is important that the smallest energy solution represents the ground state. It is the energy of the ground state that corresponds to the geometry of the ground state of a given molecule [15].

The DFT methodology is closely related to the Hartree-Fock theory in the sense that it attempts to provide a solution to the electronic state of a molecule directly from the electron density. The methodologies can be considered as substantially similar for the purposes of this discussion, however, in terms of using basis functions for orbitals and using the variational principle to find the low-energy wave function. The main difference lies in the emergence of terms responsible for both exchange and correlation in estimating the energy of the wave function, which led to a significant improvement in the description of the electronic structure [16]. Moreover, using this method, we can calculate the excitation energies.

Different functionals (for example, B3LYP) use different mathematical approximations to describe the Hamiltonian and thus estimate the energy of a given wave function [16]. A study of the literature shows that DFT is more accurate in reproducing experimental values in geometry, dipole moment, vibration frequency, etc. [17-18]. It is only important to realize that DFT is a more complete method for describing the electronic structure than that proposed from the Hartree-Fock theory and is much more complete than the semiempirical methods. However, as you might expect, incorporating more complex mathematics is also the most time consuming exercise [16].

In recent years, DFT has been used as a powerful and reliable tool for predicting more accurate molecular structure and vibration frequencies than conventional ab initio Hartree-Fock calculations [19].

Semi-empirical methods, as a rule, are well suited for calculations on molecular systems for which basis functions are optimized (for example, the heats of formations are often well reproduced) [20].

Both semiempirical and functional density methods use basis functions to represent AOs (so called basis sets), then the electronic structure of the ground state can be calculated using a mathematical procedure known as the variational principle [20].

INDO: (Intermediate Ignore Differential Overlap) The INDO method can be used for molecules containing atoms in the first row [20]. INDO includes all valence electrons.

NDDO method: (neglect diatom differential overlap): The NDDO method was described by Pople, Santry and Segal [20]. A computer program developed by Khler was applied.

AM 1: (Austrian Model 1) This was described by Dewar and colleagues. Austin's semiempirical method 1 (AM1) deals only with valence electrons [20].

PM 3: (Parametric Method 3). In 1989, James Stewart described James Stewart, the Hamiltonian used is similar to the Hamiltonian AM1.

Previously, we carried out a computer simulation of the optical properties of a representative of the thiazine series, methylene green (MG) [21]. This dye is widely used in clinical diagnostics. Based on the observations and calculations carried out within the framework of this work, specific conclusions were drawn: the absorption spectrum of the MM is formed by the phenothiazine fragment, and the nitrogen atoms of the N(CH₃)₂ and NO₂ groups do not participate in the formation of the nature of the lower electronically excited states. These states are involved in the formation of fluorescence and energy migration upon excitation. The formation of hydrogen bonds at the nitrogen atom of the central ring leads to the formation of an inactive lower singlet excited state and delocalization of the electron charge upon excitation. The growth of the structure does not lead to the appearance of an absorption band in the long-wavelength region of the spectrum. The band appears only when simulating the interaction of nitrogen and sulfur atoms in the central ring. From the analysis of the electrostatic potential values, the sites of interaction of the MM molecule with the solvent were found - the nitrogen and sulfur atoms of the central ring. The maximum isolines of the positive electrostatic potential +0.125 e pass near the sulfur atom, and the isolines of the negative electrostatic potential -0.02 e pass in the region of the nitrogen atom of the central ring. Modeling the interaction for these atoms leads to the formation of an absorption band in the 600-700 nm region. The band at 400 nm is associated with the localization of the charge on the molecular orbitals of the nitro group of the MZ. The change in intensity in this band indicates that this band is sensitive to changes in the solvent and the nitro group actively participates in the formation of the absorption spectrum in various solvents in the region of 400 nm.

6. CONCLUSION

Antibiotics are the strongest allergen. Their residual concentrations can enter the human body even with ordinary food, and their accumulation can cause not only allergic reactions, but also provoke the development of various diseases, some of which are fatal. Failure to control antibiotic residues in food can pose a health problem for many people.

The development of a fast and accurate method for the determination of antibiotics in food is an important and urgent task. Our review showed that a spectral method based on the phenomenon of fluorescence with the addition of computer modeling can be a solution to this problem.

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