Appendix 2B

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Elaboration on scientific and other academic achievements on account of application for the degree of doctor of habilitation

Bialystok 2015

1. **NAME AND SURNAME:** Wiesława Misiuk

2. **HELD DIPLOMAS, DEGREES RESEARCH - BY NAME, PLACE AND YEAR TO OBTAIN**

1972 - 1976 - Secondary School No. 1 in Bialystok

1976 - 1980 - Master's degree at the Faculty of Mathematics and Natural Sciences, Warsaw University Branch in Bialystok,

17.07.1980 - Master of chemistry, specialty teachers, graduated with honors

Thesis title: "*Using dipikryloamine to extractive-spectrophotometric determination of chlorpromazine and promethazine* "

Supervisor: Dr. Sc. Nicholas Tarasiewicz

13.06.1990 - doctoral degree in pharmaceutical sciences

Title of doctoral dissertation:

"R*eactions of certain phenothiazine derivatives with ions of titanium(IV), niobium(V), vanadium(V) and their analysis using*"

Supervisor: Dr. Sc. Nicholas Tarasiewicz

Faculty of Pharmacy, Medical University of Warsaw

3. **INFORMATION ON EMPLOYMENT IN EXISTING RESEARCH UNITS**

1980 - 1981 - Trainee assistant position at the Department of General and Inorganic Chemistry, Institute of Chemistry, University of Bialystok

1980 - 1984 - The position of assistant in the Department of General and Inorganic Chemistry, Institute of Chemistry, University of Bialystok

1984 - 1989 - The position of senior assistant in the Department of General and Inorganic Chemistry, Institute of Chemistry, University of Bialystok

1989 -1991 - Specialist scientific position in the Department of General and Inorganic Chemistry, Institute of Chemistry, University of Bialystok

1991 - 2009 - The position of assistant professor in the Department of General and Inorganic Chemistry, Institute of Chemistry, University of Bialystok

2009 - 2010 - The position of senior lecturer in the Department of General and Inorganic Chemistry, Institute of Chemistry, University of Bialystok

2010 - 2015 - The position of senior specialist in the Department of General and Inorganic Chemistry, Institute of Chemistry, University of Bialystok

4. FOREIGN RESEARCH INTERNSHIPS

X 1987 - X 1988 - Charles University, the Czech Republic, Prof. J. Zyka

5. INDICATION OF ACHIEVEMENTS as defined in the Act of 1 September 2011 (Journal of Laws No. 196, Item 1165) and of 22 September 2011 (Journal of Laws No. 204, Item 1200)

5A. Title of the scientific/artistic achievement :

Study of the interactions of selected psychotropic drugs and fluoroquinolone antibiotics with cyclodextrins

5B. **Publications included in the scientific achievements :**

The source material concerning the description of my scientific work are as follows:

A1. Misiuk W., Zalewska M., Investigation of inclusion complex of trazodone hydrochloride with hydroxypropyl-β-cyclodextrin. *Carbohydrate Polymers 77, 482-488 (2009). (IF=3.17)*

My contribution to this publication was to formulate a research problem, the choice of research methodology, performed spectroscopic measurements and their interpretation, discuss the FT-IR and NMR results, preparation of the theoretical part and writing the manuscript, the function corresponding author. My estimate the percentage of 90%.

A2. Misiuk W., Zalewska M., Study on the inclusion interactions of β-cyclodextrin and its derivative with clomipramine to spectroscopy and its analytic application. *Analytical Letters 41, 543-560 (2008). (IF=1.281)*

My contribution to this publication was to plan the research, performed by spectroscopic studies, carried out theoretical calculations and their development, interpretation and discussion of results, preparation the part of literature, participation in the preparation of the manuscript, the function corresponding author. My estimate the percentage of 80%.

A3. Misiuk W., JN Govil, Cyclodextrins, structures and properties useful in treating diseases and revitalizing body systems. *Recent Progress in Medicinal Plants, volume 29, Drug Plants III, 159-181,* Eds. J. N. Govil & V. K. Singh, Studium Press, LLC, U.S.A. 2010.

My contribution to this publication consisted of a review of the literature, an analysis of the collected material, writing the manuscript, the conception of all the schemes, the function corresponding author.

My estimate the percentage of 80%.

A4. Misiuk W. The role of assay methods in characterizing the quality of bulk pharmaceuticals. *Journal of Pharmacy & Bioallied Sciences 2, 88-92 (2010).*

My contribution to this publication consisted of the analysis of the collected material, a review of the literature, writing chapters of the manuscript, to improve the manuscript in response to the reviews, the function corresponding author.

My estimate the percentage of 100%.

A5. Misiuk W., Zalewska M., Spectroscopic investigation on the inclusion interaction hydroxypropyl-β-cyclodextrin and bupropion. *Journal of Molecular Liquids 159, 220-225 (2011). (IF=1.58)*

My contribution to this publication was to plan the research, performed spectroscopic measurements and their interpretation, discuss the NMR results and preparation of the manuscript chapters, correction the manuscript in response to the reviews, the function corresponding author .

My estimate the percentage of 85%.

A6. Misiuk W., Spectrofluorimetric study on inclusion interaction of β-cyclodextrin with duloxetine and its analytical application, *Indian Journal of Chemistry A Sec 51, 2012 , 1706-1710 (2012). (IF=0.79)*

My contribution to this publication was to fluorimetric doing research, analysis, interpretation and discussion of results, preparation of the theoretical part and writing the manuscript, the function corresponding author.

My estimate the percentage of 100 %.

A7. Misiuk W., Jasiuk E., Study of the inclusion interaction of HP-γ-cyclodextrin with bupropion and its analytical application. *Journal of Molecular Structure 1060, 272-279 (2014). (IF=1.585)*

My contribution to this publication was to execution and interpretation of spectroscopic and computational components, analysis, interpretation and discussion of results, preparation of the theoretical part and writing the manuscript, the function corresponding author.

My estimate the percentage of 85%.

A8. Misiuk W., Study of the inclusion behavior of β-cyclodextrin with ziprasidone and its pharmaceutical application. *International Journal of Pharmacy and Pharmaceutical Sciences, 7, 463-466 (2015). (IF=1.59)*

My contribution to the publication of the research was to formulate a research problem, performed a part of measurements, analysis, interpretation and discussion the results, preparation of the theoretical part and writing the manuscript, the function corresponding author.

My estimate the percentage of 100%.

A9. Misiuk W., Józefowicz M., Study on a host-guest interaction of hydroxypropyl-β-cyclodextrin with ofloxacin. *Journal of Molecular Liquids 202, 101-106 (2015). (IF=2.515)*

My contribution to this publication was to plan the research, performed spectroscopic measurements, performing calculations, analysis, interpretation and discussion of the FT-IR results, preparation of theoretical part and participation in the preparation of the manuscript chapters, to improve the manuscript in response to the reviews, the function corresponding author.

My estimate the percentage of 85%.

Summary IF of [A1-A9] publications according to the publication year is 12.511.

Scientific research presented in the publications were performed in 2008-2015 years in the Department of Inorganic and General Chemistry, Faculty of Biology and Chemistry, University of Bialystok. The research were financial by own and charter projects of University of Bialystok.

5C . **TO DISCUSS THE ABOVE –MENTIONED SCIENTIFIC WORK AND THE RESULTS OF THEIR POSSIBLE USE**

INTRODUCTION

Development of civilization in the world, especially in industrialization countries, contribute to continuous increase incidence of psychotropic diseases. Lately we can speak about social results of psychotropic diseases. Irrational organized rest, fast rate life, its irregularity, mechanization, monotonous operations exercised during work provide into psychic tonus crossing adoptative abilities of organism.

In addition every living organism, also human is exposed to activity different pathogens of microorganisms. It is observed an increase requirements on a new antibiotics making possible treatment of infection antibiotic resistant and characterized by law toxicity. Medical and chemical sciences are disposed on efficacious drugs research for patients pain alleviated.

Assessing the current state of research on psychotropic drugs and fluoroquinolone antibiotics should be noted that the rapid development of the pharmaceutical industry and the emergence of new therapeutic substances and new forms of drugs makes it necessary to develop methods for testing the quality of performance using modern research techniques such as spectroscopy, fluorimetric, immunological, chromatographic, electrochemical, flow to automate procedures and electron microscopy.

Ensuring the quality of medicinal products is an important and complex issue. In the area is observed dynamic development. Producers and government agencies are becoming more concern for the production of effective and safe medicines. An important element to ensure the quality of the medicinal product is to create the right conditions for the production and the development of appropriate methods to confirm the quality of the produced drug and its compliance with the approved specification. Effective control of the parameters affecting the quality of the finished dosage form requires specific methods, reproducible, accurate, reliable and does not generate high costs.

Constant concern and interest of the pharmaceutical industry in producing safe medicines requires compliance with good manufacturing practice (GMP) and the principles of good laboratory practice (GLP). It was noted that the quality of drugs depends mainly on proper control of the whole process of manufacturing and testing methods used in the evaluation of the entire production process and finished medicinal product.

In the European Union and Poland, registration and marketing authorization for medicinal products and medical devices related to their quality, safety and therapeutic efficacy is created by the European Agency for the Evaluation of Medicinal Products (EMEA, CPMP), the European Directorate for the Quality of Medicines (EDQM) and the International Conference on Harmonization Requirements for Medicines (ICH, EU/USA / Japan). Document describing these requirements is EudraLex (The Rules Governing Medicinal Products in the European Union), together with updates of individual documents, and the European Pharmacopoeia [1].

Quality control of drugs in the modern sense is a complex system of analyzing, evaluating and controlling all stages - from the synthesis of the compound, consider it as a drug based on multidisciplinary research, its distribution and proper ordination, to continuous monitoring of

therapeutic efficacy and side effects. Meeting these requirements is made possible by advances in chemical sciences in the use of modern control and measurement equipment, computerization and automation of processes, interdisciplinary research, as well as the implementation of scientific studies to practical applications. Multidirectional drug research requires consideration of a number of scientific fields, which are aimed at the common goal of its quality, safety and therapeutic efficacy [2,3].

It is worth noting that the active pharmaceutical ingredient has a slightly non-chemical characteristics and must meet certain quality requirements [4]. Quality requirements of active substances can be found in the guidelines of the EMEA/CVMP/1069/02, the CHMP/QWP/297/97- *Guideline on summary of requirements for active substances in the quality part of the dossier.* You should also pay attention to the new requirements on the stability of the active substance and the pharmaceutical form. The relevant data are in the *Note for Guidance on stability testing of active substances and related finished products* (EMEA/CVMP/846/99, CPMP/QWP/122/02), and the European Pharmacopoeia 6 in total monograph "Substances for pharmaceutical use." Details of the active substance manufacturing process, quality control and validation process are presented in the guidelines - *Guideline active substance master file procedure* EMEA/CVMP/134/02 or CPMP/QWP/227/02.

In studies of medicinal substances is also important economic and financial aspects. Implications of the error analysis of medicinal substances are more far-reaching than just the cost of repetition due to errors. In the European Union, independent of the manufacturer's quality control of drugs is a major concern. EDQM prepared a report to the European Parliament (document PA/PH/OMCL/2002/16), showing the increasing problems associated with poor quality of drugs currently on the European market, particularly the growing number of incorrectly manufactured finished formulations, the presence of illegal pollution, mistakes during the manufacturing process and emphasizing the role that the government plays a control system laboratories (Regulatory Role of OMCL). In Poland, for the verification of safety and quality of medicinal products and medical devices is the responsibility of the National Institute of Drug, which carries them within exercised by the Ministry of Health of control over the quality.

In accordance with the requirements of the European drugs are controlled during manufacture and release into the circulation and in certain cases during the registration process. In these studies, attention should be paid to the determination of polymorphic forms, which may depend on the bioavailability of the drug and the analysis of trace impurities. Also conducted random drug surveillance on the market and all series of medicinal products manufactured outside the European Union. Methods of analysis used in this study must be properly validated, the test

samples shall be representative (the problem of homogeneity of production batches), the results shall be elaborate by statistical analysis with estimated uncertainty. When selecting the proper techniques and procedures contemplate basically two types of criteria - which determine the quality and the outcome of the analysis on the investment and operating costs conditional on personal capabilities and needs. Modern chemical methods used in drug studies allow determination of virtually any analyte in different matrices. Analyzed a variety of systems makes it necessary to modify the previously known analytical procedures and their improvement. The situation is difficult when there is a need to develop a procedure for quality analysis system that has not been previously studied. [5,6].

Troubleshooting the chemical evaluation of the quality of active drug compounds is important in the context of examining the usefulness of these substances in the preparation of drugs and medication needs. The basic requirements of quality of medicinal products and the use of auxiliary substances are contained in the Pharmacopoeia, official, in force in the country, a set of basic requirements relating to the composition and quality of pharmaceuticals, methods for testing of raw materials and pharmaceutical preparations and selected medical devices. Pharmacopeia is an integral part of the overall system to ensure proper quality of medicines. General methods for determining the quality of medicinal products are contained in the pharmacopoeial monographs therapeutic agent. A method for analyzing the drug substance present in the solid and liquid describe specific monographs in the pharmacopoeia [7, 8].

In a comprehensive assessment of the quality of medicinal agents are important studies of pollution, due to the safety of the patient [9,10]. There are cases of withdrawal of medicinal products, due to adverse events, which were the cause of contamination present in trace amounts.

The progress of scientific thought and research techniques allows to obtain quality drugs active ingredients with high purity. European Pharmacopoeia has introduced a new feature called "transparency monograph," which relates to the identification of impurities and their arrangements acceptable levels. The pollutants identified by the methods described in the monograph, have been extended to new types of pollution, appearing, for example, when changing the process for the synthesis of the drug substance or other reasons. In the case of new pharmaceutical raw materials, information on pollution the European Pharmacopoeia Commission is obtained from the manufacturers. For substances under patent protection, information on pollution are not published, but stored in the database of the Secretariat of the European Pharmacopoeia Commission. European Pharmacopoeia determining pollutants identified individually monitored, characteristics, the presence of which has been accepted by the authorities allowing drugs on the market and detectable impurities (potential) that were not identified in the development of a

monograph, but their presence can be theoretically predicted and may occur, e.g. when upgrading process.

METHODS USED IN MEDICINES QUALITY RESEARCH

The quality control laboratories drugs should be in accordance with ISO/IEC 17025: 2001 - *General requirements for the competence of testing and calibration laboratories* and the requirements of the EDQM emerging within the European Network of Official Medicines Control Laboratories (OMCLs). Recommendations for evaluation criteria and accept the results, the specific discussion of the results do not correspond to the specifications contained in the *Evaluation and reporting of results,* document PA/PH/OMCL (2000) 52 and PA/PH/OMCL (2003) 25.

The ISO 17025 contains all the requirements to be met by testing and calibration laboratories, if they want to prove that they apply a quality system, are technically competent and capable of getting the results substantively significant. The proper operation of analytical laboratories and expertise in the countries of the European community is controlled by the audits and collaborative studies organized by OMCL (European Directorate for the Quality of Medicines).

In the quality assessment of drugs most frequently used approaches for the separation of components - high performance liquid chromatography HPLC, TLC, capillary electrophoresis CE and identification techniques - in terms of ultraviolet and visible spectroscopy UV/VIS, Fourier transform infrared FT-IR, nuclear magnetic resonance NMR, mass spectrometry MS, fluorimetric, electrochemical, X-ray diffraction to analyze polymorphic compounds of medicinal substances and electron microscopy to study the morphology of the surface.

The use of combined techniques in the analysis of drug quality contributes to obtaining more information than can be obtained with any technique used separately and they allow to save time and increase productivity analysis. However, these techniques are not without drawbacks. For example, the use of mass spectrometry as a detector in connected techniques causes destruction of the sample, cannot be recovered starting material. Moreover, closely related compounds provide very similar mass spectra due to the similar fragmentation.

Combined techniques are primarily useful in the study of pollutants medicinal substances by toxic metals. These methods are becoming an increasingly important tool for speciation analysis, which determined the separation of elements forms typically occurs in the chromatographic system - gas chromatography and high performance liquid chromatography or capillary electrophoresis,

spectroscopic methods are used as detectors while commonly used for the determination of total element content.

The capillary electrophoresis method is not very effective for separation of molecules of psychotropic drugs or antibiotics of similar weights. Application of combined CE-MS often requires the use of different types of interfaces, for example sheath fluid connection with or without a shielding fluid. The disadvantage connected to the fluid sheath, which compensates for irregularities in the flow and promotes ionization, is the need for dilution separated substances, which adversely affects the sensitivity of detection. Combination boot without liquid to counteract osmotic flow disturbances, requires a modification of the inner surface of the capillary, which is problematic and limits their use in the study of drugs.

Modern measurement techniques provide in a short time a large amount of data, the analysis of which is made possible by the use of chemometrics. Chemometrics uses mathematical methods, especially areas and departments based on probability theory, mathematical statistics, information theory, the theory of experimental design and optimization. Chemometric methods are often used in the multivariate analysis, calibration, validation, testing and identifying interference effects of chemical connections; used in pharmaceutical analysis in assessing the quality of medicinal substances and pharmaceutical forms [11-15].

I conducted research focuses on the processes of inclusion complexing dibenzoazepine, triazolopyridine, phenylethylamine, piperazine, thienylpropanamine, fluoroquinolone derivatives with cyclodextrins by spectroscopic methods FT-IR, NMR, UV/VIS and fluorimetric. FT-IR spectroscopy, one-dimensional and two-dimensional 1D and 2D NMR, UV/VIS, used in my research is the techniques, which together with the development of modern technologies in the field of new materials and techniques, data processing undergone tremendous evolution. These methods are characterized by high versatility, sensitivity and high precision, are used in quantitative analysis and basic research. Spectroscopic methods can be successfully used to identify substances and structural studies. The advantages of these methods include the availability and relatively low cost compared to the analysis of other instrumental techniques. Introduction spectrometers equipped with microprocessors for quick mathematical treatment of the spectra resulted in a huge development of these methods.

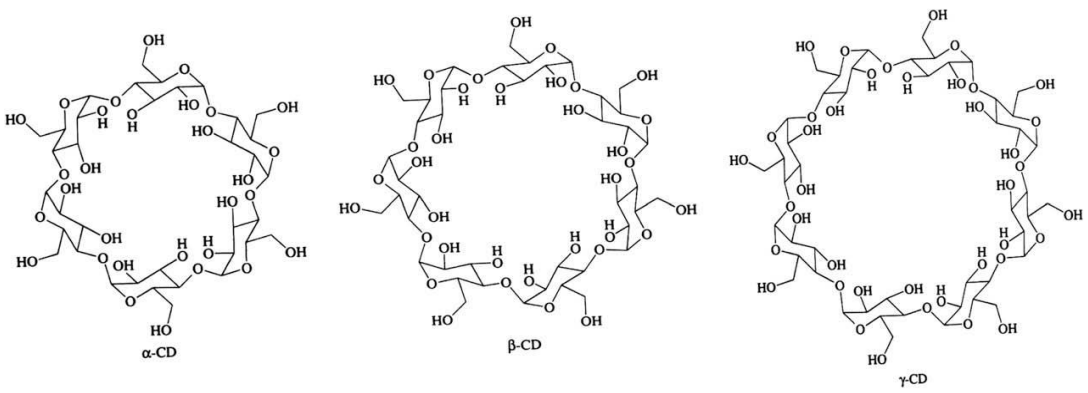
The use of computers in spectroscopic procedures are improved resolving power and accuracy, which increases certainty analytical measurements. Accuracy, precision and selectivity increased by use of improved analytical functions and amendments incorporating the effect of confounding factors. Feedback spectrometers with a computer has many benefits, such as the registration of a large number of measurement data, registering magnitude rapidly changing, control

of the device leading to automate the measurement, smoothing and filtering software to improve signal sensitivity assays, the analog signal processing in digital diagnostic device, including checking of correct operation of components, correction of measurement parameters, calculating and displaying the results, comparing the results with the reference data stored in memory storage. Spectroscopic methods are particularly easy to automate the processing of results and perform analytical operations.

Due to my previous theoretical and practical interest in the various methods used in studies of psychotropic drugs and antibiotics using cyclodextrins undertook the review studies on the latest trends in the field. As a result of these studies was reviewed in *" The role of assay methods in characterizing the quality of bulk pharmaceuticals "* [A4] presents the latest application of spectroscopic methods, chromatography and electron microscopy in studies of drugs. In the analysis of the complexes of cyclodextrins with the active substances of antidepressants, antipsychotics and fluoroquinolone antibiotics important research technique is NMR, FT-IR and electron microscopy. Based on the data analysis of these methods, you can confirm the formation of inclusion complexes and to determine their structure, the way the orientation of "guest" molecule in the cyclodextrin cavity - "host " molecule .

APPLICATION OF CYCLODEXTRINS TO CHANGE THE PROPERTIES AND STABILITY OF THE ACTIVE SUBSTANCES

Cyclodextrins (CD) structure, their physicochemical and biological properties are contributed to their wide application in drug research. Cyclodextrins are natural macrocyclic oligosaccharides composed of repeating α-D-glucopyranoside units linked by α-1,4 bonds. They are produced by enzymatic degradation of starch [16]. The compounds having six, seven and eight glucopyranoside units namely α-, β- and γ- cyclodextrins, respectively [17-19]. Cyclodextrin structure is presented in figure below

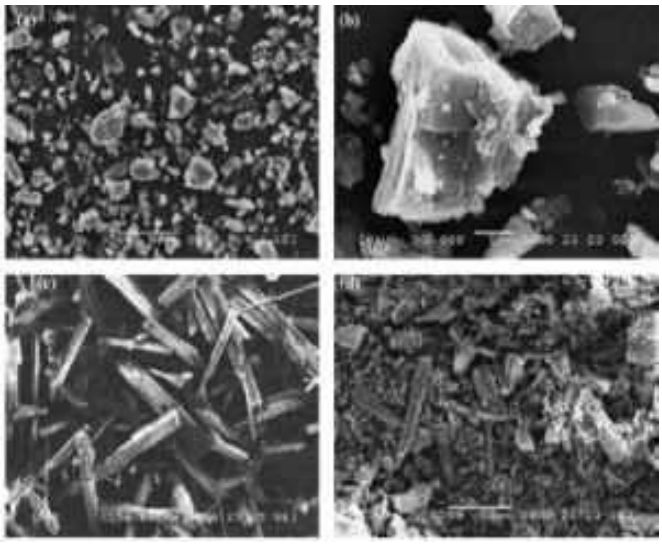


The cyclic orientation of cyclodextrin shows truncated cone structure with a hydrophobic interior and a hydrophilic exterior [20,21].

Inclusion complexes

Hydrophobic/hydrophilic character of cyclodextrins molecules are suited for ability to molecular recognition by forming host-guest complexes with different sized molecules [18]. Inclusion complexes formation depend on different factors, e.g. charge and nature of ligand and character of non-covalent force between substrate and ligand [22]. Only "guest" molecule with appreciate shape and size can include into cyclodextrin cavity. Partial inclusion are placed when complete "guest" molecule cannot include into "host" cavity. Then in cyclodextrin cavity are hydrophobic groups, hydrophilic are outside and can react with solvent molecules and hydrophilic groups of cyclodextrin [21,23].

Cyclodextrins structure, physicochemical and biological properties are useful in different disciplines of human life. The compounds are widely used in medicine, pharmacy, chemistry, agriculture, etc. Investigation of the mechanism of inclusion complexes plays an important part in supramolecular chemistry. Scanning electron microscope (SEM) methods can be assumed as a proof of solid inclusion complexes formation. SEM photographs of β-cyclodextrin (β-CD) and its complex with L-tyrosine are given in figure below



Scanning electron microscope photographs: (a) β–CD x 500, (b) β–CD x 3000, (c) L-tyrosine (TYR), (d) TYR-β-CD complex [A3]

5 D. PURPOSE, SCOPE OF RESEARCH AND DISCUSSION OF THE MAIN RESULTS

Cyclodextrins and their derivatives may be used in the production of nanoparticles and nanocapsules used primarily as drug carriers, genes, antibodies, photosensitizers in therapy and targeted as particles with an antibacterial, or antiviral. In view of the interest perspectives therapeutic applications of these structures require an analysis of their effect on the biologically active compounds of drugs and evaluate their toxicity.

The main purpose of my research was the synthesis and study of inclusion complexes cyclodextrins and their derivatives with biologically active substances selected drugs as stabilizing systems. Active substances of antidepressants, neuroleptics the new generation and fluoroquinolone antibiotics were tested as complexes with cyclodextrins. The active compounds of these drugs are susceptible to various oxidizing agents, hydrolysable, polymerizable adversely affect the physicochemical properties of the active substances and therapeutic effect. I conducted the study allow a deeper knowledge of the inclusion mechanisms of the active compounds effects of selected drugs with cyclodextrins and their impact on the physicochemical and functional properties studied drugs.

Progressive development of the pharmaceutical industry, the emergence of new therapeutic substances and new forms of psychotropic drugs and antibiotics will need to develop newer, harmonized requirements for the final product quality. The basis for assessing the quality of the drug are standards covering the requirements and test methods [1,3,4]. The problems we encounter in the analysis of therapeutic substances make clear that it can be solved by the use of cyclodextrins, whose presence contributes to changing the properties and stability of the active ingredients of medicines.

There is a tendency for the synthesis of psychotropic drugs and fluoroquinolone antibiotics that next cause effective therapeutic effect negligible impact side. Among these drugs available on the pharmaceutical market, only a part of them plays a significant role in the therapy of diseases other are complementary [24-30].

In my publications [A1, A2, A5-A9] I presented the study of therapeutic substances from the group of derivatives of dibenzoazepine (PD) - clomipramine, trazodone - from triazolopyridine (TRP), bupropion-from phenylethylamine (PEA), ziprasidone - from piperazine (PIP), duloxetine - from thienylpropanamine (TPA) and ofloxacin from fluoroquinolone (FCH) with β- and γ-cyclodextrins. In connection with the target moved studies on:

- receipt of cyclodextrin inclusion complexes with a biologically active compound and study their physicochemical properties,

- investigate the supramolecular structure of these complexes, the way the orientation of "guest" molecule in cyclodextrin cavity - "host" molecule,
- determine the mechanism of inclusion complexation of dibenzoazepine, triazolopyridine, phenylethylamine, piperazine, thienylpropanamine and fluoroquinolone derivatives with β-, γ- cyclodextrins and their hydroxypropyl-derivatives,
- applications received inclusion complexes for the development of spectroscopic and fluorimetric methods for the determination of selected active substances at μg/ml and ng/ml in the sample.

I study groups of compounds differing in the structure, it was possible to discuss the impact of the various functional groups on the physicochemical properties and stability of their complexes with cyclodextrins.

The research I conducted by spectroscopic methods NMR, FT-IR, UV/VIS and fluorometric. Among the spectroscopic techniques applied in research of cyclodextrin inclusion complexes with "guest" molecules deserves attention nuclear magnetic resonance due to their advantages. MNR technique has been used by me to study the inclusion complexes selected psychotropic drugs and fluoroquinolone antibiotics with cyclodextrins. NMR is a very valuable and convenient technique for study interactions between cyclodextrins and "guest" molecules. No other spectroscopic technique does not provide much information about the test supramolecular assembly. NMR spectra were obtained which allow to specify the structure of the inclusion complex and provide specific information on the orientation and geometric location of "guest" molecule in the cavity of cyclodextrin. While other spectroscopic techniques, e.g. UV/VIS or fluorometry can only indirectly report on the formation and structure of inclusion complexes. By means of NMR studies very quickly we obtain information about inclusions "guest" molecule into the CD cavity based on the changes of chemical shifts (ppm) of ^{1}H and ^{13}C atoms. The obtained information provide data on complex formation, its stoichiometry, durability and geometry. Through the use of ^{1}H and ^{13}C NMR received information about the partial or total inclusion "guest" molecule into the cavity of cyclodextrins. 2D NMR research provide detailed information on the impact of "guest-host" interactions, the dynamics of "guest" molecule in the cavity of cyclodextrin. NMR technique due to its advantages in comparison to other instrumental techniques promising growth in popularity of medicines quality control laboratories.

The use of cyclodextrins in pharmacy and medicine

The implementation presented my research in scientific publications [A1-A9] stems from the demand shown by the market for new innovative drugs and their formulations using cyclodextrins. Through the use of cyclodextrin inclusion complexes with active substances antidepressants, antipsychotics and fluoroquinolone antibiotics will be provided for patients previously inaccessible properties. In the pharmaceutical industry there is a great need for new, innovative medicines with few side effects, which fit within the current targeted therapy. It is a chance for these studies, particularly in the context of the development of the industry trends. Factors contributing to the development of the pharmaceutical market is the aging of the Polish society and export to other markets. By 2020, in Poland will arrive people over 60 years old. Moreover, the authorities are pushing to lower health care costs by reducing the prices of medicines. The regulators are more demanding in terms of efficiency, quality and safety of medicines, which, combined with advances have led to an increase in the cost of research in the pharmaceutical industry. To become a competitive player in the sector, pharmaceutical companies must improve the strategy to anticipate and identify key events in the development of this field. They must also be committed to the discovery of innovative solutions and use the latest technologies.

Currently, cyclodextrin inclusion complexes of substances registered as drugs are intensively studied. Mounting active substances in the cavity of cyclodextrin have different physicochemical properties than the free molecule. The administration of the drug to patients in the form of a complex with cyclodextrin has a favorable effect on the metabolism of the substance in the body. The benefits arising from the use of drugs in the form of complexes with cyclodextrins are mainly increase bioactivity, reduction of side effects and increase stability. Cyclodextrins are offset by the unfavorable taste and smell of your medicines. Furthermore, pharmaceuticals, which employ complexes with cyclodextrins are less exposed to moisture, which is important especially when highly hygroscopic active substances. These compounds are thus protects against oxidizing agents, causing their polymerization and hydrolysis. Some of the sparingly water-soluble substances are soluble when administered in cyclodextrin. Furthermore, after oral administration, in a shorter time is achieved higher drug concentration in the blood compared to the drug administered without cyclodextrin. Pharmaceuticals are so effective.

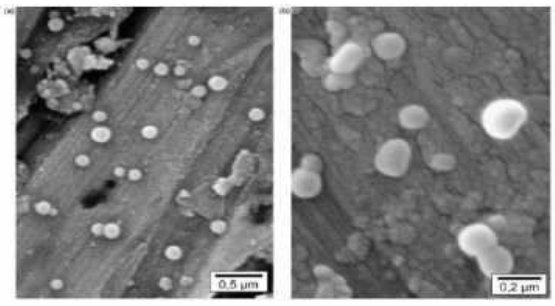
The research presented in my publications [A1-A3,A5-A9] test flow from market demand for new pharmaceuticals for the treatment of various mental disorders, especially schizophrenia and depression and various infections. It is estimated that in the adult population prevalence of

depression in the world is 12%, and an upward trend. A large group of mental illnesses are psychoses, including schizophrenia within approx. 1% of the human population and the occurrence of neurotic disorders with anxiety and insomnia often. Fluoroquinolone tested antibiotics [A9] is important in terms of therapeutic class of drugs. All living organisms, including man is exposed to microbial pathogens. Fluoroquinolone antibiotics allow the treatment of severe infections and infections are resistant to other antibiotics, and have a low toxicity. The bioavailability of psychotropic drugs and fluoroquinolone antibiotics often restricts their low solubility in water. This can be corrected through the use of carefully selected cyclodextrins. There is currently an increase in demand for psychotropic drugs and fluoroquinolone antibiotics chemically durable and effective therapeutic action. Medical science and chemistry, to above are focused on the search for new, more effective drugs that could bring relief to patients.

Cyclodextrins in biomedicine are applied in production of hydrogels useful as drug delivery systems [31-33]. Hydrogel based on hydroxypropyl-β-cyclodextrin shows ability to release monitoring of diclophenac, estradiol and sertaconazol [33]. A high solubility of γ-cyclodextrin makes possible preparing hydrogels with a lot of its amounts. It provides to improvement complexing lipophilic drug molecules.

The supramolecular structures formed between cyclodextrins and polymers have inspired progress in studies on supramolecular biomaterials for drug and gene delivery. A new concept in drug delivery system is based on using amphiphilic cyclodextrin molecules in preparing nanoparticles or nanocapsules which have considerable potential in chemotherapy. SEM photographs of amphiphilic β-cyclodextrins nanospheres are shown in figure below

(a) (b)



SEM photographs of amphiphilic β-cyklodextrins: (a) nanospheres of β-CD21C6, (b) nanospheres of β-CD14C6 [A3]

Complexes of cyclodextrins with the active substances of selected drugs

Antidepressants, antipsychotics and fluoroquinolone antibiotics addition to their effective therapeutic action is characterized by the presence of many side effects. In order to avoid adverse effects can be used administering the active substance in the form of inclusion complexes with cyclodextrins.

Binding molecules antidepressant, antipsychotic, or fluoroquinolone antibiotics in complex with cyclodextrin and its controlled release into the body entails various advantages, in particular:

• unwanted side effects are reduced, because the amount of the drug actually free and available in the medium may be lower than the dose used,

• the duration of effect may be extended, it is important because they are characterized by rapid onset of action and short half-life,

• consequently, the number of doses and frequency of administration can be reduced, which may affect the amount and intensity of side effects [35].

In the pharmaceutical industry to optimize the therapeutic properties of the drug is an important goal. For example, paroxetine used in the pharmaceutical industry in the crystalline form of the hydrochloride is poorly soluble in water. This limits the possibility to prepare liquid formulations at a given concentration of the active ingredient. Solid pharmaceutical forms of this drug have limited bioavailability [36]. In order to enhance the solubility of paroxetine hydrochloride may be used complexes with cyclodextrins or their derivatives.

In the case of fluoxetine (FLU), which functions as the "Prozac" to reduce the side effects of the drug the cyclodextrin used as compounds for continuous release of active substance [37]. Géczy [38] concluded that among the examined cyclodextrins (α-CD, β-CD, γ-CD, HP-β-CD, G2-β-CD), γ-CD can be used as the complexing agent increases the bioavailability of FLU.

Cyclodextrins are also used to increase the bioavailability and reduce the adverse effects of the antibiotic cloxacillin. Chao et al. [39] analyzed the cloxacillin inclusion complexes with β-cyclodextrin for use of this research to develop new pharmaceutical forms of the drug.

I tested active ingredients of psychotropic drugs and fluoroquinolone antibiotics with its distinctive structure exhibit a number of interesting physical, chemical and analytical properties. On the basis of preliminary tests, it was found that the β- and γ-cyclodextrins form inclusion connections with trazodone (TRD), clomipramine (CLOM), bupropion (BUP), duloxetine (DUL), ziprasidone (ZIP) and ofloxacin (OFL). A review of the world's scientific literature showed that these systems have not been previously investigated. Complexes of dibenzoazepine,

triazolopyridine, phenylethylamine, piperazine, thienylpropanamine and fluoroquinolone derivatives with cyclodextrins also protect the active substance from potential contamination. Undoubtedly, this could affect the quality of medicines, enhance their sustainability and stability and solubility in aqueous solutions, facilitate penetration into the body fluids and tissues of the patient. The effects of hydroxypropyl-β-cyclodextrin on the solubility of the active ofloxacin was studied [A9]. Based on the obtained results, it was found that the solubility of ofloxacin after complexation by HP-β-CD has increased 3.7 times compared to the alone OFL. Shown also tested the stability of complexes of duloxetine, bupropion, clomipramine, ofloxacin and trazodone with appropriate cyclodextrins at 25 ^{0}C [A1, A2, A5-A7, A9]. The measurements of absorbance or fluorescence intensity of free active substances and their inclusion complexes were performed. Based on the results obtained, it was found that analyzed inclusion complexes DUL-β-CD, BUP-HP-β-CD, BUP-HP-γ-CD, CLOM-β-CD, CLOM-HP-β-CD, OFL-HP-β-CD, TRD-HP-β-CD are stable at the time of respectively 5, 15, 15, 30, 30, 30 and 100 days at room temperature, while the same active substance in the absence of the cyclodextrin, only a few hours. The results of the study were published in [A1,A2,A5-A7,A9]. Among the studied complexes proved to be the most durable TRD-HP-β-CD. Stability of the complex TRD-HP-β-CD can prevent the competitive complexation reaction of the drug and the exchange trazodone complexed by cyclodextrn in other molecules found in the bloodstream. The results of the study were published in [A1].

β-, γ-cyclodextrin and its derivatives were used to develop procedures that may allow greater use of these methods to the study of selected psychotropic drugs and fluoroquinolone antibiotics in different pharmaceutical and biological samples. Developed fluorimetric methods for the determination of psychotropic drugs from thienylpropanamie and piperazine derivatives are characterized by a higher sensitivity, better selectivity, lower the limit of detection than a number of other literature methods. Duloxetine active substance can be determined by fluorimetric method using a β-cyclodextrin in the range of concentrations of 18.4 ng/ml-3.5μg/ml. Ziprasidone from the piperazine derivative group, used as a new generation of atypical antipsychotic drug is poorly soluble in water, which limits its bioavailability, use and marking. Use of β-cyclodextrin to complex with ziprasidone, increases its solubility and the possibility of fluorimetric determination method in the concentration range of 0.5 ng/ml-100 ng/ ml. The results of the complexation of duloxetine and ziprasidone by β-CD was published in [A6, A8]. The fact that form inclusion complexes between the test drug substances and cyclodextrins, characterized by increased water solubility of the drug substance may be used by the pharmaceutical industry to develop new technologies and easy to administer to patients dosage forms tested drugs.

The topics submitted research papers [A1-A9] fits into the current trend of research on the quality of medicinal substances associated with the provision of safe, efficient and effective psychotropic drug therapy and also fluoroquinolone antibiotic.

Exploring new inclusion complexes of medicinal substances of psychotropic drugs and fluoroquinolone antibiotics with cyclodextrins

On the market, there are now a variety of pharmaceutical products using cyclodextrins [A3,40-43]. Unfortunately, in the case of psychotropic drugs and fluoroquinolone antibiotics, there is little preparations containing these substances. The challenge for the pharmaceutical industry may be the production of new commercial preparations of these substances with the use of cyclodextrins. These preparations are more advanced as regards the pharmaceutical form ensuring favorable therapeutic effect and the safety of their use [A3,40].

Now actively participate in the development of research inclusion connections of cyclodextrins and medicinal substances of psychotropic drugs, especially antidepressants and antipsychotics and fluoroquinolone antibiotics with their introduction to the practice of pharmacy. Detailed results of the study on the inclusion complexes of antidepressants - clomipramine, trazodone, bupropion, duloxetine, antipsychotic drug - ziprasidone and the antibiotic ofloxacin using β-cyclodextrin (β- CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD) has been published in the journals of international scope- *Carbohydrate Polymers, Analytical Letters, Journal of Molecular Liquids, Indian Journal of Chemistry, Journal of Molecular Structure, International Journal of Pharmacy and Pharmaceutical Sciences* [A1, A2, A5-A9].

It should be emphasized that the characteristics of the respondents have a complex inclusion connections is necessary in view of the need to obtain a complete picture of the inclusion of the active substance of the drug into the cavity of cyclodextrins to determine its usefulness in the design of a new generation of pharmaceutical preparations are safe for patients. These studies have performed through the use of spectroscopic methods NMR, FT-IR, UV/VIS and fluorimetric.

Continuing studies on the use of inclusion complexes in the analysis of the quality of psychotropic drugs and antibiotics, received complexes of clomipramine with the β-CD and HP-β-CD, trazodone with HP-β-CD, bupropion with HP-β-CD and HP-γ-CD, duloxetine with β-CD, ziprasidone with β-CD, and ofloxacin with the HP-β-CD. The study inclusion complexation process clomipramine, trazodone, bupropion, and ofloxacin with β-cyclodextrin, hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin in a liquid phase was performed by spectroscopic

methods. Inclusion complexation of ziprasidone and duloxetine by β-cyclodextrin in the liquid phase was analyzed by fluorimetric. An important element of the study was to determine the optimal conditions for the preparation of all the analyzed complexes. The influence of pH, concentration and type of added buffer was studied. Cyclodextrins are unstable at very low pH values. Therefore, avoided the use of solutions of strong acids in the studied systems. The formation of inclusion complexes clomipramine with β-CD and HP-β-CD, trazodone with HP-β-CD and bupropion with HP-β-CD and HP-γ-CD was tested in solutions of varying pH in the range 1-8, and ofloxacin complex formation with HP-β-CD - the pH range 2-9. The maximum absorbance for the complexes CLOM-β-CD, CLOM-HP-β-CD, TRD-HP-β-CD, BUP-HP-β-CD and BUP-HP-γ-CD was observed at pH 5, while for complex OFL-HP-β-CD at a pH of 4. The formation of complexes of duloxetine and ziprasidone with β-CD was analyzed in the pH range of 2-10 and 4-8, respectively. Maximum fluorescence for DUL-β-CD and ZIP-β-CD was observed at pH 6.4 and in the pH range 5-7, respectively.

Also analyzed the effect of different buffers - Tris-HCl, H_3BO_3-KCl-NaOH, Britton-Robinson, KH_2PO_4-NaOH on inclusion complexing reaction conditions in the test systems. Optimum complexation results were obtained using Britton-Robinson buffer.

Obtained results of this study show that the formation of inclusion complexes TRD-HP-β-CD, CLOM-β-CD and CLOM-HP-β-CD, BUP-HP-β-CD and BUP-HP-γ-CD, DUL- β-CD, ZIP-β-CD and OFL-HP-β-CD also depends on the concentration of cyclodextrins. The influence of the concentration of β-CD, HP-β-CD, γ-CD and HP-γ-CD in the studied systems was also investigated. The optimal concentration of cyclodextrin were chosen using the maximum value of the absorbance or fluorescence during the formation of the tested complexes. The results are published in [A1,A2-A5-A9].

An important step in the research was to determine the complexation constants characterizing the studied systems. These constants were determined using the method of Benesi-Hildebrand. The values of these constants for systems TRD-HP-β-CD, CLOM-β-CD, CLOM-HP-β-CD, BUP-β-CD, BUP-γ-CD, DUL-β-CD, ZIP-β-CD and OFL-β-CD are respectively $9.63x10^3$, $9.42x10^3$, $9.58x10^3$, $4.30x10^3$, $3.50x10^3$, $5.83x10^3$, $1.49x10^3$ and $1.30x10^3$ l/mol. The obtained complexes have different values complexation constants. Inclusion complexes of ZIP- β-CD and OFL -HP-β -CD are characterized by not all to high values complexation constants, but in the case of potential drugs is advantageous because it can relatively easily be released in the body. It has been shown that the stability constant of the complex formed by the bupropion with hydroxypropyl-β-cyclodextrin [A5] may be successfully varied by using different cyclodextrin derivative - hydroxypropyl-γ-cyclodextrin [A7]. Control stability constant of the complex is

particularly important in the case of drug delivery and allows the selection of optimal conditions of cyclodextrin derivative to drug administration. It also allows you to plan the amount of drug released and the time of its release. Detailed results for the determination of various complexation constants in the studied systems are presented in publications [A1,A2,A5-A9].

When determining the stoichiometry of the investigated complexes, determined the molar ratio of reagents by the continuous variation Job's method and confirmed by Scatchard and Benesi-Hildebrand. Based on the obtained data, it was found that the molar ratio TRD: HP-β-CD, CLOM: β-CD and CLOM: HP-β-CD, BUP: HP-β-CD and BUP:HP-γ-CD, DUL: β-CD, ZIP: β-CD and OFL: HP-β-CD in the analyzed complexes is 1: 1.

An important aim of the study was to carry out the characteristics of supramolecular structures analyzed complexes using various spectroscopic methods - FT-IR, ^{1}H NMR, ^{13}C NMR, 2DNMR. In the processes of inclusion of molecular structural factors play an important role, because the majority of the investigated processes must be based on a thorough knowledge of the structure of components and of the complexes formed. The UV/VIS absorption spectra, FT-IR spectra, NMR nuclear magnetic resonance and fluorescence spectra, which confirmed the formation of inclusion connections were registered. The UV/VIS absorption spectra for CLOM, TRD, BUP, DUL and complexes CLOM-β-CD, CLOM-HP-β-CD, TRD-HP-β-CD, BUP-HP-β-CD and HP-BUP-γ-CD, DUL-β-CD and OFL-HP-β-CD performed in the spectral range 200-400 nm. In the registered spectral range absorbance values of β-CD, HP-β-CD, HP-γ-CD are zero. The absorbance of inclusion complexes CLOM-β-CD, CLOM-HP-β-CD, TRD-HP-β-CD, BUP-HP-β-CD and BUP-HP-γ-CD, DUL-β-CD and OFL-HP-β-CD are higher than the absorbance of the alone active substance without bound in a complex with cyclodextrin. The wavelengths at which the absorption maximum is observed for the tested complexes and the same active substance are usually the same or slightly different (~ 5 nm).

In the case study DUL, ZIP and their complexes with β-CD used a more sensitive fluorimetric method. The use of UV/VIS spectroscopy in studies of complexes DUL-β-CD and ZIP-β-CD did not provide relevant results confirming their creation. The fluorescence spectra DUL, ZIP, β-CD and complexes DUL-β-CD, ZIP-β-CD was made in the range of 300-500 nm. Based on the obtained results, it was found that the fluorescence intensity of inclusion complexes DUL-β-CD, ZIP-β CD in comparison to the same active substance DUL or ZIP significantly increased. The position of the emission maximum of the inclusion complex and the same active substance is the same or slightly different (~ 5 nm).

Collected data of fluorimetric and UV/VIS presented in publications [A1,A2,A5-A9] confirmed the formation of inclusion complexes CLOM-β-CD, CLOM-HP-β-CD, TRD-HP-β-CD, BUP- HP-β-CD, BUP-HP-γ-CD, DUL-β-CD, ZIP-β-CD and OFL-HP-β-CD.

For more detailed information on the structure of the complexes studied TRD-HP-β-CD, BUP-HP-β-CD, BUP-HP-γ-CD and OFL-HP-β-CD and the mechanism of formation of these connections was used spectroscopic method FT-IR. Studies FT-IR spectroscopy was carried out in the spectral range 4000-400 cm^{-1}. Pills of all analytes done with spectrally pure KBr. FT-IR spectra of the active substances trazodone, bupropion, and ofloxacin, hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin and their complexes with cyclodextrins performed and interpreted. The spectra of these compounds are abundant in the band, hence the accurate analysis is difficult.

Based on the analysis of the data obtained, it was found that in FT-IR spectrum of inclusion complex of BUP-HP-γ-CD, stretching vibration band of the C-Cl present in the range of 1134-1080 cm^{-1} disappears in the spectrum of BUP. Furthermore, band stretching vibration of C = O observed at 1694 cm^{-1}, in the FT-IR spectrum of the complex does not disappear. Based on a detailed analysis of FT-IR data on BUP, HP-γ-CD complex BUP-HP-γ-CD set in [A7] could conclude that the fragment BUP molecule containing a benzene ring with substituent -Cl enters into the HP-γ-CD cavity.

Analysis of the FT-IR data on the TRD, HP-β-CD complex TRD-HP-β-CD gathered in [A1] provides information that the stretching vibration band of the C-Cl occurring at 1099 cm^{-1} in the spectrum of TRD, in the spectrum of inclusion complex disappears. Furthermore, the FT-IR spectrum of the complex TRD-HP-β-CD band is observed at 1705 cm^{-1} corresponding to the stretching vibration of C=O. Band observed at 2464 cm^{-1} corresponding to the stretching vibrations of the third row = N^- an amino group exists both in the FT-IR spectrum of the complex TRD-HP-β-CD and the TRD. On the basis of the details of the FT-IR data on the TRD, HP-β-CD and TRD-HP-β-CD complex described in [A1] may suggest that penetrates into the cavity of the cyclodextrin a part of TRD molecule, benzene ring with –Cl substituent.

From the analysis of the FT-IR data on the OFL, HP-β-CD, OFL-HP-β-CD complex included in the publication [A9], it can be observed an increase in the intensity of the band stretching vibration of C=O group at 1716 cm^{-1} in the spectrum of the complex relative its intensity in the spectrum of the same ofloxacin. In the FT-IR spectrum of ofloxacin are characteristic bands for the vibration of C=O, COOH, > N-CH_3, -CH_3, aromatic backbone. Vibrations coming from –CH_3 ofloxacin occurring at 1457 and 1408 cm^{-1} are stored in the FT-IR spectrum of the complex. This indicates that –CH_3 group not involved in the formation of the complex OFL-HP-β-CD. In

addition, the band corresponding to the vibration -F, N-CH_3, C-O-O are stored in the FT-IR spectrum of the complex. It was also observed slight shift stretching vibration bands of -OH groups of cyclodextrin towards lower wavenumbers - bathochromic shift. It reveals a weak interaction between the carbonyl group of ofloxacin and the - OH groups derived from HP-β-CD. Based on the details of the FT-IR on the OFL, HP-β-CD and OFL-HP-β-CD complex [A9] can be concluded that penetrates into the cavity of the cyclodextrin a part of OFL molecule containing an aromatic ring with the C=O group.

Sample values of the wave number bands of bupropion, hydroxypropyl-γ-cyclodextrin and the inclusion complex of BUP-HP-γ-CD in the FT-IR spectra are shown in Table 1 and reference [A7].

Table 1. Wavenumbers (cm^{-1}) and assignments for bands observed in FT-IR spectra of BUP, HP-γ-CD and BUP-HP-γ-CD inclusion complex.

		Infrared bands (cm^{-1}) and assignments
BUP	**HP-γ-CD**	**BUP-HP-γ-CD inclusion complex**
3070: ν (C-H) from aromatic ring	3403: ν (O-H)	3403: ν (O-H)
2980 to 2854: ν (C-H) from CH_2	2928: ν (C-H)	2936: ν (C-H)
1694: ν (C=O)	1420: δ (C-H) from CH_2	1694: ν (C=O)
1551:ν(C=C) from aromatic ring	1375: δ (C-H) from CH_2; δ O-H	1594: ν (C=C) from aromatic ring
1457,1430,1405 and 1384: δ (C-H)	1334: coupled δ (C-C-H), δ (C-O-H),δ (H-C-H)	1334: coupled δ (C-C-H), δ (C-O-H),δ (H-C-H)
bending from CH_3	1154 and 1084: ν (C-O), ν (C-C), δ (C-O-C)	1156 and 1083: ν (C-O), ν (C-C), δ (C-O-C)
1134 and 1080: ν (C-Cl)	1031: δ (O-C-H),δ (C-C-H), δ (C-C-O)	1031: δ (O-C-H),δ (C-C-H), δ (C-C-O)
800,780,752, 739, 706 and 672: δ (C-H)	943: skeletal vibration involving α-1,4 linkage	943: skeletal vibration involving α-1,4 linkage
from aromatic ring	853: δ (C-C-H), ν (C-O), ν (C-C)	739: δ (C-H) from aromatic ring
	from anomeric vibration	

„ν" - stretching vibration band, „δ"- deformation vibration bands

Detailed data on FT-IR spectroscopy studies of complexes TRD-HP-β-CD, BUP-HP-β-CD, BUP-HP-γ-CD, OFL-HP-β-CD are presented in publications [A1,A5,A7,A9].

In order to confirm the nature of the investigated inclusion complexes dibenzazepine, triazolopyridine, and phenylethylamine derivatives with β-cyclodextrin, hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin was performed to study ^{1}H NMR and ^{13}C NMR.

Based on chemical shift values identified the individual signals of the test compounds and of inclusion complexes.

Examples of the chemical shift of signals derived from the protons and carbon atoms in the spectra of ^{1}H and ^{13}C NMR trazodone and its inclusion complex TRD-HP-β-CD are summarized in Table 2 and publication [A1].

Table 2. Chemical shifts (ppm) in NMR spectra trazodone and its inclusion complex TRD-HP-β-CD.

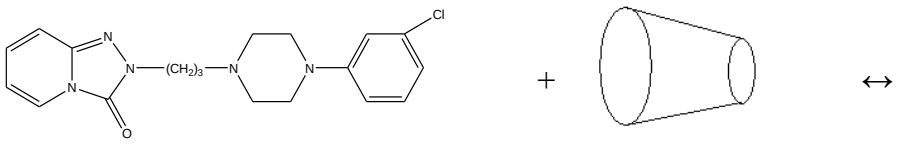
No. H/C	TRD		Inclusion complex TRD-HP-β-CD (1:1)		Δδ	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	7,73	123,15	7,72	123,16	-0,01	0,01
2	6,72	112,53	6,67	112,51	-0,05	-0,02
3	7,30	132,26	7,26	132,24	-0,04	-0,02
4	7,16	114,19	7,13	114,20	-0,03	0,01
5	-	142,90	-	142,90	-	-
6	-	149,02	-	149,04	-	0,02
7	4,09	42,90	4,06	42,99	-0,03	-0,01
8	2,27	23,02	2,23	23,03	-0,04	-0,01
9	3,21	53,95	3,18	53,96	-0,03	0,01
10	3,37	51,46	3,36	51,49	-0,01	0,03
11	3,39	46,35	3,38	46,50	-0,01	0,15
12	-	150,24	-	150,50	-	0,26
13	6,98	116,79	6,94	116,83	-0,04	0,04
14	-	134,54	-	134,53	-	-0,01
15	6,91	121,37	6,88	121,26	-0,03	-0,11
16	7,22	130,68	7,20	130,69	-0,02	0,01
17	6,89	115,31	6,86	115,23	-0,03	-0,08

From the data obtained by ^{1}H and ^{13}C NMR on the TRD, HP-γ-CD and TRD-HP-β-CD complex can be inferred for which the hydrogen atoms and carbon atoms is the largest chemical shifts (ppm). Based on the observed changes in chemical shifts may be implied by the formation of an inclusion complex TRD-HP-β-CD [A1].

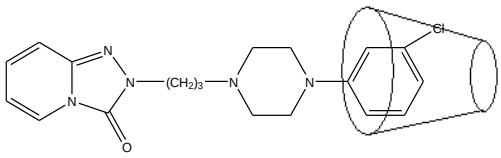
Detailed analysis of the ^{1}H NMR spectra and ^{13}C NMR of CLOM, TRD, BUP, β -CD, HP- β-CD, HP-γ-CD and complexes of CLOM-β-CD, CLOM-HP-β CD, TRD-HP-β-CD, BUP-HP- β-CD and BUP-HP-γ-CD confirms the formation of inclusion complexes studied. The results allow for a deeper analysis of inclusion connections forming [A1,A2,A5,A7].

In order to determine how the inclusion of molecule of trazodone, clomipramine, and bupropion in the cavity of cyclodextrin is also used 2D NMR technique. The results of these tests and their analysis are presented in my publications [A1,A2,A5].

Based on the comprehensive results of ^{1}H and ^{13}C NMR and 2D NMR (COSY, HSQC, ROESY) nuclear magnetic resonance and FT-IR infrared spectroscopy, specified how a "guest " molecule enters in the "host" molecule of cyclodextrin. It was found that the particles of the active compounds tested are located at different depths in the cavities of cyclodextrins. It was concluded that the part of trazodone molecule, which contains a substituent -Cl include into the cavity hydroksylopropylo-β-cyclodextrin [A1]. The bupropion complex with hydroxypropyl-β-cyclodextrin are formed by the inclusion into a cyclodextrin cavity a part of bupropion molecule containing a benzene ring [A5]. Clomipramine connection with β-cyclodextrin or hydroxypropyl-β-cyclodextrin are formed by the inclusion in the CD cavity a benzene ring without a substituent -Cl [A2]. Exemplary scheme of inclusion complex formation of trazodone with hydroxypropyl-β-cyclodextrin is shown in figure below



TRD HP-β-CD



Inclusion complex TRD/HP-β-CD

The morphology of the structure ziprasidone ZIP, β-CD and inclusion complex of β-ZIP-CD was studied by scanning electron microscope SEM. The data are presented in [A8]. SEM images observed at irregular crystal structure of ZIP and spherical form characteristic for β-CD. SEM image of the complex ZIP-β-CD shows morphological changes in its structure. Less spherical

forms of β-CD and structures specific to the ZIP were observed. Occurring morphological changes may suggest interaction between molecules of ZIP and β-CD. The structure of the inclusion complex is imaged by SEM is different from the same ZIP and β-CD. The data obtained by scanning electron microscopy infer the complex formation by the inclusion of ZIP into the β-CD cavity.

Study of the structure of inclusion complexes and explain the mechanisms of inclusion of the active compound molecules in the cavity of cyclodextrins contributes to a deeper knowledge of the processes governing these phenomena. This allows you to monitor the physicochemical and therapeutic properties of modern formulations with cyclodextrins, also for the benefit of society and civilization. In these studies used chemicals and solvents with low aggressiveness, and reduced harmful effect on humans, so they do not contribute to the deterioration of the environment.

An important achievement of the study is also to determine the relationship between the structure and the compound activity (QSAR). To this aim, values of partition coefficient (P) of the active substance in octanol-water system, which best matches the arrangement of polar and non-polar phases in a biological system. It is a system comprising octanol saturated with water and water saturated with octanol. Such solvents can serve as a model of body fluids or human skin cells. Tests were performed for trazodone and bupropion and their inclusion complexes with hydroxypropyl-β-cyclodextrin. Based on the data set for the coefficients P trazodone and its inclusion complex with HP-β-CD, and for bupropion and its BUP-HP-β-CD complex. Log P values for TRD (guest) and TRD-HP-β-CD complex are respectively 0.87 and 0.48. Log P for BUP and BUP-HP-β-CD complex is 0.82 and 0.39 respectively. Based on the obtained data, it was found that hydrophobicity of the trazodone and bupropion is quite low. However, this property is modified by complexation with hydroxypropyl-β-cyclodextrin. The relative hydrophobicity of the TRD and BUP determined by the complexation of β-HP-CD and presented the equation Δ logP = logP (guest) - logP (complex); Δ logP is 0.39 and 0.44 for the TRD for BUP. As a result of trazodone and bupropion encapsulation by HP-β-CD, their hydrophobic properties and thus bioavailability increased.

The study shows that the cyclodextrin improves the solubility of the pharmacologically active substances of psychotropic drug from triazolopirydine and phenylethylamine derivatives. Increasing drug solubility and the ability to pass through biological barriers, may contribute to reducing the dose. The same can be expected to increase the efficacy and safety of pharmaceutical preparations of some psychotropic drugs from triazolopirydine and phenylethylamine derivatives.

The research presented in [A1-A9] indicate the applicability of cyclodextrins in the preparation of medicines containing tested dibenzoazepine, triazolopyridine, phenylethylamine, piperazine, thienylpropanamine and fluoroquinolone derivatives. The results obtained on the

physicochemical properties of inclusion complexes analyzed, their supramolecular structure, reaction mechanisms complexation of active substances with cyclodextrins and their derivatives may also contribute to create a basis for the development of new drug carrier systems of psychotropic drugs from studied groups and fluoroquinolone antibiotics. The presence of cyclodextrins affects the increase in the solubility and stability of the active test compounds, which may contribute to their increased bioavailability.

The use of cyclodextrins to improve the quality of audit procedures selected antidepressants, antipsychotics and fluoroquinolone antibiotics by fluorimetric and spectroscopic methods, can be a good solution proposed by me in publications [A1-A9]. Inclusion complexes of active substances of drugs with β-, γ- cyclodextrins and their derivatives have been used for the development of fluorimetric and spectroscopic methods for the determination of the active compounds tested. The conducted my research shows that the developed method using cyclodextrins are sensitive, precise and accurate. Exemplary assay linearity TRD, CLOM and DUL using cyclodextrins are respectively 5-30 mg/ml, 17.6-70 mg/ml and 18.4ng/ml-3.5 mg/ml, the correlation coefficients 0.9998, 0.9997 and 0.9998, and the detection limits are equal to 0.27 mg/ml, 2.04 mg/ml and 6.71 ng/ml. It has been found that the active substance of trazodone, clomipramine, bupropion, duloxetine, ziprasidone, and ofloxacin in the presence of cyclodextrin used is stable from a few to a few dozen days [A1,A2,A5-A9]. Furthermore, sensitivity of methods for determining the trazodone and bupropion using HP-β-CD, expressed by the molar absorption coefficient (ε) is greater than the methods for the determination of the active substance itself. Molar absorption coefficient values are respectively $1.28 \cdot 10^4$ and $1.18 \cdot 10^4$ L mol^{-1} cm^{-1} for methods based on TRD-HP-β-CD and BUP-HP-β-CD. For the methods of TRD and BUP determination without HP-β-CD, values of ε are respectively $8.2 \cdot 10^3$ and $7.86 \cdot 10^3$ L mol^{-1} cm^{-1}. Procedures for determining the therapeutic substances of studied psychotropic drugs and fluoroquinolone antibiotics using cyclodextrins can be competitive in relation to the methods proposed by the European Pharmacopoeia [1], which has been documented in publications [A1-A9].

Due to my previous theoretical and practical interest in different layouts cyclodextrins, I made the theoretical studies and the review of the latest research and applications of cyclodextrins in supramolecular chemistry. As a result of these studies was reviewed in "*Cyclodextrins, structures, properties useful for treating diseases and revitalizing body systems*" [A3] discusses the latest developments in the use of cyclodextrins in the construction of various supramolecular systems - inclusion complexes, aggregates, rotaxanes, polirotaxanes and other, connectivity methods used in these studies and the use of these systems in medicine and pharmacy. Of these systems deserve attention rotaxanes and polirotaxanes.

Rotaxanes a supramolecular systems, which, because of their possible use in the synthesis machinery and molecular switches, are now very widely studied in various research centers worldwide. Cyclodextrins, due to their macrocyclic construction, are most commonly used to synthesize of rotaxane. A typical synthesis rotaxane as follows: molecule monosubstituted α-cyclodextrin (α-CD) is threaded to fit into the cavity diphenyloacethylene chain and is locked in this embodiment with two large blocking groups. Depending on the size of the -R substituent can be observed change in the free rotation substituted α-CD relative to the chain passing through the interior. The rotaxane properties can be used to build sensors used in the environmental and biological studies [A3].

Polirotaxanes like rotaxanes, using, in its molecular structure cyclodextrins are being intensively studied because of their possible applications. Polirotaxanes arise in the case of using a long chain polymer, such as PEG (polyethylene glycol), PEO (polyethylene oxide), PNIPA (poly (N-isopropylacrylamide) as the thread, after which they can move freely cyclodextrin molecule. Due to the specific construction polirotaxanes can be used as molecular switches, insulated molecular wires or "smart" materials. Also polirotaxanes in biomedical research in the near future may be promising supramolecular materials. Various reports in the literature on the possible uses polirotaxanes as drug carriers, in particular in the targeted tumor therapy and gene therapy.

5E. A summary of the most important achievements of research in postdoctoral work cycle

The results of my research [A1-A9] published in 2008-2015 made available to a wide group of researchers in the country and the world. The study focused on the processes of inclusion complexation of cyclodextrins with the active substances of psychotropic drugs from the groups of derivatives of dibenzoazepine, triazolopyridine, phenylethylamine, piperazine, thienylpropanamine and fluoroquinolone antibiotic. Test results discussed in detail in each publication, provided a number of important information about the conditions of inclusion complexation reaction, design cyclodextrin complexes with specific molar masses and the physicochemical and structured. This enabled the extension of the scope of research to find uses for the complexes obtained. These studies significantly expand knowledge of the complexes of β- and γ-cyclodextrins with biologically active compounds selected psychotropic drugs and fluoroquinolone antibiotics. Cyclodextrins significantly improves the quality of the active ingredient of the drug, increases its durability and resistance to various factors destabilizing. A significant increase in the signal intensity of the active substance in the presence of cyclodextrin can be the basis for the development of highly sensitive fluorimetric assays. These methods can be

competitive in comparison to other literature methods. All publications [A1,A2,A5-A9] were carried out in terms of their practical utility in the manufacture of the new generation pharmaceutical preparations of psychotropic drugs tested and antibiotics with cyclodextrins application.

The results presented in publications [A1-A9] provide new, valuable information that can contribute to development in the field concerning study of inclusion complexes cyclodextrin-psychotropic drug and cyclodextrin-fluoroquinolone antibiotic by spectroscopic methods and thus the development of civilization in this area.

The most important achievement of research work:

1. Obtained so far no described in the world scientific literature inclusion complexes of cyclodextrins with active substances - clomipramine, trazodone, bupropion, duloxetine, ziprasidone and ofloxacin, and examined selected their physicochemical properties. Received complexes TRD-HP-β-CD, CLOM-β-CD, CLOM-HP-β-CD, BUP-HP-β-CD, BUP-HP-γ-CD, DUL-β-CD and OFL- HP-β-CD of specified stoichiometric composition of 1: 1. It has been found that the complexes investigated have a high stability in time from a few to a few dozen days, and the same active substances not complexed are stable only a few hours.

2. Complexation constants characterizing the studied systems were determined. Stability constant control allows you to set the optimum cyclodextrin derivative to the conditions of administration, which is very important for drug delivery. The highest values were obtained for systems TRD-HP-β-CD, CLOM-β-CD, CLOM-HP-β-CD. It has been found that the value of the constant clomipramine complexation by a HP-β-CD is greater than by β-CD. It was further found that the stability constant of the complex of bupropion with hydroxypropyl-β-cyclodextrin can be successfully varied by using different cyclodextrin derivative – hydroxypropyl-γ-cyclodextrin.

3. Spectroscopy research by FT-IR, ^{1}H NMR, ^{13}C NMR and 2D NMR provided important information on the structure of the complexes TRD-HP-β-CD, CLOM-β-CD, CLOM-HP-β-CD and BUP-HP-β-CD. It has been found that molecules of the active compounds tested are located at different depths in the cavities of cyclodextrins. The use of methods of 2D NMR helped determine how the orientation of "guest" molecule in the cavity of cyclodextrin - "host" molecule.

4. The mechanism of the inclusion complexation of the active substances tested with selected cyclodextrins was proposed.

5. Application of the inclusion complexes to elaboration of new sensitive spectroscopic and fluorimetric methods for the determination of examined active substances at level μg/ml and ng/ml in samples.

A very important direction of scientific papers as possible to take in the near future is to explore the use of cyclodextrins to develop procedures medicinal use of other drugs.

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6. DISCUSSION OF OTHER SCIENTIFIC AND RESEARCH ACHIEVEMENTS

Since the work in the Department of General and Inorganic Chemistry, Institute of Chemistry, Faculty of Biology and Chemistry, University of Bialystok in 1980 I started research activities. I conducted the research on the analysis of the use of organic compounds - derivatives of phenothiazine of the substituents at positions 2 and 10. I took the development of methods for the determination of phenothiazine derivatives used in medicine as psychotropic drugs. Introduction to methods for the determination phenothiazines using the latest analytical techniques annual internship allowed me to research at the Charles University in 1987-88 years. I conducted a series of studies of oxidation same phenothiazines and certain of its derivatives using various spectroscopic techniques UV/VIS, ASA, NMR and chromatographic GC, HPLC. Part of the research was the subject of my doctoral dissertation. In 1990, the decision of the Council of the Faculty of Pharmacy, Medical University of Warsaw obtained a doctoral degree in pharmaceutical sciences, based on a doctoral dissertation defended Fri. "Reactions of certain phenothiazine derivatives with the ions of titanium (IV), niobium (V), vanadium (V) and their analysis using", made under the direction of D.Sc. Nicholas Tarasiewicz.

Results of my research have been published in international scientific journals and presented at national and international conferences and scientific meetings. My previous academic achievements includes 75 scientific reports - 32 original papers, 8 review papers, 35 abstracts reports presented on national and international conferences. In my output is the authorship of 2 chapters in two books.

The total impact factor according to the year of the publication of my scientific papers is 32.95, after including a doctoral degree - 31.71. For all the scientific achievements I received 710 points of MNISW. Number of citations of publications according to the Web of Science database has 261. In 29 scientific publications I am the first author. Hirsch index of the Web of Science database is equal to 9.

Starting in 1980 I presented the results of my research at the Annual Scientific Assemblies of the Polish Chemical Society, Scientific Assemblies Polish Pharmaceutical Society, Second Multidisciplinary Conference of Drug Sciences, Symposium: Food-Drug-Health, conference "Modern methods for the analysis of drugs, narcotics, poisons and stimulants", Third Conference "Biologically active compounds - the activity, the structure, synthesis". Additionally, the results of my research were presented at international conferences - 9th European Conference of Analytical Chemistry (1996), 6th International Symposium on Kinetics in Analytical Chemistry (1998), XIX Slovak - Czech Spectroscopic Conference (2008).

Also I participated in conferences, such as the VII Polish Analytical Chemistry Conference - *Analyst in the development of civilization* (Torun 2005), *3rd International Forum on Innovative Technologies for Medicine* (Bialystok 2009), "*Towards applicability - equipping of Center Synthesis and BioNanoTechno Analysis University of Bialystok*" (Bialystok 2014).

After obtaining a doctoral degree in 1990 I conducted research on biologically active compounds from the group of thioxanthene, dibenzoazepine, dibenzooxepine and dibenzocycloheptadiene using spectroscopic method, HPLC and TLC chromatography and molecular modeling. The aim of this study was to develop and refine the spectroscopic methods used to analyze selected compounds in terms of the possibilities :

- determination of the content of active substance in the presence of the degradation product,
- research purity, stability of the active compound and its ion - associative connections, the inclusion and oxidation products,
- identification of impurities of the active substance, degradation products and their labeling,
- automation methods for the determination of certain active substances.

Developed a manual spectroscopic methods for the determination of the active compounds of certain drugs have been automated. I applied for this purpose FIA flow injection technique. This technique thanks to the advantages - short time of determination, low consumption of reagents, micro quantity of sample, the possibility of miniaturization, has been used to automate and carry out serial determinations of phenothiazine derivatives. I developed the FIA methods allowed the determination of 80 promazine and thioridazine samples, and 35 piperazine samples within 1 hour. The results are published in national and international journals.

List of publications post-doctoral (beyond those which are the subject of habilitation)

1. Misiuk W., Tarasiewicz M., Spectrophotometric determination of promethazine and diethazine hydrochlorides with ammonium metavanadate. *Pharmazie 48, 66-67 (1993).*
2. Misiuk W., Tarasiewicz M., Application of thiocyanate complex of niobium(V) in spectrophotometric investigations of promazine hydrochloride in pure form and in pharmaceutical preparations. *Acta Pol. Pharm. 52, 373-378 (1995).*
3. Misiuk W., Tarasiewicz M., Spectrophotometric determination of perazine and thioridazine in pharmaceutical preparations. *Pharmazie 51, 62 (1996).*
4. Misiuk W., Tarasiewicz M., Application of thiocyanate complex of titanium(IV) in extractive - spectrophotometric determination of some phenothiazines. *Acta Pol. Pharm. 54, 115-118 (1997).*

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24. Misiuk W., Sensitive spectrophotometric methods for quantitative determination of chlorprothixene in pharmaceutical dosage form. *Pak. J. Pharm. Sci. 19, 87-94 (2006).*
25. Misiuk W., Tykocka A., Sensitive extractive spectrophotometric methods for the determination of nortriptyline hydrochloride in pharmaceutical formulations. *Chem. Pharm. Bull. 55, 1655-1661 (2007).*

In the current research project I conduct study on cyclodextrins connections with different groups of drugs - antipsychotics and cephalosporin antibiotics. Complexation constants and the stoichiometry of the studied inclusion complexes were determined. In order to optimize the process of inclusion, thermodynamic parameters of ΔG and ΔH are studied. For the analysis of inclusion complexes and determine the no chemical nature of the interactions is used molecular modeling (suitable computer programs and databases). The study is also obtained supramolecular structure of the complexes and morphology of the surface.

As part of the research topics I am actively collaborating with various units of the University of Bialystok, such as the Department of Natural Products, Department of Analytical Chemistry, Department of Environmental Chemistry, Center BioNanoTechno.

In addition to working with the team at the Institute of Chemistry of the University of Bialystok I was carrying out research in collaboration with various scientific centers in the country and abroad, prof. Nemcova (Charles University in Prague, Czech Republic), prof. Rychlovsky (Charles University in Prague, Czech Republic), prof. Szakova (Agrotechnical Czech University in Prague), prof. Govil (Scientific Agricultural Research Institute, India), prof. Moniuszko-Jakoniuk (Medical University of Bialystok, Poland).

I also reviewed scientific publications for Journals:

from Philadelphia List:

Journal of Pharmaceutical and Biomedical Analysis (1), IF=2.829; Journal of AOAC International (12), IF=1.385; Carbohydrate Polymers (14), IF=3.916; Talanta (3), IF=3.511; Spectrochimica Acta A (2), IF=2.129; Journal of Hazardous Materials (1), IF=4.331; International Journal of Analytical Chemistry (1), IF=0.904; Analytical Biochemistry (3), IF=2.305; Analytical Letters (1), IF=0.981; Journal of Inclusion Phenomena and Macrocyclic Chemistry (1), IF=1.426; Bioorganic & Medicinal Chemistry Letters (1), IF=2.951; Letters in Organic Chemistry (1), IF=0.648; Chemistry Central Journal (4), IF=1.66; Food Analytical Methods (2), IF=1.802; Drug Testing and Analysis (1), IF=2.816; Journal of Spectroscopy (1), IF=0.831; Arabian Journal of Chemistry (2), IF=2.684;

beyond Philadelphia List:

Chemical Industry & Chemical Engineering Quarterly (1), ISRN Analytical Chemistry (6), *African Journal of Pharmacy and Pharmacology (1).*

In addition, I reviewed the projects No. POIG.01.01.02-12-093/09 and No. POIG.01.01.02-00-069/09 on research and priority axis of the Operational Program Innovative Economy in Action 1.1. " *Support for scientific research for the development of a knowledge-based economy".* I have also been a reviewer of the project WDN - POIG.0103.02-00-046/11/2012 .

While working at the University of Bialystok, I received several awards of the Rector of the University of Bialystok for scientific activity.

I am also a member of editorial and scientific boards of international journals :

"*Journal of Pharmacy and Bioallied Sciences",*

„*ISRN Analytical Chemistry",*

"International Scholarly Research Notices",

„International Journal of Pharma and Bioscience" (IF = 5.121).

I am also a member of Polish Chemical Society and was a member of Polish Society of Toxicology until 2004.

Wiesława Misiuk