Preface

The topic of this book is the electroanalytical chemistry of nucleic acids and proteins, development of electrochemical sensors and their application in biomedicine and in the new fields of genomics and proteomics. A substantial part of the book is devoted to the electrochemistry of DNA and RNA (Chapters 2 and 3) and to the development of sensors for detecting DNA damage (Chapters 12 and 13) and DNA hybridization (Chapters 3–11). After intensive coverage of several crime cases by media the popularity of DNA can be compared to that of movie stars. For many of those who know something more about the nature of DNA, it is the most interesting and most important of all molecules. It is the molecule of life. Chromosomal DNA is the largest, naturally occurring, well-defined molecule. Its relatively regular structure is closely related to the DNA functions, such as storage of genetic information, its replication and copying into RNA. Moreover, DNA (and RNA) can form excellent sensing recognition layers and many of its properties can be utilized in the development of the DNA sensors. It is the nucleotide sequence (containing the genetic information) in specific segments of the human genome, which stays in the center of attention of DNA analysis for biology, medicine, pharmacy and a number of areas of practical life. Determination of the average nucleotide sequence of the human genome (containing 3×10^9 base pairs) was a difficult and expensive task, which took several years. Now we want to have information about nucleotide sequences in specific parts of the human genome of individuals in hours or minutes. It appears that electrochemical detection can greatly contribute to such a goal.

The knowledge of nucleotide sequence in the human genome represents one of the pillars of 21st century medicine, but it does not tell us what proteins are being made where, in what amounts, under what conditions, whether and how they are postsynthetically modified, etc. Such information should be provided by proteomics. The term "proteome" (coined about 10 years ago) represents total protein complement of the genome. Among the main activities of proteomics are: (a) identification of all the proteins made in the given cell, tissue or organism; (b) determination of how the proteins interact; (c) resolving the three-dimensional structures of the proteins to find the spots where binding of drug might affect their activity. At present, 2-D gel electrophoresis, mass spectrometry and X-ray crystallography are the most important methods in proteomics (see Appendix to Chapter 19 for details).

Proteins greatly differ from DNA in their physico-chemical properties, structures and particularly in multiplicity of their functions. Their electrochemical analysis is thus more complicated, requiring multiple approaches, which take into consideration the properties of individual proteins. We may thus ask a question: Can electrochemistry extend the arsenal of methods useful in proteomics? The great potential of electrochemical analysis in proteomics,

summarized in this book, suggests that the answer to this question should be positive.

This book will tell you about recently developed highly sophisticated methods of electrochemical analysis of nucleic acids and proteins and the development of biosensors. In addition it will summarize the ways, which in the past century led to the present state of electrochemical analysis of nucleic acids and proteins.

The story began several years after the First World War in Prague (Czechoslovakia).

In 1922, Jaroslav Heyrovský invented polarography, a new electroanalytical method working with spontaneously renewed mercury dropping electrode. Shortly afterwards (1924) he constructed in collaboration with M. Shikata the first automatically recording instrument – the polarograph. The ability of proteins to catalyze hydrogen evolution at mercury electrodes, manifested by d.c. polarographic signals, was discovered only 8 years after the invention of polarography (Chapters 18 and 20). In the 1930s polarography of proteins was closely connected with the names of J. Heyrovský and R. Brdička. Polarography of proteins soon found application in medicine, particularly in oncology, and for a number of years, "Brdička's reaction" was intensively studied as a tool in cancer diagnostics (Chapter 20). In 1959, J. Heyrovský was awarded the Nobel Prize in chemistry.

Owing to the development of new methods in protein analysis, the interest in the polarographic catalytic signals of proteins gradually weakened, and since the 1970s the attention of electrochemists turned to direct electrochemistry of a limited number of redox-active center containing proteins. This approach resulted in a very interesting branch of protein electrochemistry, the present state of which is reflected in this book (Chapters 14–17). The development of electrochemical immunosensors represents an important step toward proteomics (Chapter 14). In addition it appears that the ability of proteins to catalyze hydrogen evolution measured by modern electrochemical methods (Chapters 18 and 19) may become useful in biomedicine and proteomics (Chapter 19).

In the 1930s the nature and biological role of proteins was much better understood than those of nucleic acids. The role of DNA as a material of heredity was discovered in 1944, and its double-helical structure, closely connected to its biological functions such as storage of genetic information, replication and transcription, was discovered in 1953 by Watson and Crick. Five years later the first paper was published showing that DNA and RNA are polarographically active (Chapters 1 and 3). Further research revealed that the polarographic signals of native double-stranded DNA greatly differed from that of denatured single-stranded DNA, suggesting that bases are hidden in the interior of the native DNA molecule, while in denatured DNA they are accessible for the electrode processes. These results were in good agreement with the Watson–Crick DNA double-helical structure as well as with the concepts of DNA denaturation and renaturation, developed in the beginning of the 1960s. In contrast, these results were in contradiction to the DNA structure, proposed by L. Pauling (1953), with the sugar phosphate backbone inside the molecule and bases located on the molecule surface. Regrettably, when L. Pauling proposed his incorrect DNA model no data on polarography of DNA were yet available.

Classical d.c. polarography was poorly suitable for the analysis of chromosomal DNA, and thus from its very beginning the electrochemical analysis of DNA was carried out with the oscillopolarography at controlled alternating current (invented by J. Heyrovský in 1941), which (due to its cyclic mode) might be considered as a predecessor of cyclic voltammetry (CV) and of the present constant current chronopotentiometry. Starting from 1966 oscillographic polarography of DNA was gradually replaced by differential pulse polarography as well as by other methods, such as a.c. polarography, CV, square-wave voltammetry and constant current chronopotentiometry. These methods combined with mercury electrodes showed great sensitivity for changes in DNA conformation (Chapter 3), including minor changes resulting from damage to DNA by various chemical and physical agents (Chapters 3 and 12). In the second half of the 1970s oxidation of adenine and guanine residues in DNA and RNA at carbon electrodes was observed, marking the beginning of application of solid electrodes in nucleic acid electrochemistry (Chapters 2, 3 and 7). Introduction of electroactive markers into DNA research dates back to the beginning of the 1980s, while first DNA-modified electrodes were prepared about 5 years later.

Up to the beginning of the 1990s electrochemistry of nucleic acids was a domain of a mere handful of laboratories in Europe. Progress in genomics and particularly in the Human Genome Project in that time stimulated enormous interest in new methods capable to unravel the genetic information stored in the nucleotide sequence of DNA. Only after the construction of DNA arrays (chips) with optical detection the attempts to develop DNA chips with electrochemical detection (which is simpler and should be less expensive) have become popular among electrochemists. Since the middle of the 1990s the electrochemistry of nucleic acids and the development of DNA sensors have become a booming field involving large number of laboratories all over the world. Similar increase in the interest in electrochemistry of proteins can be expected if the electrochemical analysis meets the requirements of proteomics. This book shows that such expectations are not unrealistic.

Electrochemistry of nucleic acids and proteins should not be a domain of electrochemists alone. It requires interdisciplinary approaches and teams including physicists, chemists, biologists as well as biotechnologists, both experimentalists and theorists. The book is thus intended for a wide variety of readers unified by their interest not only in electrochemistry of nucleic acids and proteins but also in modern biotechnologies, nanotechnologies, surface chemistry, bioelectronics, etc. It is hoped that the book will spark imagination in students and young scientists to create new tools for science and medicine of the 21st century.

Helpful assistance of Dr. Zdenek Pechan and Mrs. Petra Mittnerová and other colleagues in handling all Chapters, Figures, Permissions, etc. is gratefully acknowledged.

Brno, July 2005 Emil Paleček, Joseph Wang