

## 4. Conclusion

This Thesis represents a contribution to the search for new analytical methods applicable on environmental and biological samples. The current state-of-art concerning the formation, occurrence, and biological activity of fluorene and its nitro and oxo derivatives was summarized in Chapter 1. Further experimental work was focused on assessment of new approaches in the detection of the studied compounds. Attention was paid mainly to nitrofluorenes, fluorenone and nitrofluorenones, namely **2-nitrofluorene**, **2,7-dinitrofluorene**, **9-fluorenone**, **2-nitro-9-fluorenone**, and **2,7-dinitro-9-fluorenone** and the possibilities of their determination using polarographic and voltammetric methods.

Interactions of calf thymus dsDNA with 2-nitrofluorene and 2,7-dinitrofluorene at the DNA/SPCPE were investigated and subtle DNA damage under conditions of direct DNA-analyte interaction at room temperature and damage to DNA bases under condition of electrogeneration of short-lived radicals of nitrofluorenes at human body temperature were found.

The obtained results can be summarized as follows:

- Modern polarographic and voltammetric methods at mercury electrodes developed for determination of trace amounts of 9-fluorenone and 2,7-dinitro-9-fluorenone (and other NPAHs generally) in concentration ranges from  $2 \times 10^{-8}$  to  $1 \times 10^{-5}$  mol L<sup>-1</sup> (for 9-fluorenone) and from  $2 \times 10^{-9}$  to  $1 \times 10^{-5}$  mol L<sup>-1</sup> (for 2,7-dinitro-9-fluorenone) offer a sensitive, inexpensive, independent, and reliable alternative to more frequently used chromatographic methods.
- Solid phase extraction can be successfully used for preliminary separation and preconcentration of 2,7-dinitro-9-fluorenone from drinking and river water samples with over 82 % extraction efficiency.

- m-AgSAE in combination with modern voltammetric techniques is a suitable sensor for the determination of submicromolar concentrations of 2-nitrofluorene, 2,7-dinitrofluorene, 9-fluorenone, 2-nitro-9-fluorenone, and 2,7-dinitro-9-fluorenone. It provides high stability and reproducibility, although the achieved  $L_Q$ s are about one order of magnitude higher compared to DPV at HMDE. However, amalgam electrodes can replace mercury electrodes in cases where higher robustness and easy operation is required or unsubstantiated fear of “toxic” mercury disables its use as electrode material. For 2-nitrofluorene, even lower  $L_Q$  ( $\sim 2 \times 10^{-9} \text{ mol L}^{-1}$ ), was reached using AdSDPV at m-AgSAE in comparison to HMDE. The m-AgSAE in combination with DPV offers verified possibility of determination of tested nitro derivatives of fluorene and 9-fluorenone in model samples of drinking and river water.
- Proposed mechanism of redox electrode reactions of studied compounds at different electrode types provides overall view of their electrochemical behavior. That can contribute to better understanding of the mechanism of their interaction with living systems.
- Voltammetric detection of damage to DNA using DNA/SPCPE biosensor represents simple electroanalytical methodology and shows the potential of this disposable biosensor for the investigation of genotoxic effects of chemical compounds of environmental and health interest. The type of association of 2-nitrofluorene and 2,7-dinitrofluorene with the surface attached dsDNA under *in vitro* conditions is the intercalative association. The toxicity of the compounds under study can be caused by this interaction and by their redox activation.

