BENZO[*A*]PYRENE ITS INFLUENCE ON HUMAN ORGANISM AND NEW NANOPHOTONIC DETECTION METHOD

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EXTENDED ABSTRACT

Benzo[a]pyrene (BaP) is representative of polycyclic aromatic hydrocarbons (PAHs) family, the substance of the first hazard class. In an environmental, BaP accumulates mainly in a soil and less in a water. It comes from soil to plants and human tissues and continues to move on in the food chain in living organisms where at each stage the BaP concentration is increasing sufficiently. To human organism BaP can come through skin, respiratory organs, digestive system and transplacental infections. Besides that BaP is the most typical chemical carcinogen in environmental, it is dangerous to humans even at low concentrations, since its metabolites are mutagenic and highly carcinogenic and has the property for bioaccumulation. Being chemically relatively stable, BaP can migrate for a long time from one object to another. As a result, many objects and process in the environmental objects which do not have the ability to synthesize the BaP, are the secondary sources of its production.

Content control of BaP in environmental can be accomplished by different assay among which the most wide-spread is liquid chromatography. Known methods possess both positive and negative characteristics the last are connected with assay complexity, not allowing of their used in a field conditions, duration, high cost. So new technologies especially based on nanotechnologies and nanomaterials are in great demand both for BaP and other hazardous organic PAHs compounds. Having in mind that BaP as most of PAH has high fluorescence yield in visible spectrum and is capable to emit electrogenerated chemiluminescence (ECL), it is quite possible to use this well-known assay method for both direct and indirect definition [1]. At the same time mentioned ECL methods of BaP definition provide not enough low limit of detection (LOD). Using luminescent nanomaterials such as semiconductor quantum dots (SCQD) as highly efficient detector elements in appropriate nanophotonic sensor can provide assay for BaP detection in surrounding objects water in the first turn with rather low LOD (≤10 nmol/l). The proposed combined photonic (electrochemiluminescent), nanotechnology (sensor's electrode modification) and electrochemical (analytical signal excitation) techniques are possessing a number of advantages which are discussed in the given paper.

Keywords: benzo[a]pyrene, electrochemiluminescence, environmental objects, luminescence, nanotechnology, nanophotonic sensor.

1. INTRODUCTION

1.1. Benzo[a]pyrene and its influence to the living organisms

The problem of food safety in the world community became relevant. Especially important are issues of detection, detention and further standardization of permissible levels of carcinogenic substances in food and environmental water. Of particular interest are such known carcinogen compounds as PAHs containing a system of condensed nuclei.
In present work for the development of improved nanophotonic assay method as a PAH representative benzo[a]pyrene (3,4-benzpyrene), $C_{20}H_{12}$, a five-ring hydrocarbon was choused (See Figure 1).

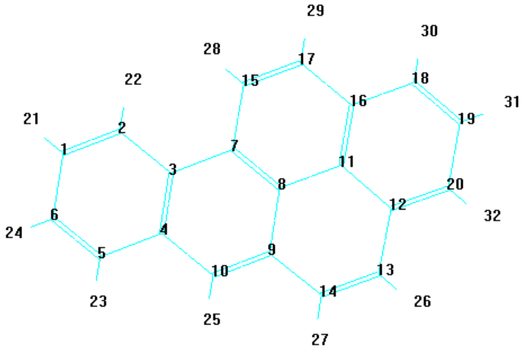


Figure 1: Benzo[a]pyrene molecular structure.

BaP and PAHs as a whole are wide spread environmental contaminants. People may be exposed to BaP from air, water, soil, cigarette and other plant product smoke, food and so on through inhalation, ingestion and skin contact (See Figure 2).

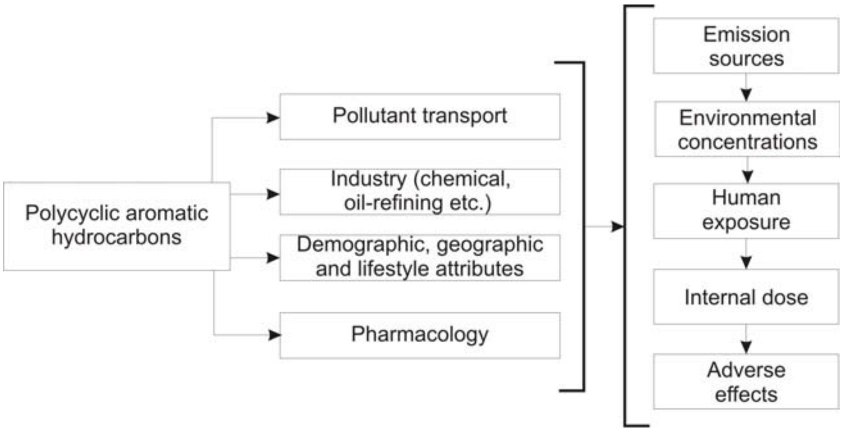


Figure 2: Process of PAHs infiltration into human organism and development of adverse effects.

BaP is slightly soluble in water and is poorly volatile. Humans may be exposed to BaP in food, tobacco and other plant smoke, and some occupational environments, and through contacts with BaP containing products such as coal tar, coal tar-based shampoo, asphalt and creosote-treated wood. Besides, some concentration of BaP is present in drinking water. The maximum/median intake level for the BaP in µg/day per person is 0,36/0,05 [2]. Penetration pathways of BaP in drinking water are shown in Figure 3.

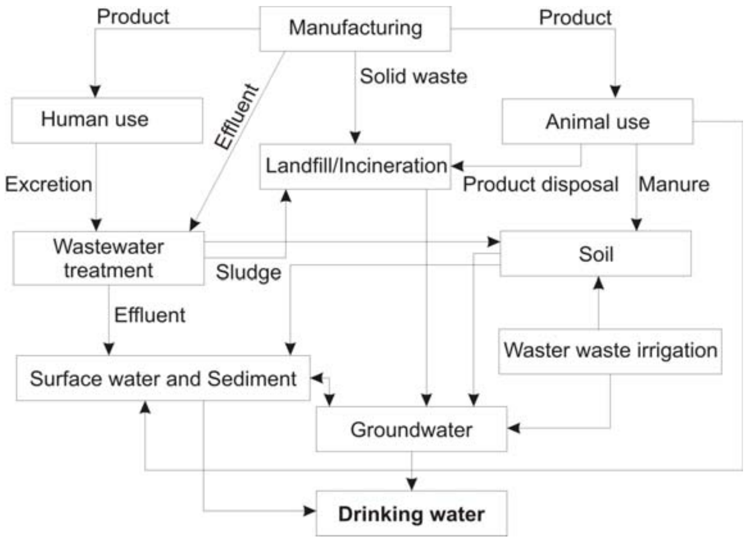


Figure 3: Penetration pathways of BaP in drinking water.

The low solubility of BaP in water results in its partitioning between soil (≈80%), sediment (≈17%), water (1%) and air (less than 1%), suspended sediment and biota [3].

BaP can lead to structural changes in DNA and as a consequence to the development of diseases with a genetic basis [4]. BaP has caused tumors in laboratory animals when administered in the diet, applied to their skin or inhaled for a long period of time. Humans exposed to mixtures of PAHs and other compounds at high concentrations over long periods of time can also develop cancer.

Thus, BaP is highly toxic, carcinogenic and mutagenic substance classified with the 1th hazard class.

In many countries it is possible to observe the excess of maximum allowable concentration (MAC). In addition, MAC to pollutants in different countries may differ. Standards of European Community (EC), United States of America (USA), Commonwealth of Independent States (CIS) and recommendations of World Health Organization (WHO) on the most toxic substances in water are provided in Table 1 [5]. As can be seeing, the most severe requirements apply to highly toxic substance benzo[a]pyrene. This necessitates the efficient control of its content and spreading.

Table 1: Standards on the most toxic substances in water

Substances	Maximum allowable concentration, mg/dm^3			
	EC	USA	CIS	WHO
Benzo[a]pyrene	0,01	0,2	0,005	0,7
Benzene	1	5	10	0,7
Vinyl chloride	0,5	2	-	10
Dichloroethane	3	5	-	30
Arsenic	10	50	50	10
Nitrites	500	1000	-	3000

1.2. Detection methods of benzo[a]pyrene in liquid phase

Currently, for control of toxic substances in an environmental objects various physical and chemical methods of analysis are being used - optical (spectrophotometric, fluorescent), chromatographic (gas, liquid, thin-layer), electrochemical (voltammetric, potentiometric, electrochemical), chromatography, mass spectrometry, chemiluminescent etc. However, none of these methods are universal.

The concentration of BaP in the water varies depending on the type of water source: surface water, groundwater and drinking water. Surface water such as river water and coastal water can be heavily contaminated with BaP, because low precautions or the dumping of industrial waste. PAH levels in uncontaminated groundwater are usually in the range of 0÷5 ng/ml [6].

Currently, there are several extensively used methods for determining BaP, among which one of the most widely used is HPLC method, Table 2 [6].

Table 2: Standard methods for BaP determination

Method	LOD / time	Principle of method
High-Performance Liquid Chromatography (HPLC)	0,2 ng/ml / from 15 minutes to few hours	The method consists in the separation of the mixture components between two phases
Immunochemical analysis (ICA), immuno-enzyme analysis , fluorescence polarization immunoassay (FPIA)	0,9 ng/ml (for FPIA)	Immunochemical reaction between antibody (Ab) and antigen (Ag)
Biological test methods	70 ng/ml / from 3 to 30 days	Based on the response of living organisms (fungus, bacterium, algae etc.) on the adverse effects of pollutants.
Chemical test methods	0,2 mg/ml / about 10 min	Reaction with chromogenic chemicals.

However, the HPLC method has some essential disadvantages. Firstly, traditional methods of analysis are rather expensive (complicated equipment); secondly, they require a lot of time to fulfill an assay with necessary preliminary sample preparation (solid-or liquid extraction, concentration). In addition, there is a large consumption of organic solvents, which raises the problem of their utilization, without prejudice to the environment.

Immunochemical methods of analysis of trace amounts of organic compounds are a rapidly growing field of analytical chemistry. ICA is based on the specific binding of determined substances with the appropriate antibodies. ICA has good response and sensitivity. However, it has some disadvantages: high demands to personal competence and lack of analysis specificity, which in some cases makes problematic a rigorous quantification of the analyte. Furthermore, methods of radioimmunoassay [7], enzymoimmunoassay [8 - 11], and electrochemical methods [12 - 13] for BaP detection in ground and groundwater were developed.

As was point out in extended abstract session the known methods of BaP determining in water have some significant disadvantages such as the duration of the procedure of sample preparation and analysis, complexity, large amount of supplies of chemical reagents needed for analysis, low LOD of BaP in water solutions.

So new methods of BaP determination are in demand, which would not be so laborious and applicable for the efficient and rapid analysis of surrounding objects.

In this paper, we propose a new nanophotonic method and sensor for the BaP detection, which is practically free from the above mentioned disadvantages of known methods.

2. ELECTROCHEMILUMINESCENT DETECTIONG METHOD

Electrochemiluminescence or electrogenerated chemiluminescence is a kind of non-optically excited luminescence produced during electrochemical reactions in solutions. ECL has several important advantages such as low LOD ($<10^{-10}$ mol/l) and high selectivity making it as powerful analytical method [1]. ECL assay can be direct and indirect [1]. The latter is more wide-spread and can be used both for detection of substances, possessing a very weak and unstable or no ECL analytical signal during an assay procedure. Detector luminophores that are necessary for such assay can be divided into three groups: a) metallorganic complexes; b) organic, such as PAHs or pyrazoline derivatives and c) semiconductor nanoparticles such as SCQD [14].

In present work, as luminescent material, we proposed to use semiconductor quantum dots (SCQD) – modern nanodimension material.

The principal stage in proposed assay method is charge-transfer recombination between ionic forms of analyte A (BaP) and SCQD (or, in short, QD) as detector elements with emission of analytical ECL signal (Figure 4).

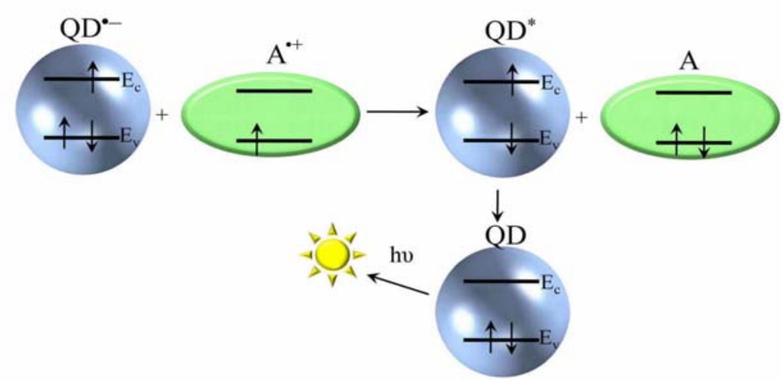


Figure 4: Scheme of detection process in a nanophotonic sensor instrument.

Reactants are oxidized and reduced, correspondingly on electrodes of a given sensor. During electric current flow through sensor's electrodes electron-transfer reactions between electrode and corresponding particles – analyte *A* or *QD* are taking place. It follows by formation of QD's anion-radicals $(CdTe/TGA)^{\bullet-}$ TGA - thioglycolic acid on its working electrode (WE) and analyte cation-radicals $A^{\bullet+}$ on sensor auxiliary electrode, a SiC plate with dimensions equal to WE one. The WE represents specially prepared optically transparent semiconductor ITO plate modified by thin-layer ordered film of QD's plotted by known methods – Langmuir-Blodgett [15] or spin-coating. As a result of reaction between $A^{\bullet+}$ and $(CdTe/TGA)^{\bullet-}$ (Fig. 4) the singlet-excited forms of QD's $(CdTe/TGA)^{*}$ can be formed. This QD's state is not stable, thus charge carriers move from conducting to valence band with light quanta emission.

The latter represents analytical signal of a system under consideration. The sensor's tuneability for a given analyte is rather precise because mentioned analytical signal is depended upon electrolysis potential and detector's elements parameters – material, diameter, type of the cover and shell, and, also, detector emission properties, such as quantum efficiency, emission spectrum (band gap), etc.

Proposed method and sensor instrument are very selective and sensitive also. Using of semiconductor QD's enhance ECL analytical signal on several orders of magnitude. So for substance in question (BaP) LOD is of the order of 1 nmol/l and less. This is quite enough for its detecting in liquid solutions.

3. MODELLING OF MAIN SENSOR PROSSESSES

3.1. Calculated of the benzo[a]pyren

Before the ECL assay in a sensor cell, BaP as analyte ought to be oxidized and converted into the cation-radical form. So the determination of BaP ionization potential (IP) directly correlated with potential of BaP oxidation. IP can be calculated as the difference between the potential energies of the BaP ground state and the BaP radical cation. Using quantum chemical calculations in the Gamess 6.0 software with a set of basis functions 6-31G, the following data were obtained (Table 3).

Table 3: Calculation results of BaP in vacuum

	HOMO, eV	LUMO, eV	ΔE, eV	IP, eV
BaP (basic state)	-5,543	4,656	10,199	7,36
BaP (cation-radical)	-9,967	-3,730	6,237	

HOMO - highest occupied molecular orbital;
LUMO - lowest unoccupied molecular orbital;
ΔE = LUMO – HOMO.

As can be seen from Table 3, IP has quite low value; so conclusion about BaP cation-radicals formation and their relative stability can be made.

4. RESULTS

4.1. Materials

As detection carcinogenic substance benzo[a]pyrene was used, this was prepared by double recrystallization from nonpolar solvents. The base electrolyte is a salt of tetrabutylammonium perchlorate (TBAP, Aldrich). In this work next solvents were used - bidistilled water, benzene (chemically pure) and dimethylformamide (DMF, chemically pure, Aldrich). SCQDs were synthesized in Chernivtsy National University in laboratory of Professor L. Scherbak and were used as obtained.

4.2. Electrochemiluminescent detection of benzo[a]pyrene

Sample containing 1 l of BaP test water solution were mixed with 10 ml of benzene and shaken for several minutes. Than necessary separation solutions using a funnel was carried out. 5 ml of obtained concentrate (solution BaP in benzene) was transferred by pipette in three-electrode ECL cell from home-made "ELAN-2m" apparatus for electrochemical and ECL assay and 5 ml of electrolyte - 0.05 M TBAP in DMF was added. Then this solution was electrolyzed and the analytical signal - ECL intensity I_{ecl} was recorded. The BaP concentration was determined using a pre-constructed calibration curve. For this 1 mM solution of the corresponding polyacene (BaP) dissolved in an electrolyte consisting of a mixture of DMF - benzene (1:1) and 0.05 M TBAP was prepared. Using dilution the concentration up to 1 nM of BaP in solution was achieved, then appropriate solutions transferred to the ECL cell and the ECL intensity was recorded. Each measurement of the analytical signal must be accomplished for at least three times then the mean and relative standard deviations can be calculated and a plot of calibration curve $\lg I_{ecl} = f(\lg c_a)$, where c_a –concentration can be built (Figure 4).

For creation of nanophotonic sensor intended for BaP content measurements in different liquid solutions water including we used above-mentioned construction. Obtained preliminary results points out upon possibility of obtaining rather low LOD (≤10 nmol/l) with such sensor device.

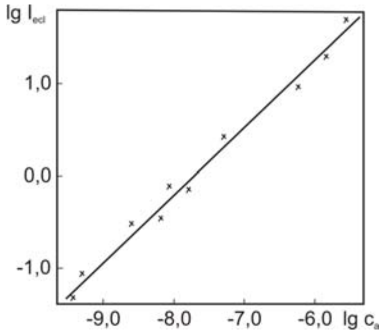
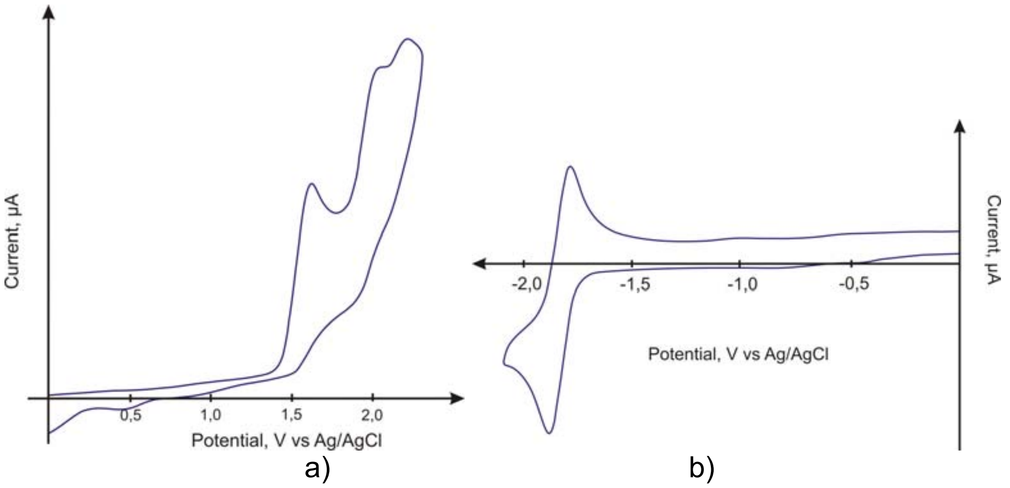


Figure 4: Calibration curve BaP in solution (DMF – benzene = 1:1). Cathode ECL on fixed electrode, polarization rate 100 mV/s $\lg I_{ecl}$ $\lg c_a$



a) b)

Figure 5: Voltammograms (a – oxidation; b – reduction) of 0,05 M BaP in $2 \cdot 10^{-4}$ M Ru + DMF- benzene (1:1) + 0,05 TBAP on glasscarbon electrode.

5. CONCLUSIONS

Proposed new assay procedure and sensor instrument which based on nanomaterials (quantum dots) and electrochemiluminescent method can considerably simplify precise BaP detection in water. Using modern semiconductor nanoparticles with high optical emission instead of standard luminophors provided higher assay metrological characteristics. As opposed to organic luminophors, SCQDs emission positions are tunable in a wide emission range from UV to NIR due to the quantum size effect and can be used widely as multicolored probes and luminescent labels in ecology assays.. Also multifarious water-soluble SQDS modified with different compounds synthesized during execution of the present work show biocompatibilities and cellular toxicities which can bring their wider applications antibioterrorism task including.

6. ACKNOWLEDGMENT

The authors gratefully acknowledge the support for this research by Science and Technology Center in Ukraine Project #4494, 5067 (Project Manager: Rozhitskii M.M.)

REFERENCES

1. Rozhitskii N.N. (1992), Electrochemiluminescence Analysis of Solutions, *J. Anal. Chem.*, **V.47, No. 11-12**, 1288-1310.
2. Polynuclear aromatic hydrocarbons in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality (2003), World Health Organization, WHO Press, 32.
3. Hicks H.E. (1996), The Great Lakes: A historical overview, *Toxicol. Ind. Health*, **12(2/3)**, 303-313.
4. Verhofstad et al. (2010), Benzo(a)pyrene induces similar gene expression changes in testis of DNA repair proficient and deficient mice, *BMC Genomics*, **11**, 333.
5. Kielhorn J., Boechuke A. (1998), World Health Organization Press, 123 – 152.
6. James F. Lawrence (1984), Liquid chromatography in environmental analysis, *Humana Press*.
7. Herikstad B.V., Ovrebo S., Haugen A. and Hagen I. (1993), Determination of polycyclic aromatic hydrocarbons in urine from coke-oven workers with a radioimmunoassay, *Carcinogenesis*, **14**, 307-309.
8. Székács A., Le H.M., Knopp D. and Niessner R. (1999), A modified enzyme-linked immunosorbent assay (ELISA) for polyaromatic hydrocarbons, *Anal. Chim. Acta*, **399**, 127-134.
9. Scharnweber T., Fisher M., Suchánek M., Knopp D. and Niessner R. (2001,. Monoclonal antibody to polycyclic aromatic hydrocarbons based on a new benzo[a]pyrene immunogen, *Fresenius J. Anal. Chem*., **371,** 578-585.
10. Li K., Woodward L.A., Karu A.E. and Li Q.X. (2000), Immunochemical detection of polycyclic aromatic hydrocarbons and 1-hydroxypyrene in water and sediment samples, *Anal. Chim. Acta*, **419**, 1-8.
11. Matschulat D., Deng A., Niessner R. and Knopp D. (2005), Development of a highly sensitive monoclonal antibody based ELISA for detection of benzo[a]pyrene in potable water, *Analyst*, **130 (№7)**, 1078-1086.
12. Chuang J.C., Pollard M.A., Chou Y.L., Menton R.G., Wilson N.K. (1998), Evaluation of enzyme-linked immunosorbent assay for the determination of polycyclic aromatic hydrocarbons in house dust and residential soil., *Sci. Total Environ*., **V. 224. № 1-3**, 189-199.
13. Nording M., Haglund P. (2003), Evaluation of the structure/cross-reactivity relationship of polycyclic aromatic compounds using an enzyme-linked immunosorbent assay kit, *Anal. Chim. Acta*., **V. 487. № 1**, 43-50.
14. Wujian M. (2008), Electrogenerated Chemiluminescence and Its Biorelated Applications, *Chem. Rev*., **35**, 125.
15. Zholudov Y, Snizhko D et al (2008), Aqueous electrochemiluminescence of polycyclic aromatic hydrocarbons immobilized into Langmuir–Blodgett film at the electrode *Electrochimica Acta*, **54**.