РЕЗЮМЕТА НА НАУЧНИТЕ ТРУДОВЕ

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представени за участие в конкурс за заемане на академичната длъжност „доцент" по област на висше образование 5. Технически науки, професионално направление 5.11. Биотехнологии, научна специалност „Технология на биологично активните вещества (вкл. ензими, хормони, белтъчини)" обявен от Университет „Проф. д-р Асен Златаров" - Бургас и обнародван в ДВ бр. 36 /3.05.2019 г.

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1. **Becheva, Z., Ivanov, Y., Gabrovska, K., Godjevargova, T., Rapid immunofluorescence assay for staphylococcal enterotoxin A using magnetic nanoparticles, International Journal of Food Science and Technology, 2019, 54, 916-922. *IF- 1,797.***

Abstract: A competitive immunoassay for staphylococcal enterotoxin A (SEA) detection in milk was developed, using immobilised antibody onto magnetic nanoparticles (MNPs). MNPs were prepared and then modified to introduce amino groups on them. The morphology and size of the obtained both unmodified and modified MNPs were characterized using TEM analyses. Monoclonal anti-SEA antibody was immobilized onto the modified MNPs (MNP-Ab). Staphylococcal enterotoxin A was conjugated with fluorescent dye ATTO620NHS. The characteristics of fluorescence conjugate were examined. The amount of MNP-Ab and concentration of the fluorescent conjugate used for competitive immunoassay were optimized: 0.25 mg and 53 μg mL $^{-1}$, respectively. The detection limit of developed immunoassay was determined – 0.23 ng mL $^{-1}$ SEA in spiked milk samples. The immunoassay takes only 30 min, the magnetic separation is fast (<10 s) and the volume of the sample for analysis is very small (200 μL).

2. **Milka Atanasova, Galina Yordanova, Ruska Nenkova, Yavor Ivanov, Tzonka Godjevargova, Dinko Dinev, Brewing yeast viability measured using a novel fluorescent dye and image cytometer, Biotechnology and biotechnological equipment, 2019, ISSN: 1310-2818 (Print) 1314-3530 (Online).**https://doi.org/10.1080/13102818.2019.1593053, ***IF-1,227.***

Abstract: This study presents an automated, image-based cytometry method for determination of total counts and viability of yeast cells by using a newly synthesized DNA fluorescent dye PO-TEDM-1 and a new Easycounter YC instrument. The synthesized polycationic asymmetric monomethine cyanine dye PO-TEDM-1 penetrates only into dead cells. The new fluorescent dye has high nucleic acid sensitivity and rapid interaction kinetics. The optimal concentration of the fluorescent dye for staining dead cells was 1 mg mL^{-1}. The Easycounter YC system was used to determine the total cell count and viability of *Saccharomyces carlsbergensis*. The actual viability measured using the proposed method significantly correlated with the theoretical viability (R^2 of 0.9988). The optimal linear interval was from $1x10^5$ to $1x10^7$ cells mL^{-1}. The coefficient of variation with Easycounter YC in the optimal range was 2.5–4%, whereas that of the manual hemocytometry method, in the same range was higher, 15–23%. We tested the procedure in a study of the total cell count and viability of yeast cells from the propagators in beer production as a function of the dilution. The proposed method can be used in assays involving simple cell counting and quality assurance in sample bioprocessing.

3. Z. R. Becheva, K. I. Gabrovska, Y.L. Ivanov and T. I. Godjevargova, Magnetic Nanoparticle Based Immunofluorescence Assay for Determination of Aflatoxin B1. Journal of Analytical Chemistry, 2019 (под печат), писмо от редактора. ***IF-0,971***

Abstract: A rapid sensitive immunofluorescence assay for aflatoxin B_1 (AFB_1) determination was developed using magnetic nanoparticles (MNPs). MNPs were prepared by thermal co-precipitation of Fe^{2+} and Fe^{3+} in aqueous solution. MNPs were functionalized with (3-aminopropyl)triethoxysilane. Polyclonal anti-AFB_1 antibody was immobilized with optimum amount (40 μg/mg MNPs). Fluorescein isothiocyanate (FITC) conjugate of AFB_1 was prepared. A competitive immunoassay of AFB_1 was performed. The AFB_1 in the sample and AFB_1-FITC fluorescent conjugate was competed for binding to the immobilized antibody on MNPs. The magnetic nanoparticles were removed with a permanent magnet. The fluorescence intensity of the unbound conjugate AFB_1-FITC in the supernatant was measured. The excess of the conjugate is directly proportional to the concentration of AFB_1 in the sample. The developed method is very rapid – 30 min, and sensitive – limit detection 0.9 pg/mL AFB_1, with linear range 1 – 100 pg/mL.

4. Yaneva, M., Ivanov, Y., Todorov, N., Godjevargova T., Magnetic-nanoparticles-based fluorescent immunoassay for individual and simultaneous determination of dichlorvos and paraoxon in milk, Food and Agricultural Immunology, 2018, 29(1), 228-243. *IF-2,568.*

Abstract: A sensitive and rapid magnetic-nanoparticles-based fluorescent immunoassay (MNPs-FIA) for individual and simultaneous determination of dichlorvos (DDVP) and paraoxon in untreated raw milk was developed. The conjugates DDVP–cBSA (cationized bovine serum albumin) and paraoxon–BSA (bovine serum albumin) were labeled by fluorescent dyes. The optimal amount of immobilized polyclonal anti-DDVP and anti-paraoxon antibodies on MNPs (0.375 mg Ab-MNPs) and optimal concentrations of DDVP–cBSA–FITC (18 μg/mL) and paraoxon–BSA–FITC (22 μg/mL) for MNPs-FIA were determined. The calibration curves of individual and simultaneous immunoassays of DDVP and paraoxon were investigated. The IC50 value of the individual paraoxon assay in raw cow's milk was 60 ng/mL and linear range 2–300 ng/mL. The IC50 value of the individual DDVP assay was 70 ng/mL and linear range 5–300 ng/mL. The IC50 values of the paraoxon and DDVP (100 and 120 ng/mL respectively) in simultaneous assay were higher than those of the individual ones, and their linear ranges were 10–400 ng/mL.

5. **Yaneva, M.Y., Ivanov, Y.L., Godjevargova, T.I., Preparation of Polyclonal Antibodies with Application for an Organophosphorus Pesticide Immunoassay, Analytical Letters, 2017, 50(8), 1307-1324,** ***IF - 1,206.***

Abstract: Haptens of dichlorvos and paraoxon were conjugated to the carrier proteins of bovine serum albumin. The obtained conjugates were characterized by infrared and ultraviolet–visible spectroscopy. The binding ratios of dichlorvos and paraoxon-to-carrier proteins were also evaluated. The number of hapten molecules per protein molecule of dichlorvos–cationized bovine serum albumin conjugate was higher
than for paraoxon–bovine serum albumin conjugate. The sheep polyclonal antibodies were produced against the dichlorvos and paraoxon. New multipolyclonal antibodies were obtained and characterized following the immunization of a 1:1 mixture of the immunogens for the simultaneous determination of dichlorvos and paraoxon by the immunoassay. An indirect enzyme-linked immunosorbent assay was used to characterize the reactivity of the antibodies to hapten conjugates. The multiantibodies showed lower affinities
than the separate antibodies, but their affinities were sufficient for an immunoassay for the simultaneous determination of the analytes. The detection limit and linear range for the determination of dichlorvos and paraoxon alone and together were determined. The recovery was characterized to determine dichlorvos and paraoxon fortified in model solutions and milk. These results demonstrate the potential of this immunoassay for the quantitative screening of dichlorvos and paraoxon.

6. **Nenkova, R.D., Ivanov, Y.L., Godjevargova, T.I., Influence of different nanoparticles on electrochemical behavior of glucose biosensor, AIP Conference Proceedings, 2017, 1809,020037.** ***IF-1,653.***

Abstract: The influence of nanosized particles on the glucose oxidase loading and the performance of amperometric glucose bionsensors were studied. Four enzyme electrodes (Pt/PAN/GOD, Pt/PAN/NZ/GOD, Pt/PAN/NZ/MNP/GOD, Pt/PAN/NZ/MWNT/GOD) were prepared by cross-linking of glucose oxidase (GOD) on nanocomposite material.Nanocomposites were prepared by entrapping nanozeolite (NZ), multiwalled carbon nanotubes (MWNT) and magnetic nanoparticles (MNP) in polyacrylonitrile (PAN) film. Cyclic voltammetric kinetic studies have been carried out with the four biosensors and the surface concentration of the adsorbed electroactive species on the electrodes was estimated. The highest enzyme concentration on the electrode surface corresponded to the electrodes prepared by nanozeolite separate (Pt/PAN/NZ/GOD) and combined with multi-walled carbon nanotubes (Pt/PAN/NZ/MWNT/GOD). The sensitivity of these two biosensors was the highest and that is in accordance with the greater amount of the adsorbed electroactive species on the electrodes (0.373 mol.cm^{-2}). This was indication that a good synergistic effect happened when MWNTs and NZ were combined and these greatly improve the electron transfer ability of the sensor interface.Amperometric measurement of the two glucose oxidase electrodes (Pt/PAN/NZ/GOD and Pt/PAN/NZ/MWNT/GOD) with best results was carried out. The linear concentration interval of the Pt/PAN/NZ/MWNT/GOD biosensor was up to 3 mM, the detection limit - 0.02 mM glucose and the storage stability - 81% of its initial current response after 30 days.

7. **Godjevargova, T.I., Ivanov, Y.L., Dinev, D.D., Multiplex fluorescent immunoassay device based on magnetic nanoparticles, AIP Conference, 2017, 1809,020018.** ***IF-1,653.***

Abstract: Immunofluorescent analyzer based compact disc for simultaneous detection of 3 antibiotics in the same milk sample is consisting of two parts: CD-based immunofluorescence kit and optoelectronic fluorometer. Kit consists of 2 parts: Lyophilized immobilized antibodies on supermagnetic nanoparticles in Eppendorf tubes and CD-based microfluidic disk, in which are formed five chamber systems for simultaneous detecting of 5 separate samples. Each system consists of 2 chambers connected by a special micro channel acting as a hydrophobic valve. In the first chamber lyophilized conjugates of 3 antibiotics with accordingly 3 different fluorescent dyes are placed. The second chamber is for detection of fluorescent signal. The optoelectronic fluorometer comprises of: integrated thermostatic block; mechanical-detecting unit (fluorometer) and block

with controlling and visualizing electronics. The disc gets into a second block of the analyzer, where centrifugation is performed and also reporting of the fluorescent signals. This unit comprises a rotor on which the disc is fixed, permanent electromagnet in the form of a ring inserted under the disc and module of 3 LED diodes with emission filters for the relevant wavelengths corresponding to the used fluorescent dyes and 1 integrated photodiode, in front of which is mounted filter with 3 spectral peaks. The signal from the photodiode is detected by the electronic unit which is sensitive "lock-in" amplifier, the engine rotor management, control of thermostatic device and management of periphery of the analyzer, consisting of display and communications with computer.

8. **Mita L, Forte M., Rossi A., Adamo C., Rossi S, Mita D., Guida M., Portaccio M., Godievargova T., Ivanov Y., Samir M. and Eldin M., Removal of 17-α- Ethinylestradiol from Water Systems by Adsorption on Polyacrylonitrile Beads: Isotherm and Kinetics Studies, Peertechz J Environ Sci Toxicol, 2017, 2(1): 048-058.** ***IF-2,491.***

Abstract: An investigation on the removal of 17-a-Ethinyl Estradiol (EE2) from aqueous solutions using Polyacrylonitrile (PAN) beads has been carried out under closed conditions. The kinetic and equilibrium results obtained for EE sorption with different initial concentrations have been analyzed. Experimental data at equilibrium have been correlated with the Langmuir, Freundlich, Tempkin, and Dubinin–Radushkevich (D–R) isotherm models. The applicability of the isotherm equations to the adsorption system has been compared by means of the correlation coefficients. The adsorption data resulted fitted well by the Freundlich isotherm model. Kinetic analysis was performed with three different types of kinetic adsorption models using the pseudo-first-order, pseudo-second-order, and simple Elovich models. Analysis of the kinetic data indicated that the EE2 adsorption was a second-order process. Diffusion mechanisms have been analyzed by means of the diffusion rate equations inside particulate of Dumwald–Wagner and intraparticle models. The actual rate-controlling step involved in the EE sorption process was determined by further analysis of the sorption data by the kinetic expression given by Boyd. All together these results allowed understanding the adsorption mechanism of the process and have shown the usefulness in using PAN beads in removing EE2 from synthetic aqueous solutions, also at concentrations higher than those measured in the environment.

9. **Forte, M., Mita, L., Perrone, R., Rossi S, Argirò M, Mita DG, Guida M, Portaccio M, Godievargova T, Ivanov Y, Tamer MT, Omer, A.M., Mohy Eldin, M.S. , Removal of methylparaben from synthetic aqueous solutions using polyacrylonitrile beads: kinetic and equilibrium studies,**

Environmental Science and Pollution Research, 2017, 24(2), 1270-1282. ***IF-2,800.***

Abstract: The removal of methylparaben (MP), a well-known endocrine disruptor, from aqueous solutions using polyacrylonitrile (PAN) beads has been studied under batch conditions, at room temperature and at different initial MP concentrations. The kinetic and equilibrium results have been analyzed. Kinetic modeling analysis has been carried out with three different types of adsorption models: pseudo-first-order, pseudo-second-order, and Elovich model. Kinetic data analysis indicated that the adsorption was a second-order process. The MP adsorption by PAN was also quantitatively evaluated by using the equilibrium adsorption isotherm models of Langmuir, Freundlich, Dubinin-Radushkevich (D-R), and Temkin and the applicability of the respective isotherm equations has been compared through the correlation coefficients. Adsorption data resulted well fitted by the Freundlich isotherm model. Data of MP adsorption have also been used to test different adsorption diffusion models. The diffusion rate equations inside particulate of Dumwald-Wagner and the intraparticle diffusion model have been used to calculate the diffusion rate. The actual rate-controlling step involved in the MB adsorption process was determined. The kinetic expression by Boyd gave the right indications. All together, our results indicate that PAN beads are a useful tool to remediate water bodies polluted by endocrine disruptors.

10. Vasileva, N., Ivanov, Y., Damyanova, S., Kostova, I., Godjevargova, T., Hydrolysis of whey lactose by immobilized β-galactosidase in a bioreactor with a spirally wound membrane, International Journal of Biological Macromolecules, 2016, 82, 339-346. ***IF-3,909.***

Abstract: The β-galactosidase was covalently immobilized onto a modified polypropylene membrane, using glutaraldehyde. The optimal conditions for hydrolysis of lactose (4.7%) by immobilized β-galactosidase in a batch process were determined 13.6 U enzyme activity, 40◦C, pH 6.8 and 10 h. The obtained degree of hydrolysis was compared with results received by a free enzyme. It was found, that the lactose hydrolysis by an immobilized enzyme was 1.6 times more effective than the lactose hydrolysis by a free enzyme. It was determined that the stability of the immobilized enzyme was 2 times higher in comparison with the stability of free enzyme. The obtained immobilized system β-galactosidase/polypropylene membrane was applied to produce glucose–galactose syrup from waste whey. The whey characteristics and the different preliminary treatments of the whey were investigated. Then the whey lactose hydrolysis in a bioreactor by an immobilized enzyme on a spirally wound membrane was performed. The optimal membrane surface and the optimal flow rate of the whey

through the membrane module were determined, respectively 100 cm^2 and 1.0 mL min^{-1}. After 10 h, the degree of lactose hydrolysis was increased to 91%.The operation stability was studied. After 20th cycle the yield of bioreactor was 69.7%.

11. **Dimcheva, N., Horozova, E., Ivanov, Y., Godjevargova, T., Self-assembly of acetylcholinesterase on gold nanoparticles electrodeposited on graphite, Central European Journal of Chemistry, 2013, 11(11), 1740-1748.** ***IF-1,425.***

Abstract: The immobilisation of AChE enzyme through chemisorption on Au-modified graphite was examined with view of its prospective application in the design of membraneless electrochemical biosensors for the assay of enzyme inhibitors. The developed immobilisation protocol has been based on a two-stage procedure, comprising: i) electrodeposition of gold nanostructures on spectroscopic graphite; followed by ii) chemisorption of the enzyme onto gold nanoparticles. Both the coverage of the electrode surface with Au nanostructures and the conditions for enzyme immobilisation were optimised. The proposed electrode architecture together with the specific type of enzyme immobilisation allow for a long-term retaining of the enzyme catalytic activity. The extent of inhibition of the immobilised acetylcholinesterase enzyme by the organophosphorous compound monocrotophos has been found to depend linearly on its concentration over the range from 50 to 400 nmol mL^{-1} with sensitivity 77.2% inhibition per 1 μmol mL^{-1} of monocrotophos.

12. **Gabrovska, K.I., Ivanova, S.I., Ivanov, Y.L., Godjevargova, T.I. , Immunofluorescent Analysis with Magnetic Nanoparticles for Simultaneous Determination of Antibiotic Residues in Milk, Analytical Letters, 2013, 46(10), 1537-1552.** ***IF-1,206.***

Abstract: An analytical system for simultaneous detection of three antibiotic residues in milk (penicillin, tetracycline, and sulfadimethoxine sodium salt) was developed. The method was based on a fluorescence immunoassay. Magnetic nanoparticles (MNPs) were prepared and then coated with 3-(aminopropyl) triethoxysilane (APTES). Antibodies, against penicillin, tetracycline, and sulfadimethoxine, were immobilized on APTES-MNPs. Two immobilization methods were used: a random and an oriented method by protein A. The fluorochrome-antigen conjugates, containing FITC, ATTO 590, and ATTO 630 were obtained. Three separate immunofluorescent analyses were investigated for

penicillin, tetracycline, and sulfadimethoxine, first in buffer and then in milk. Linearity values of standard curves in milk were: for penicillin 4–15 ng.mL^{-1}, for tetracycline 50–500 ng.mL^{-1} (using random immobilization) and 100–500 ng.mL^{-1}(using oriented immobilization), and for sulfadimethoxine 100–500 ng.mL^{-1}. The linearity and sensitivity of calibration curves of determination of penicillin, tetracycline and sulfadimethoxine in milk were very close to the obtained results for separate determination of three antibiotics. It was shown that the multiple immunofluorescence analysis for antibiotic residues in milk without preliminary treatment is effective and rapid.

13. **Ivanova S., Ivanov Y., Godjevargova T., Urea Amperometric Biosensors based on Nanostructured Polypyrrole and Poly Ortho-Phenylenediamine, Open Journal of Applied Biosensor, 2013, 2, 12-19, Web of Science.** ***IF-0,54***

Abstract: Urea Amperometric biosensor was obtained on the base of nanostructured polypyrrole (PPy) and poly ortho- phenylenediamine (POPDA). The optimal conditions for monomer electropolymerization were determined. The effect of supporting electrolyte and number of deposition cycles on the OPDA and Py electropolymerization were studied. It was proved that POPDA and PPy were affected by pH changes and responded to the ammonium, product of urease catalyzed reaction. SEM images of the modified Pt/PPy electrode were presented. The cycle voltammograms and chrono amperometric curves of Pt/POPDA/urease and Pt/PPy/urease electrodes were studied. A good linear relationship was observed for Pt/POPDA/urease electrode in a concentration range from 6.7 to 54 mM urea. For Pt/PPy/urease elec-trode the linear relation in the range from 0.02 to 0.16 mM urea was determined. The entrapped carbon nanotubes (CNT) in PPy film and the bipolymer layers were prepared for construction of Pt/PPy/CNT/urease, Pt/POPDA/PPy/urease and Pt/PPy/POPDA/urease biosensors. Obviously, the addition of POPDA to the composition of the two biosensors (Pt/PPy/POPDA/urease and Pt/POPDA/PPy/urease) reduced their sensitivity to urea. Pt/PPy/CNT/urease and Pt/PPy/ urease biosensors were 173 and 138 times more sensitive to urea than biosensor without PPy (Pt/POPDA/urease bio-sensor). It was found, that the performance of Pt/PPy/CNT/urease electrode was the best from the five obtained biosensors: linear range of urea concentrations—from 0.02 to 0.16 mM; sensitivity—15.22 μA/mM and detection limit— 0.005 mM urea.

14. **Vasileva, N., Iotov, V., Ivanov, Y., Godjevargova, T., Kotia, N., Immobilization of β-galactosidase on modified polypropilene membranes, International Journal of Biological Macromolecules 2012, 51(5), 710-719.** ***IF-3,909.***

Abstract: A new immobilized system: β-galactosidase-modified polypropylene membrane was created. It was obtained 13 different carriers by chemical modification of polypropylene membranes by two stages. The first stage is treatment with $K_2Cr_2O_7$ to receive carboxylic groups on membrane surface. The second stage is treatment with different modified agents ethylendiamine, examethylenediamine, hydrazine dihydrochloride, hydroxylamine, o-phenylenediamine, p-phenylenediamine, N,N-dibenzyl ethylenediamine diacetate to receive amino groups. The quantity of the amino groups, carboxylic groups and the degree of hydrophilicity of unmodified and modified polypropilene membranes were determined. β-Galactosidase was chemically immobilized on the obtained carries by glutaraldehyde. The highest relative activity of immobilized enzyme was recorded at membrane modified with 10% hexamethylenediamine (Membrane 5) – 92.77%. The properties of immobilized β-galactosidase on different modified membranes – pH opt, temperature optimum, pH stability and thermal stability were investigated and compared with those of free enzyme. The storage stability of all immobilized systems was studied. It was found that the most stable system is immobilized enzyme on Membrane 5. The system has kept 90% of its initial activity at 300^{th} day (pH = 6.8; 4◦C). The stability of the free and immobilized β-galactosidase on the modified membrane 5 with 10% HMDA in aqueous solutions of alcohols – mono-, diol and triol was studied. The kinetics of enzymatic reaction of free and immobilized β-galactosidase on the modified membrane 5 at 20 °C and 40 °C and at the optimal pH for both forms of the enzyme were investigated. It was concluded that the modified agent – hexamethylenediamine, with long aliphatic chain ensures the best immobilized β-galactosidase system.

15. **Ivanov, Y., Marinov, I., Portaccio, M., Lepore M., Mita, D.G., Godjevargova, T. Flow-injection system with site-specific immobilization of acetylcholinesterase biosensor for amperometric detection of organophosphate pesticides, Biotechnology and Biotechnological Equipment, 2012, 26(3), 3044-3053.** ***IF-0,622.***

Abstract: A flow-injection system with integrated amperometric biosensor featuring an easily replaceable immobilzed acetylcholinesterase (AChE) membrane was studied. The amperometric biosensor was constructed on the basis of site-specific immobilization of AChE on a hybrid polymer membrane with integrated multi-walled carbon nanotubes. Multistage modification of the membrane and

immobilization of the enzyme was proved by Fourier transform infrared spectroscopy. The optimum flow-rate of the flowinjection analysis (FIA) system was 0.5 mL/min. It gave a linear response to acetylthiocholine chloride from 2 μM to 100 μM, with an average RSD of 3.0% (n = 6). The sensitivity of the constructed biosensor was 0.093 $\mu A/\mu M \cdot cm^2$. The $K_{m\ app}$ value of the immobilized AChE was 1.15 mM and the linear correlation coefficient $R^2 = 0.9949$. The method had a low detection limit for three organophosphorus pesticides (OPs) in model pesticide solutions –paraoxon ethyl (0.9×10-12 M), monocroptophos (1.8×10^{-12} M) and dichlorvos (2.0×10^{-12} M). This indicated that the action of multi-walled nanotubes and controlled site-specific enzyme immobilization ensured high electrocatalytic activity and selectivity of the biosensor towards pesticides. It was found that the biosensor can be reused 15 operation cycles. After storage for 30 days the enzyme membrane retained over 80% of its initial response. The FIA system was used for detection of anti-cholinesterase activity of two binary OP mixtures. The results for paraoxon + monocroptophos and paraoxon + dichlorvos showed that the total inhibition activity was not simply additive, but was lower than the sum of the individual inhibition values. Moreover, the difference between the sum of the individual inhibition values and the real results for the mixture was bigger for the binary system paraoxon and dichlorvos (7-10%) compared with that for paraoxon and monocroptophos (5-7%). The developed biosensor system is an ideal tool for monitoring of organophosphate pesticides.

16. Marinov, I., Ivanov, Y., Vassileva, N., Godjevargova, T., Amperometric inhibition-based detection of organophosphorus pesticides in unary and binary mixtures employing flow-injection analysis, Sensors and Actuators, B: Chemical, 2011, 160(1), 1098-1105. ***IF-3,535.***

Abstract: The present work is focused on the application of an acetylthiocholine (ATCh) biosensor in a flowinjection system for the detection of organophosphorus pesticides. The optimal operating conditions of the flow-injection system were determined: flow-rate – 0.5 $mL.min^{-1}$, substrate concentration – 100 μM, incubation and reactivation time–10 min. A calibration plot was obtained for ATCh concentration ranging from 20 to 200 μM. A linear interval was detected along the calibration curve from 20 to 100 μM with a correlation coefficient $R^2 =$ 0.996. The sensitivity of the constructed biosensor was calculated to be 0.083 μA μM^{-1} cm^{-2}. The application of the flow-injection system for detection and quantification of three organophosphorus pesticides – paraoxon ethyl, monocrotophos and dichlorvos in unary solutions and in binary mixtures was investigated as well. The inhibition curves for each pesticide was plotted and the linear intervals were determined along with the corresponding equations and

detection limits – 0.87×10^{-11} M for paraoxon, 1.08×10^{-11} M for monocrotophos and 1.22×10^{-10} M for dichlorvos. The bimolecular inhibition constants ki were calculated by performing amperometric measurements of the residual enzyme activity after incubation for 10 min in a series of samples with varying pesticide concentrations (from 2 to 100 μM). The highest inhibition potency was observed for paraoxon (2.3×10^{5} M^{-1} min^{-1}), and the lowest – for dichlorvos (3.5×10^{4} M^{-1} min^{-1}). The flow-injection system was used in the detection of anti-cholinesterase activity of two binary mixtures – paraoxon + monocrotophos and paraoxon + dichlorvos. It was interesting to observe that the total anti-cholinesterase activity of the mixtures was lower than the anti-cholinesterase activity of paraoxon with the same concentration in the sample. The storage stability of the enzyme membrane was considerably improved with respect to our previous work. After storage for 30 days, the enzyme membrane retained over 90% of its initial response. The halflife storage time of the enzyme membrane (50% residual activity) was almost tripled – from 25 to 75 days.

17. Nicolucci, C., Rossi, S., Menale, C., Godjevargova T, Ivanov Y, Bianco M, Mita L, Bencivenga U, Mita, D.G., Diano, N., Biodegradation of bisphenols with immobilized laccase or tyrosinase on polyacrylonitrile beads, Biodegradation, 2011, 22(3), 673-683. ***IF-2,77.***

Abstract: The biodegradation of waters polluted by some bisphenols, endowed with endocrine activity, has been studied by means of laccase or tyrosinase immobilized on polyacrylonitrile (PAN) beads. Bisphenol A (BPA), Bisphenol B (BPB), Bisphenol F (BPF) and Tetrachlorobisphenol A (TCBPA) have been used. The laccase-PAN beads system has been characterized as a function of pH, temperature and substrate concentration. The biochemical parameters so obtained have been compared with those of the free enzyme to evidence the modification induced by the immobilization process. Once characterized, the laccase-PAN beads have been employed in a fluidized bed reactor to determine for each of the four bisphenols the degradation rate constant (k); the t_{50}, i.e., the time to obtain the 50% of degradation, and the removal efficiency (RE_{90}) after 90 min of enzyme treatment. The same parameters have been measured for each of the four pollutants with the same fluidized bed bioreactor loaded with tyrosinase-PAN beads. The internal comparison, i.e., in each of the two catalytic systems, has shown that both enzymes exhibit a removal efficiency in the following order BPF>BPA> BPB> TCBPA. The external comparison, i.e., the comparison between the two catalytic system, has shown that the catalytic power of laccase were higher than that of tyrosinase. The operational stability of both catalytic systems resulted excellent, since they maintained more than 80% of the initial activity after 30 days of work.

18. Velichkova, Y., Ivanov, Y., Marinov, I., Rajendran R., Kamini N. Dimcheva N., Horozova, E., Godjevargova T., Amperometric electrode for determination of urea using electrodeposited rhodium and immobilized urease T., Journal of Molecular Catalysis B: Enzymatic, 2011, 69(3-4), 168-175. *IF-2,753.*

Abstract: An amperometric biosensor was developed for determination of urea using electrodeposited rhodium on a polymer membrane and immobilized urease. The urease catalyzes the hydrolysis of urea to NH_4^+ and HCO_3^- ions and the liberated ammonia is catalytically and electrochemically oxidized by rhodium present in the rhodinized membrane on the Pt working electrode. Three types of rhodinized polymer membranes were prepared by varying the number of electrodeposition cycles: membrane 1 with 10 deposition cycles, membrane 2 with 40 cycles and membrane 3 with 60 cycles. The morphologies of the rhodinized membranes were investigated by scanning electron microscopy and the results showed that the deposition of rhodium was like flowers with cornices-like centers. The influence of the amount of electrodeposited rhodium over the electrode sensitivity to different concentrations of ammonia was examined initially based on the cyclic voltammetric curves using the three rhodium modified electrodes. The obtained results convincingly show that electrode with rhodinized membrane 1, which contain the lowest amount of electrodeposited rhodium is the most active and sensitive regarding ammonia. It was found that the anodic oxidation peak of ammonia to nitrogen occurs at 0.60 V. In order to study the performance of urease amperometric sensor for the determination of urea, experiments at constant potential (0.60 V) were performed. The current–time experiments were carried out with urease rhodinized membrane 1 (10 cycles). The amperometric response increased linearly up to 1.75 mM urea. The detection limit was 0.05mM. The urea biosensor exhibited a high sensitivity of $1.85\mu A.mM^{-1}\ cm^{-2}$ with a response time 15 s. The Michaelis–Menten constant K_m for the urea biosensor was calculated to be 6.5mM, indicating that the immobilized enzyme featured a high affinity to urea. The urea sensor showed a good reproducibility and stability. Both components rhodium and urease contribute to the decreasing of the production cost of biosensor by avoiding the use of a second enzyme.

19. Katya Gabrovska, Javor Ivanov, Ioana Vasileva, Nedyalka Dimova, Tzonka Godjevargova, Immobilization of urease on nanostructured polymer membrane and preparation of urea amperometric biosensor, International Journal of Biological Macromolecules, 2011, 48,620-626. *IF-2,453.*

Abstract: A new matrix for enzyme immobilization of urease was obtained by incorporating rhodium nanoparticles (5% on activated charcoal) and chemical

bonding of chitosan with different concentration (0.15%; 0.3%; 0.5%; 1.0%; 1.5%) in previously chemically modified AN copolymer membrane. The basic characteristics of the chitosan modified membranes were investigated. The SEM analyses were shown essential morphology change in the different modified membranes. Both the amount of bound protein and relative activity of immobilized enzyme were measured. A higher activity (about 77.44%) was measured for urease bound to AN copolymer membrane coated with 1.0% chitosan and containing rhodium nanoparticles. The basic characteristics (pH_{opt}, T_{opt}, thermal, storage and operation stability) of immobilized enzyme on this optimized modified membrane were also determined. The prepared enzyme membrane was used for the construction of amperometric biosensor for urea detection. Its basic amperometric characteristics were investigated. A calibration plot was obtained for urea concentration ranging from 1.6 to 23mM. A linear interval was detected along the calibration curve from 1.6 to 8.2mM. The sensitivity of the constructed biosensor was calculated to be 3.1927$\mu A.mM^{-1}$ cm^{-2}. The correlation coefficient for this concentration range was 0.998. The detection limit with regard to urea was calculated to be 0.5mM at a signal-to-noise ratio of 3. The biosensor was employed for 10 days while the maximum response to urea retained 86.8%.

20. Ivanov, Y., Marinov, I., Gabrovska, K., Dimcheva, N., Godjevargova, T., Amperometric biosensor based on a site-specific immobilization of acetylcholinesterase via affinity bonds on a nanostructured polymer membrane with integrated multiwall carbon nanotubes integrated multiwall carbon nanotubes, Journal of Molecular Catalysis B: Enzymatic, 2010, 63(3-4), 141-148. ***IF-2,33.***

Abstract: Acetylcholinesterase (AChE) was immobilized on chemically modified poly-(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) membranes in accordance with three different methods, the first of which involved random enzyme immobilization via glutaraldehyde, the second one—site-specific enzyme immobilization via glutaraldehyde and Concanavalin A (Con A) and the third method—modified site-specific enzyme immobilization via glutaraldehyde in the presence of a mixture of multiwall carbon nanotubes and albumin (MWCNs+ BSA), glutaraldehyde and Con A. Preliminary tests for the activity of immobilized AChE were carried out using these three methods. The third method was selected as the most efficient one for the immobilization of AChE and the prepared enzyme carriers were used for the construction of amperometric biosensors for the detection of acetylthiocholine (ATCh). A five level three factorial central composite design was chosen to determine the optimal conditions for the enzyme immobilization with three critical variables: concentration of enzyme, concanavalin A and MWCNs. The design illustrated that the optimum values of the

factors influencing the amperometric current were CE: 70U.mL^{-1}; $C_{Con\ A}$: 1.5mg.mL^{-1} and C_{MWCN}: 11mg.mL^{-1}, with an amperometric current 0.418 μA. The basic amperometric characteristics of the constructed biosensor were investigated. A calibration plot was obtained for a series of ATCh concentrations ranging from 5 to 400 μM. A linear interval was detected along the calibration curve from 5 to 200 μM. The correlation coefficient for this concentration range was 0.995. The biosensor sensitivity was calculated to be 0.065μA. μM^{-1} cm^{-2}. The detection limit with regard to ATCh was calculated to be 0.34 μM. The potential application of the biosensor for detection and quantification of organophosphate pesticides was investigated as well. It was tested against sample solutions of Paraoxon. The biosensor detection limit was determined to be 1.39×10^{-12} g.L^{-1} of Paraoxon, as well as the interval (10^{-11} to 10^{-8} g.L^{-1}) within which the biosensor response was linearly dependant on the Paraoxon concentration. Finally the storage stability of the enzyme carrier was traced for a period of 120 days. After 30-day storage the sensor retained 76% of its initial current response, after 60 days—68% and after 120 days—61%.

21. Marinov, I., Ivanov, Y., Gabrovska, K., Godjevargova, T. , Amperometric acetylthiocholine sensor based on acetylcholinesterase immobilized on nanostructured polymer membrane containing gold nanoparticles, Journal of Molecular Catalysis B: Enzymatic, 2010, 62(1), 66-74. *IF-2,33.*

Abstract: Poly(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) membranes were chemically modified and loaded with gold nanoparticles. Acetylcholinesterase was immobilized on the prepared membranes in accordance with two distinctive procedures, the first of which involved immobilization of the enzyme by convection, and the other by diffusion. The prepared enzyme carriers were used for the construction of amperometric biosensors for detection of acetylthiocholine. Two sets of experiments were carried out. The first set was designed so that to evaluate the effects of the gold nanoparticle deployment and the immobilization procedures over the biosensor effectiveness. The other set of experiments was conducted in order to determine the influence of the individual components of the enzyme mixture, containing gold nanoparticles, acetylcholinesterase, bovine serum albumin and glutaraldehyde, over the current output of the constructed acetylthiocholine biosensors. The optimum composition of the mixture was determined to be as follows: enzyme, 0.1U.ml^{-1}; gold nanoparticles, 0.50 ml (per 1ml enzyme mixture); albumin, 0.5% and glutaraldehyde, 0.7%. On the basis of the experimental results, the most efficient enzyme membrane was selected and used for the preparation of an acetylthiocholine biosensor. Its basic amperometric characteristics were

investigated. A calibration plot was obtained for ATCh concentration ranging from 10 to 400 μM. A linear interval was detected along the calibration curve from 10 to 170μM. The sensitivity of the constructed biosensor was calculated to be 0.066 $\mu A.\mu M^{-1}$ cm^{-2}. The correlation coefficient for this concentration range was 0.996. The detection limit with regard to ATCh was calculated to be 1.80 μM. The potential application of the biosensor for detection and quantification of organophosphate pesticides was investigated as well. It was tested against sample solutions of Paraoxon. The biosensor detection limit for Paraoxon was determined, 7.39×10^{-11} g l^{-1}, as well as the concentration interval (10^{-10} to 10^{-7} g l^{-1}) within which the biosensor response was linearly dependant on Paraoxon concentration. Finally the storage stability of the enzyme carrier was traced for a period of 50 days. After storage for 20 days the sensor retained 75% of its initial current response and after 30 days −25%.

Научна публикация в нереферирани списания с научно рецензиране или в редактирани колективни томове

22. Ivanov Y., Godjevargova T., Immunochemical Assays for Determination of Organophosphorus Pesticides in Milk, Dairy and Vet Sci J 11(3): JDVS.MS.ID.555811, 2019, ISSN: 2573-2196, Open access.

Abstract: The detection of pesticide residues is an important task in ensuring the safety of milk. In the last decade, organophosphorus insecticides have been used, but they also pose a serious risk because of their high toxicity. For fast screening of milk, immunochemical methods of analysis are appropriate. Enzyme-linked immunosorbent assay is the most common assay mode and base of many commercialized assay kits. Residues of some organophosphorus compounds in milk and dairy products determined by ELISA are shown. For fast screening of pesticides, the lateral flow strips are also described. The advantages of a new developed method MNPs-based immunoassay for determination of phosphorus pesticides in milk was described and some publications were represented. In recent years, simultaneous identification of more than one pesticide is increasingly perceptual, that way the examples for multi-immunoassays for determination of organophosphorus pesticides was presented.

23. Becheva Z., Ivanov Y., Godjevargova T., Fluorescence immunassays for staphylococcal Enterotoxin A, Proceedings of 159th The IRES International Conference, 18-23, Barcelona, Spain, 11th-12th April, 2019, ISBN 978-93-88786-88-1, www.worldresearchlibrary.org

Abstract: Staphylococcal food poisonings are one of the main food-borne outbreaks worldwide. Staphylococcal enterotoxins are thermostable exotoxins that contaminate a wild range of foods, like milk and meat. The most common of the enterotoxins is staphylococcal enterotoxin A (SEA). In the present study fluorescence immunoassays for SEA were developed.Two fluorescent conjugates were prepared, one with SEA and the other with the SEA-specific polyclonal antibody. They were used as fluorescent markers in two different immunoassays: fluorescent linked immunosorbent assay (FLISA), and magnetic nanoparticle (MNP) – based fluorescence immunoassay (FIA). The linear range of the FLISA wasfrom 0.01 pg/mL to 1 μg/mL SEA, whereas the MNP-based FIA had linearityfrom 0.5 pg/mL to 10 ng/mL SEA, both in buffer solutions.However, the direct FLISA method is time-consuming (more than 2 h), thus the developed MNP-based FIA is preferable (30 min). That is due to the large relative surface area of the MNPs which facilitate the immunoreactions (Ab-SEA). The assay with MNPs was used for SEA detection in milk samples. It was found that the linear range of SEA in milk samples was almost the same.

24. Atanasova M., Ivanov Y., Study of the metabolic activity of Saccharomyces yeasts in brewing and bioethanol industry, Proceedings of 159th The IRES International Conference, Barcelona, Spain, 24-28, 2019, ISBN 978-93-88786-88-1, www.worldresearchlibrary.org

Abstract: The metabolic activity of brewing yeasts and yeasts used in bioethanol production was investigated using carboxyfluorescein diacetate (cFDA). cFDA penetrates the cell membrane, then active esterase enzymes inside the cell cleaved acetate residues, and the released fluorescein stains the cell in green. The optimal pH of two type of cell staining was determined – pH 4. The optimal temperature for esterase hydrolization of cFDA of brewing cells was 37 ^{0}C, and for bioethanol cells – 40^{0}C. The cFDA optimal concentration for metabolic activity determination of the brewing yeast was found to be 50 μg/ml, and for yeasts in bioethanol production was found to be 200 μg/ml. The incubation time for cell staining was varied with both the strains. It is noteworthy that at 5 minutes the number of stained cells of the brewing yeast Saccharomyces carlsbergensis is 2 times greater than that of the bioethanol producing species. At 10 minutes for both strains the number of stained cells reaches the maximum. At 20 minutes, the released fluorescein from esterase hydrolysis of cFDA begins to leak from the brewing yeast cells and their count decreases. For bioethanol cells the leakage begins at 40 minutes. The metabolic activity of cells during the cultivation of two investigated strains was studied. The same samples were simultaneously tested for determination of total cell count, dead cell count and viable cell count. The results convincingly show that for different cell types the penetration of cFDA into the

cells and the leakage of the released fluorescein is different and the conditions of the assay need to be specified.

25. IvanovY., Atanasova M., Godjevargova T., Yeast viability assessment during bioethanol production, Proceedings of 159th The IRES International Conference, Barcelona, Spain, 29-33, 2019, ISBN 978-93-88786-88-1, www.worldresearchlibrary.org

Abstract: The viability of cell suspensions from bioethanol production was studied, using new fluorescent dye Sofia Green. The DNA binding dye Sofia Green was compared to one of the most commonly used nucleic acid binding dyes propidium iodide. The comparison of emission spectra of Sofia Green and propidium iodide before and after interaction with DNA was made. The emission intensity of Sofia Green before binding to DNA was 9 time lower than emission intensity of propidium iodide. The emission amplification of Sofia Green after the interaction with DNA was 15 times more than emission amplification of propidium iodide. The new dye has a low background and high emission amplification. Sofia Green dye was used to stain samples taken from Fermentor 3 of local factory for bioethanol production. The fermentation was carried out with corn mash and Saccharomyces cerevisiae yeast with high ethanol tolerance. It can be seen that the observed impurities from corn mash were not stained with Sofia green and were not visible on the microscopic fluorescence image. Propidium iodide had a significant background and impurities from corn mash were stained and give false positive results. A series of experiments were carried out to determine the total count, the count of dead cells and cell viability in samples taken from bioethanol production, from the pre-fermentor and from seven consecutive fermentors using fluorescence image cytometer EasyCounnter YC and Sofia Green dye. The highest viability was observed in the pre-fermentor - 84.3%. Then viability gradually decreases with the increasing number of fermenters from 81% to 58%, in the last fermenter. From first to fourth fermenter the viability decreased only with 5%. The results showed that the Sofia Green dye can be used for direct yeast cell staining in corn mash samples and give precise cell viability results.

26. Ivanov Y., Staining of yeast cells with different fluorescent dyes, SCIENCE AND TECHNOLOGIES, IX, 2019.

Abstract: In order to optimize fermentation processes, it is important to characterize the physiological and metabolic activity of the yeast cells throughout the procedure. Four fluorescent dyes were compared: red fluorescent dyes propidium iodide (PI) and thiazole orange cyanide (TO3CN) and green fluorescent dyes thiazole orange (TO) and new cyanine dye Sofia Green. The absorbance spectra were observed and the emission spectra before and after binding to DNA

were compared. The excitation and emission peak for each dye was determined. The rate of emission enhancement after binding to DNA was established. The dyes were combined and applied for live/dead staining of *Saccharomyces cerevisiae cells* and monitored by fluorescence microscopic image. Propidium iodide and Sofia Green dyes stained only dead cells. TO and TO3CN stained both dead and live cells. A double staining procedure was carried out. Propidium iodide was used in combination with TO dye and Sofia Green combined with TO3CN dye. The optimal concentration of the dyes for the cell staining was determined: 4 μg/ml for PI, 1 μg/ml for TO, 0.1 μg/ml for Sofia Green and 10 μg/ml for TO3CN. The optimal incubation time for cell staining was evaluated. A drop from the suspension was applied on a slide and was observed under a fluorescence microscope. The use of such combination of fluorochromes that selectively permeate in live or dead cells could lead to the development of rapid procedure for yeast viability investigation during fermentation in different industrial and food processes.

27. Atanasova M., Ivanov Y., Gabrovska K., Determination of the number and viability of white blood cells in capillary and venues blood, SCIENCE AND TECHNOLOGIES, IX, 2019.

Abstract: Determination of the number of white blood cells (WBCs) and their viability in capillary or venous blood is an important indicator for clinical diagnosis of patients. A method has been developed to determine the cell number and viability of white blood cells in fresh capillary or venous blood by a new EASYCOUNTER BC fluorescence microscope (produced by Milkotronic LTD). Concentration and viability of WBCs in capillary and venous blood can be measured without the removal of red blood cells from the sample, as opposed to the often used microscopic counting in which the erythrocyte lysis is obligatory condition. A new DNA dye Sofia Green is used. The dye permeates only in dead cells because their cell membrane is compromised. Living cells do not stain because their cell membrane is intact. Therefore, in order to determine the total number of leukocytes, it is necessary to add white blood cell lysis solution to make them permeable to the Sofia Green dye. In this case all white blood cells are stained by the Sofia Green dye. The optimal working range for measuring WBCs with the EASYCOUNTER BC was determined. The coefficient of variation (CV, %) of the results obtained with EASYCOUNTER BC in the optimal working range varied between 3-4%. For comparison the CV of the results obtained by microscopic counting was significantly higher, in the range 12-26%. This shows that the EASYCOUNTER BC fluorescence microscope possess better reproducibility and accuracy and could be used for rapid clinical diagnosis.

28. Ivanov Y., Determination of total number of somatic cells and bacteries in raw milk by automatic fluorescent imaging cytometr LACTOSCAN SCC, SCIENCE AND TECHNOLOGIES, IX, 2019.

Abstract: The total number of somatic cells (SCC) is a recognized indicator of cow health and milk quality. SCC is an indicator of the presence of infection and inflammation of the milk of the dairy cows, called mastitis. The total number of bacteria in milk is also an important indicator of milk quality. Individual methods for the express and objective diagnosis of somatic cells and bacteria in milk have been developed. Methods were performed using Lactoscan SCC fluorescence imaging cytometer. The Lactoscan SCC is portable, using LED optics and CCD capture technologies. To stain the somatic cells and bacteria, a new fluorescent DNA dye Sofia Green, which has high emission intensity and a very low background, is used. A comparison of the new fluorescent dye with the commercial dye propidium iodide was made. Optimal conditions for determining the total number of somatic cells and bacteria - concentrations of lysis buffer and fluorescent dye were determined. An important factor in determining the number of bacteria is the clarification of the milk sample. Different methods are used to clarify the milk - chemical, enzymatic, physical methods. The purpose of this treatment is to lysate somatic cells, to dissolve fat globules and proteins and to prepare bacterial cells to penetrate the dye to bacterial cell DNA. The degree of clarification of the milk sample is monitored by measuring its optical density. The coefficient of variation of all results has been calculated. It moves in the range of 2-6%. The data obtained convincingly shows the good technical capabilities of the new apparatus for determining the total number of somatic cells and the total number of bacteria.

29. Godjevargova T., Ivanov Y., Atanasova M., Becheva Z., Zherdev A., Magnetic nanoparticles based fluorescence immunoassay for food contaminants, Food Science and Applied Biotechnology, 2019, 2, (1), 38-45.

Abstract: Nanotechnology provides exciting new possibilities for advanced development of new analytical tools and instruments for bioanalytical applications. Magnetic nanoparticles (MNPs) have attracted much research interest in the past decade because they have good biocompatibility and can be readily separated from reaction mixtures with the aid of an external magnetic field. The heterogenic fluorescent immunoassays for determination of different analytes (antibiotics, pesticides, progesterone, aflatoxins, enterotoxins) using MNPs were developed. MNPs were prepared by thermal co-precipitation of Fe2+ and Fe3+ in aqueous solution. MNPs were functionalized with (3-aminopropyl) triethoxysilane. Two

different analyses were made for proving the successful modification of MNPs – FTIR and TEM. The corresponding antibodies were immobilized on modified MNPs. Then corresponding antigen - fluorescent dye were synthesized. The next step was purification of resulting conjugates by gel filtration chromatography and proving by UV-Vis and Fluorescence analyses. The 9 different separate competitive MNPs based immunoassays were developed – for penicillin, sulphonamide, tetracycline, progesterone, aflatoxin M1 and B1, enterotoxin A, paraoxon and dichlorvos. Then multiplex fluorescent immunoassay device based on magnetic nanoparticles for determination of penicillin, sulphonamide and tetracycline in milk sample was developed. Device ensured fast, selective and cheap antibiotic analysis. Very high analytical characteristics for all three antibiotics were received - very low LODs and wide linear range. Practical applications: The developed immunofluorescent methods are used for determination of low toxin concentrations in foods. Magnetic nanoparticles used as carrier for antibody immobilization accelerated the mass-exchange processes and reduced the analysis time. Combining immunofluorescence analysis with an automated device makes the method very practical for ensured food safety.

30. Yaneva M., Ivanov Y., Godjevargova T., Zvereva E., Immunofluorescence assay of pesticides on the base of immobilized multi-polyclonal antibody, Scientific works of university of food technologies, Food Science and Applied Biotechnology, 2019, 2, (1), 46-53.

Abstract: The sensitive competitive immunofluorescence method for simultaneous determination of paraoxon and dichlorvos with immobilized multi-polyclonal antibody on magnetic nanoparticles was developed. The multi-polyclonal antibody was obtained after the immunization of mixture of two prepared immunogens dichlorvos-cBSA and paraoxon-BSA (1:1). The immunogens dichlorvos-cBSA and paraoxon-BSA were synthesized preliminary. Multi-polyclonal antibody against dichlorvos and paraoxon was covalently coupled on magnetic nanoparticles. The competitive fluorescence conjugates dichlorvos-cBSA-FITC and paraoxon-BSA-ATTO 620 were synthesized. Two typical calibration curves of immunofluorescence assay for determination of dichlorvos and paraoxon in buffer solutions were obtained. The linear interval from 2 to 200 $ng.mL^{-1}$ for these two pesticides was determined. Then the calibration curves for dichlorvos and paraoxon were obtained in cow milk solutions. The linear range of pesticides in cow milk was determined (from 5 to 300 $ng.L^{-1}$) and the detection limit for paraoxon (3.5 $ng.mL^{-1}$) and dichlorvos (4 $ng.mL^{-1}$) was found. The obtained results for cow milk samples were compare with results in UHT, pasteurized cow milk,

sheep and goal milk. Quite different are the results when analyzing paraoxon and dichlorvos in standard solutions prepared in sheep's milk. The linear working range for the two pesticides is between 7 and 300 ng.mL^{-1} .It is obviously, that there is a shift of all analytical characteristics up to higher values. The reason for this is the high fat content of sheep's milk. The obtained results were showed that the developed method was 2 time more sensitive than method with the results obtained with mixture (1:1) of two separated antibodies – anti-paraoxon and anti-dichlorvos. These results confirmed the potential of the immunoassay for quantitative simultaneous screening of both dichlorvos and paraoxon. Practical applications: The use of immunofluorescence assay based on immobilized multi-polyclonal antibody on magnetic nanoparticles allows us to detect simultaneous paraoxon and dichlorovos in raw milk and other dairy products.

31. Atanasova M., Ivanov Y,.Godjevargov L, Godjevargova T., Preparation of functionalized magnetic nanoparticles, Annual of Assen Zlatarov University, Burgas, Bulgaria, 2018, v.XLVII 84-88.

Abstract: Magnetic nanoparticles (MNPs) have gained a lot of attention in biomedical and industrial applications due to their biocompatibility, easy of surface modification, high surface area, large surface-to-volume ratio and easy separation under external magnetic fields. In this work magnetic nanoparticles were prepared using thermal co-precipitation of Fe^{2+} and Fe^{3+} in aqueous solution. The influence of pH, and stirring rate was observed. The stirring rate was varied from 1300 to 1500 rpm and pH was varied from 13 to 14. The mean size of the particles was determined by transmission electron microscopy (TEM) analysis. The selected kind of particles were functionalized using three different modifying agents: (3-aminopropyl)triethoxysilane (APTES); tetraethyl orthosilicate (TEOS) and APTES; sodium oleate and chitosan. Amino groups after functionalization were determined by colorimetric method using Traut's and Ellman's reagents. The modifications of nanoparticles were proved by Fourier-transform infrared spectroscopy and by differential scanning calorymetry analysis. The mean size of modified MNPs was determined by TEM analysis. Bovine serum albumin was immobilized on the surface of the functionalized MNPs. The protein immobilization capacity of the MNPs was evaluated, as the concentration of bovine serum albumin was measured before and after immobilization by the Bradford method. MNPs functionalized with APTES turned out to be the most appropriate carrier for protein immobilization. APTES-functionalized MNPs showed a high degree of homodispersity, high protein immobilization capacity and possibility to performing an assay in pseudo-homogeneous mode.

32. Becheva Z., Ivanov Y., Godjevargova T., Comparison of Alexa 488, DR110 and FITC conjugated to antibody for microscopic assays, PROCEEDINGS, Biotechnologies and food technologies, 2018, 57, 10.2, 194-198, Reports Awarded with "Best Paper" Crystal Prize'18.

Abstract: The fluorescent dyes DR110 and Alexa 488 were obtained. Synthetic fluorescent dyes that are conjugated to antibodies are useful tools in microscopic imaging. Alexa 488, DR110 and fluorescein 5(6)-isothiocyanate (FITC) were compared in applications using various conjugates with anti-sheep IgG antibody. Antibody-fluorescent dye conjugates with variety degree of labelling were obtained. Their fluorescence characteristics were observed by fluorescence spectrophotometer and fluorescence microscope. Brightness, photobleaching and background of the fluorescent conjugates were examined. Alexa 488 labeled antibody has brighter fluorescence and negligible photobleaching and background in microscopic assays, then DR110 and last FITC dye.

33. Becheva Z, Gabrovska., K., Ivanov Y. Enhancement of immunoassay`s fluorescence and detection sensitivity of neutrophils by using antibodies multiple labelled with dye/DNA conjugate, Annual of Assen Zlatarov University,Burgas, 2017, XLVI (I), 31-36.

Abstract: A simple method of attaching multiple fluorescent labels on an antibody with a dye/DNA conjugate to increase the immunoassay sensitivity was suggested. In the work, bovine neutrophil fragments adsorbed on the surface of a 96-well plate were detected by its immunoreaction with biotinylated anti-bovine neutrophil antibody. A 30 base pair double-stranded oligonucleotide terminated with biotin was attached to the antibody through the biotin/streptavidin/biotin interaction. Multiple labeling of the antibody was achieved after a fluorescent DNA probe was added into the solution and bound to the oligonucleotide. By comparison with fluorescein-labeled streptavidin, the assay with the dye/DNA label produced up to 2-fold increase in fluorescence intensity, and consequently about 7-fold lower detection limit. The multiple labeling method uses readily available reagents, and is simple to implement.

34. Atanasova M., Ivanov Y., Studing of characteristic of fluorescent dye YO-Dam-1. PROCEEDINGS OF UNIVERSITY OF RUSE, секция Biotechnology and food technology, 2015, 54, 10.2, 70-74.

Abstract: The absorption and emission characteristics of new fluorescent dyes were determined. YO-Dam-1 has an absorption maximum at 478nm.The complex YO-Dam-1-DNA has an emission maximum at 511nm and a higher fluorescent

intensity compared with the dyes propidium iodide and YOYO 1. The rate of binding of the YO-Dam-1 DNA is very large. Equilibrium is achieved after 30s.

35. Ivanov Y., Gabrovska K., Godjevargova T., Determination of Organophosphorous Pesticide in fruit samples using a nanostructured Acetylcholinesterase Amperometric biosensor, PROCEEDINGS OF UNIVERSITY OF RUSE, 2014, 53, 10.2, 14-19.

Abstract: Organophophorous pesticides are some of the most widely used insecticides in citrus fruit, tomato and apple cultures. Biosensors based on the inhibition of acetylcholinesterase have been used for detection of pesticides in different samples. Due to its permitted use in such cultures, the methodology proposed was applied in order to evaluate the occurrence of matrix effects in the electroanalytical determination of paraoxon residues directly in the samples from apple, tomato and orange, without pretreatment or clean up steps. The results show that the biosensor has the potential for monitoring of pesticides in foods.

36. Иванова С., Иванов Я., Габровска К., Годжевъргова Ц., Получаване на модифицирани магнитни наночастици и приложението им за имобилизация на биоагенти, Научни трудове на Русенския университет, 2012, 51, 9.2, 59-64.

Abstract: Preparation of modified magnetic nanoparticles and their application for immobilization of bioagents. Modified magnetic nanoparticles with chitosan and 3-aminopropyltriethoxysilane were obtained.The amount of aminogroups on the nanoparticle surface was determined.The optimal concentration of two modified agents wasestablished that provides the highest degree of immobilization of albumin.

37. Gabrovska K., Marinov I., Ivanov Y., Comparative evaluation of the effectiveness of the immobilized acetylcholinesterase onto PAN membranes and electrospun nanofibres, 2009, Proceeding LVI, (1), 319-324, Plovdiv.

Abstract: The two different modifie,d with chitosan carrfers - PAN membranes(PANCHl) and PAN electrospun nanofibres (PANnfCHI) for acetylcholinesterase (AChE) immobilization were used. Both the amount of bound protein and relative activity of immobilized enzyme were measured. A higher activity (about 87%) was measured for AChE bound to PANnfCHl. The basic characteristics (pHopt, Topt, thermal, storage and operation stability) of immobilized enzyme were also determined. The obtained resu/fs indicated that both PANnfCHI and PANCHI membrane are suitable carriers for AC\E immobitization.

38. Габровска К., Василева Н., Маринов И., Иванов Я., Годжевъргова Ц., Изследване на работните характеристики на биосензор с имобилизирана ацетилхолинестераза за определяне на концентрацията на пестициди, Научни трудове, РУ" А. Кънчев", 2009, 48, 9, 148- 153.

Abstract: Study of operating characteristics of biosensor with immobilized Achetylcholinesterase for detection of pesticide concentration: Polyacrylonitrile (PAN) membrane, modified with NaOH and with entrapped gold nanoparticals (GNPs) was used for immobilization of acetylcholinesterase (AChE). The biosensor for determination of pesticides was constructed from this immobilized system. The electrochemical behaviors of PAN + GNPs + AChE/Pt electrode (voltamperometric curve; amperometric response and amperometric current-time) was studied. The calibration plot of acetylthiocholin and Paraoxon was investigated. The inhibition of AChE by different concentration of Paraoxon was determined.