ABSTRACT

Master`s degree work Tretiak D. V. on “Urea determination biosensor based on inorganic heterostructures” – K.: Igor Sikorsky Kyiv Polytechnic Institute, 2017. 114 p., 18 fig., 36 tab., literature – 38.

Actuality consists in the necessity to develop a simple, easy to use, accurate and inexpensive device to measure urine in analytical samples in various application fields.

Research purpose is detection of the possibilities of using the biosensor based on enzyme immobilized on a solid inorganic carrier as a device for measuring the urea content in samples.

Object of research is biosensor based on enzyme immobilized on a solid inorganic carrier as a device for measuring the urea content in samples.

Urea is widely distributed in nature and its analysis has considerable interest in clinical practice and agricultural chemistry. It is known to be an important marker for evaluating uremic toxin levels. The normal level of urea in serum is from 15 to 40 mg/dl (2.5–7.5 mM/l). In patients suffering from renal insufficiency, urea concentrations in serum vary from 180 to 480 mg/dl and, at increasing levels above 180mg/dl, hemodialysis is required. Apart from clinical applications, urea monitoring is used in food science and environmental monitoring. As the principal component of non-protein nitrogen in cow milk, urea is utilized as an indicator of protein-feeding efficiency. Urea also plays a strategic role in the marine nitrogen cycle, including sources of excretion by invertebrates, fish and bacteria decomposition. Urea estimation is important during environmental monitoring.

Many methods are available for urea estimation, including gas chromatography, calorimetry, and fluorimetry. However, these methods suffer from complicated sample pretreatment steps and are unsuitable for on-site monitoring. It is predicted that biosensors can be utilized to overcome some of these problems.

Biosensors (Fig. 1) are analytical devices that incorporate biological materials such as enzymes, nucleic acids (DNA and RNA), microorganisms, whole cells,

antibodies, cell receptors or biologically derived materials, which in most cases are specific to an analyte in intimate contact with a physico-chemical transducer.

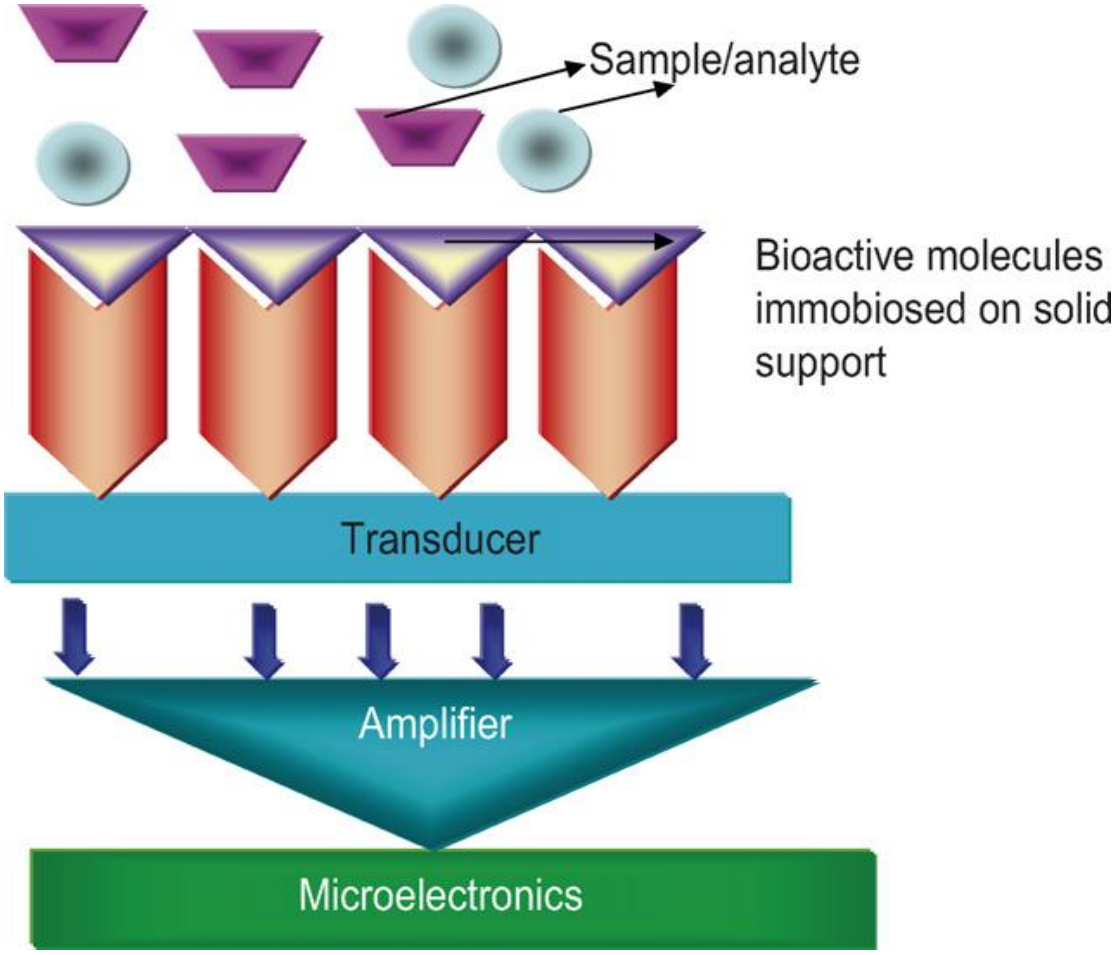

Figure 1. Chart of biosensor

Enzymatic biosensors (Fig. 2) utilize the biospecificity of an enzymatic reaction. Enzymatic urea biosensors utilize biochemical reactions. In other words, analyte and enzyme result in a product that can be detected and quantified using a transducer (amperometric/ potentiometric/optical thermal/piezoelectric).

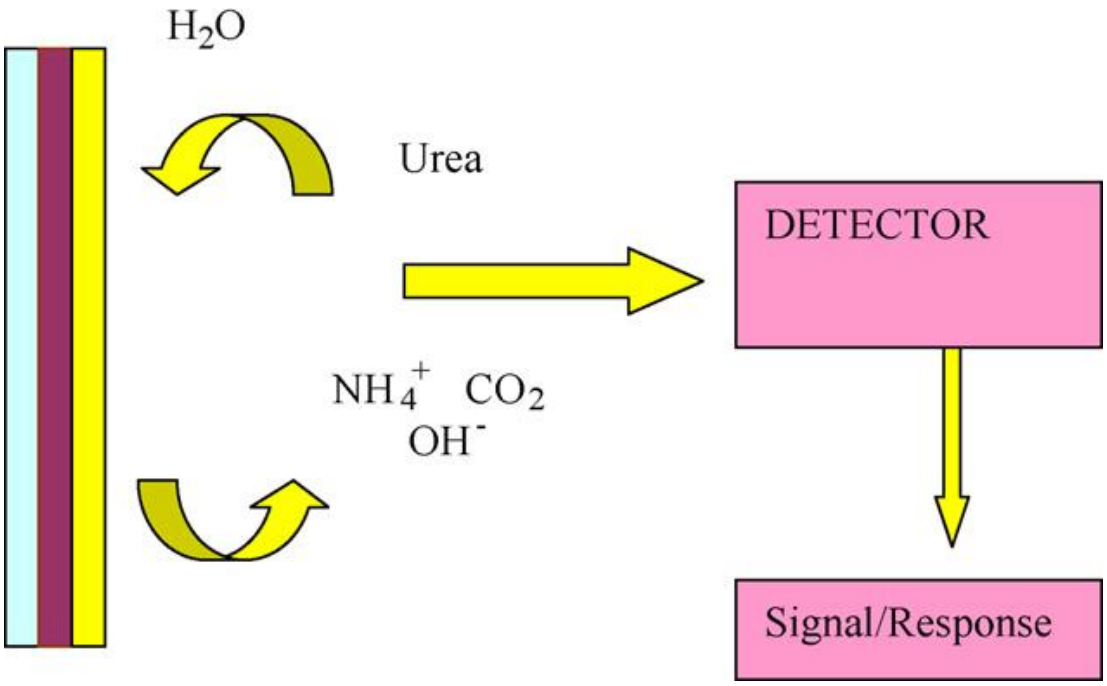

Figure 2. Schematic of a typical urea biosensor

Measuring scheme of investigated urea biosensor has the following form (Fig. 3).

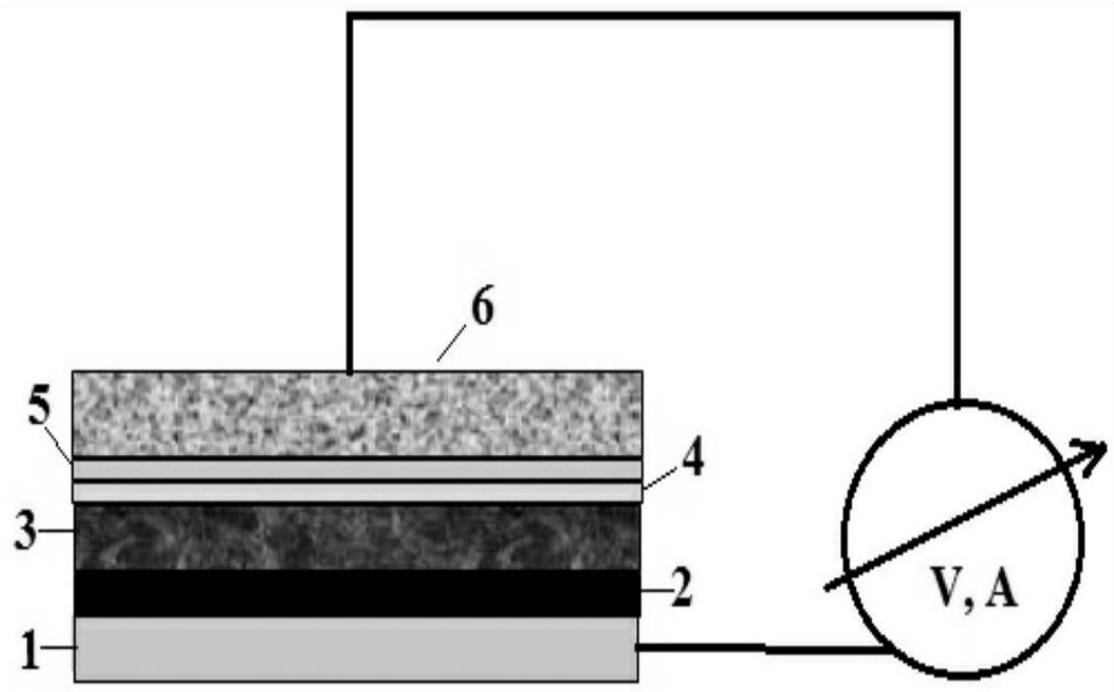

Figure 3. Measuring scheme of investigated urea biosensor

Au - electrode is used as a current collector to the enzyme electrode modified by heterostructure deposited on a substrate of graphene. Kits using immobilized inorganic carrier are moistened profusely with a solution of KCl, for electrolytic contact.

Analytical signal values are recorded as current density in the cell over the time, which is installed in the system after successive adding to the system heterostructure - graphene aliquots of urea with different concentrations.

Inorganic materials of various origin, nature and structure are used as carriers for enzyme immobilization. These are natural silicates and aluminosilicates, namely bentonite, the same modified by phosphate - ions, kaolin and aerosil.

There has been a series of experiments on measuring response curves of 3% solution of urea investigated solid carriers.

In response curve of 3% solution of urea biosensors based on kaolin (Fig. 4) and aerosil (Fig. 5) stable signal response is not observed, so subsequent study, they did not participate. In response curve of 3% solution of urea biosensors based on bentonite (Fig. 6) and modified bentonite (Fig. 7) has a steady response, so biosensors with the above solid carriers have been selected for calibration to determine disability.

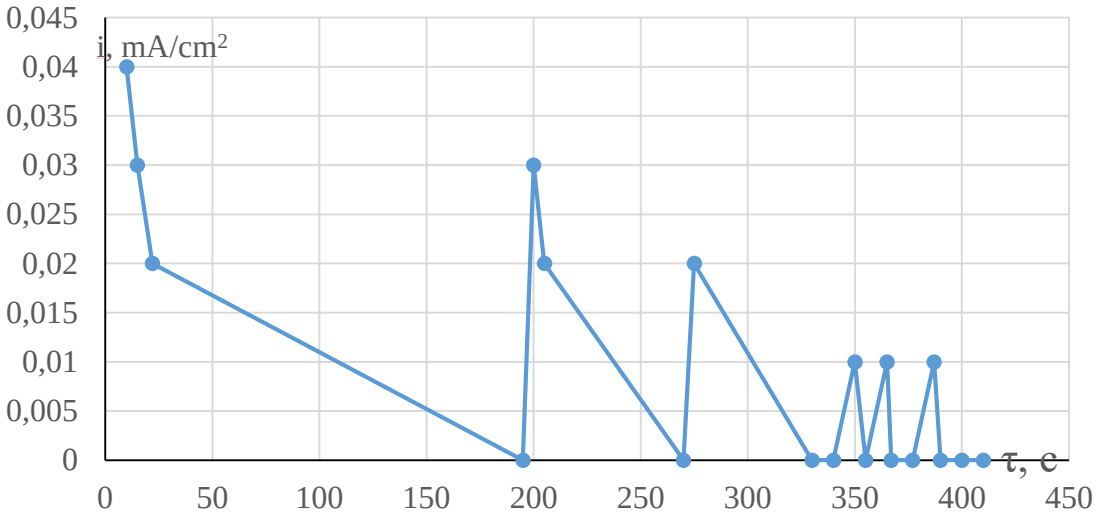

Figure 4. Response curve of 3% solution of urea biosensors based on kaolin

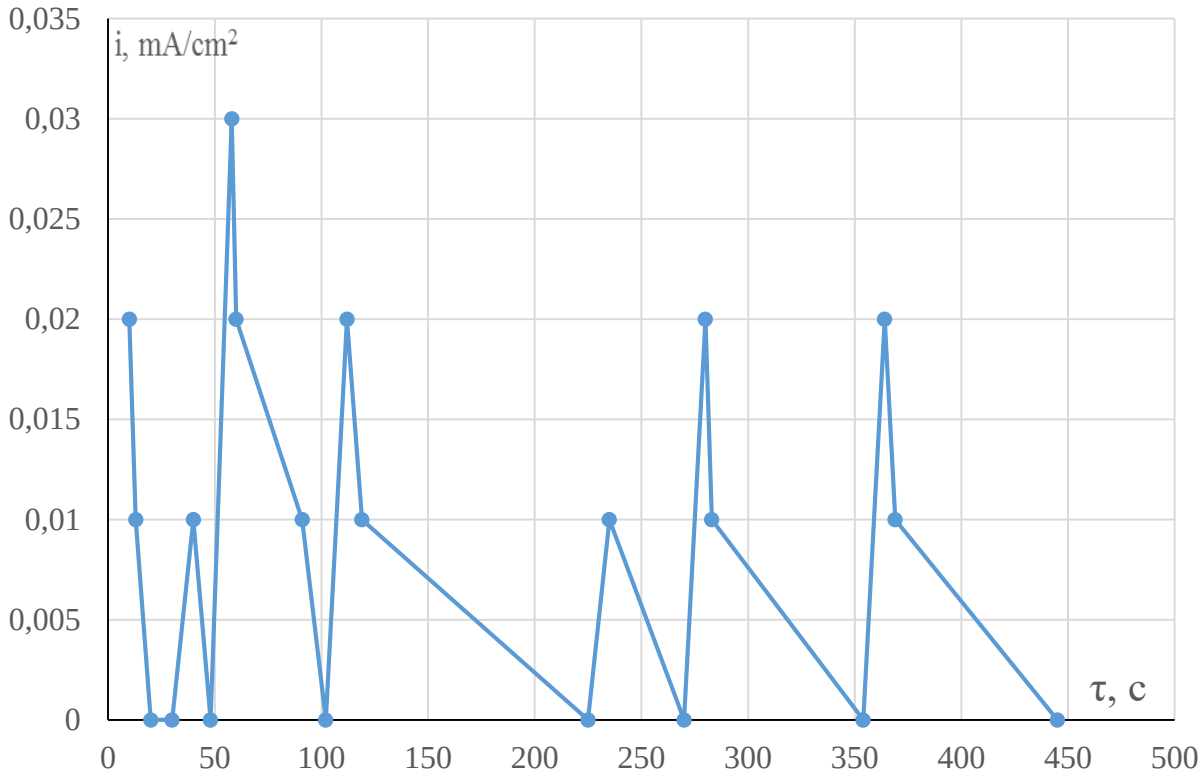

Figure 5. Response curve of 3% solution of urea biosensors based on aerosil

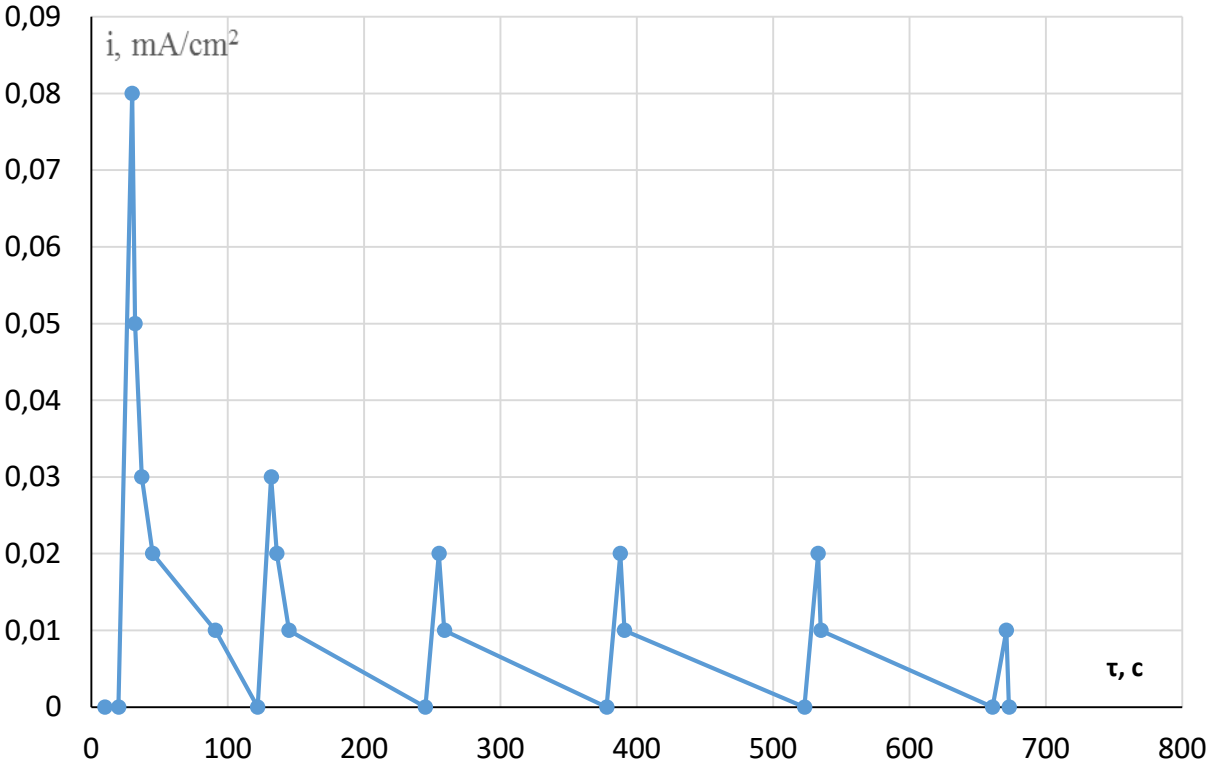

Figure 6. Response curve of 3% solution of urea biosensors based on bentonite

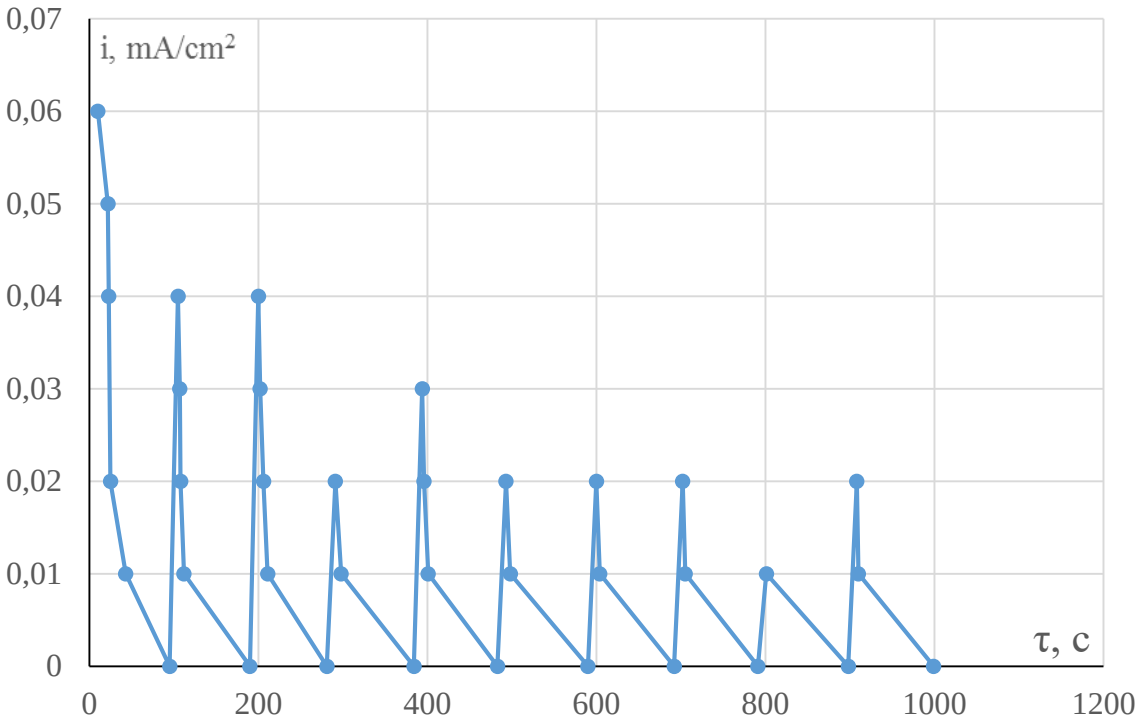

Figure 7. Response curve of 3% solution of urea biosensors based on modified bentonite

In the calibration curve of urea biosensor based on bentonite (Fig. 8) is not observed expressive linear dependence of current response signal from the urea concentration in the sample solution, so this solid carrier does not satisfy the requirements for performance of industrial design urea biosensor. Calibration curve of urea biosensor based on modified bentonite (Fig. 9) shows a clear linear dependence of current signal from the concentration of urea.

The essential difference in the behavior of bentonite and its modified form related to the ability of alumosilicate to form suspension electrode in water in the presence of dissolved oxygen.

Protons, adsorbed on the particles surface (as in the case of modified bentonite), stabilize the formed H_2O_2. It leads to using modifications to increase the actual surface of the particles of the suspension that participates in the absorption, exchange and charge transfer, leading to regulation of the catalytic material functions.

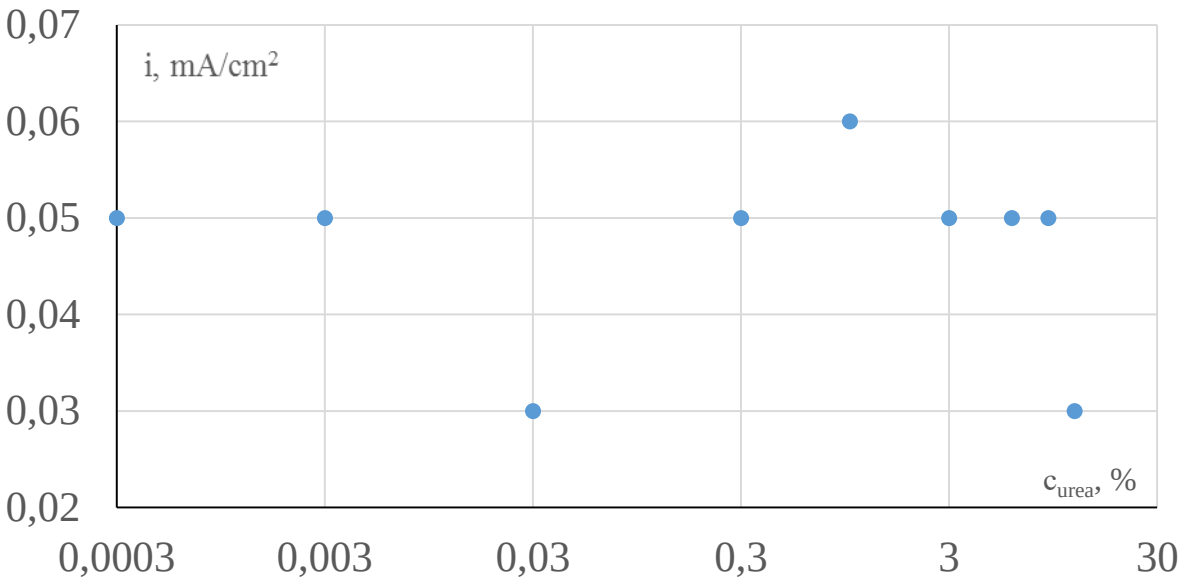

Figure 8. Calibration curve of urea biosensor based on bentonite

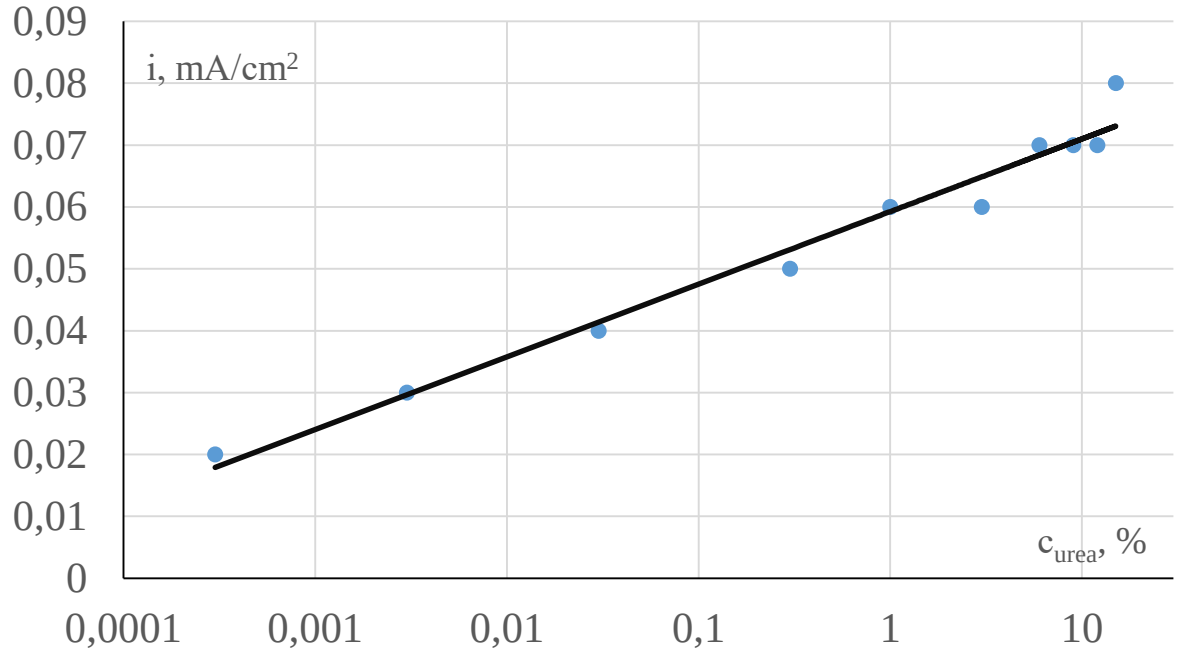

Figure 9. Calibration curve of urea biosensor based on modified bentonite

Considering above results of research were developed technology of manufacturing of urea biosensors based on modified bentonite for their industrial production with productivity 0,5 m^2/hr (Fig. 10).

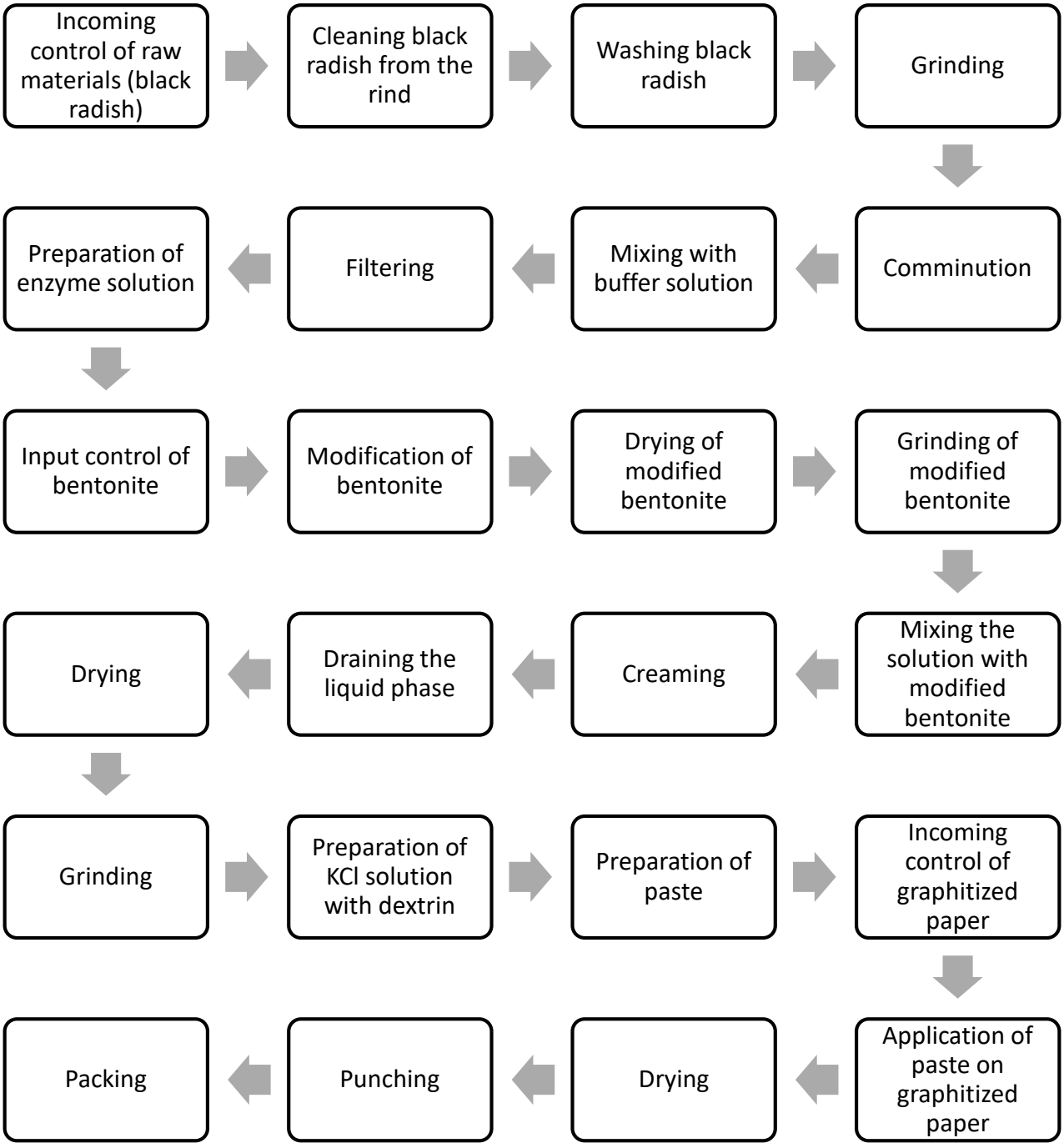

Figure 10. Technology of manufacturing of urea biosensors based on modified bentonite

During the economic and organizing justification chosen optimal kind of labor movement (consecutive) which satisfies the necessary productivity and requires a minimum number of workers and equipment.

Economic indicators of developed project were calculated (Tab. 1).

Table 1. Economic indicators of developed project

№	Economic indicators	Value
1	Number of staff (the list)	4 employees
2	Annual production output	251 batch (1 batch = 4 m^2/hr)
3	Investment	2300372 UAH
4	Cost	1074952 UAH/year
5	Profitability	51,7 %
6	Market price	1631500 UAH
7	Profit	556548,5 UAH/year
8	Period of investment return	4 year
9	The cost of production assets: • fixed assets; • floating assets.	 1306300 UAH 994071,5 UAH

Based on the above results designed startup project, which includes the following steps:

1. Description of project idea
2. Technological audit of project idea
3. Analysis of market opportunities of startup project
4. Development of market strategy of project
5. Development of marketing program of startup project

Considering the results of the analysis, the implementation of the project is viable.

Conclutions:

1. In this master thesis work has been investigated urea biosensor based on enzyme immobilized on a solid inorganic carrier and considered possibilities of its use.

2. Research conducted for urea biosensors based on black radish enzyme immobilized on solid inorganic carriers of various origins (bentonite, modified bentonite, kaolin and aerosil). Research results that the best performance demonstrated biosensor based on modified bentonite. This is is due to the structural features of the surface of the carrier.
3. Based on the results of the research developed technological process of manufacturing the active mass of urea biosensor with a capacity of 0.5 m^2/hr. Calculated the expenses of raw materials and water needed to implement the process.
4. According to the technological scheme conducted economic and organization accounts. Developed organization structure of the company, the type of movement of labor, conduct technical control, balance consumption of floating assets, defined technical and economic indicators.
5. Developed startup project, which follows that the project competitive and has all of necessary facilities for the implementation of the project on the market.

Keywords: biosensor, urea, bentonite, immobilization, black radish, startap-project.