ratio of 1:1. According to the calibration graph (Fig. 4b), we found the amount of acetic acid in milk. The specific activity of the enzyme is 11 mmol/mL.

C. Application to Real Samples

The passage of pesticides to milk from pasture depends on many factors, such as the quantity of the ingestion, absorption, metabolism of pesticides, and excretion by animals in production. Several reports about milk are contaminated by pesticides [6], [7], even though most chemicals can be found in animals' urine and feces. Therefore, real-time detection of pesticides is crucial as sample conditions can vary depending on the day and time of collection and shipping to the lab. Hence, there is a real need for sensors that can make it possible to monitor and field-check detection. Researchers have developed portable biosensors that can be implemented in water, tea, juice, and milk with simple operation techniques, which are more desirable [4], [6]-[9], [24]. To determine the presence or absence of an enzyme inhibitor (malathion) in milk transducer with the polymer film formed on it containing the immobilized enzyme is immersed in a prepared milk sample. In the absence of an enzyme inhibitor (malathion), the pink color turns to yellow; if it is present, the color remains pink. The test-system's performance was carried out according to the following scheme: malathion was added to the milk sample in the following amounts: 0.03; 0.05; 0.07; 0.09, and 0.12 mg/kg. Then the activity of cholinesterase in this milk was investigated. The dependence of the enzyme activity on the concentration of pesticide in milk is presented in Fig. 5. As can be seen from Fig. 4b, the activity of the enzyme decreases sharply when reaches the pesticide maximum residual level content in milk. The detection limit of the biosensor showed to be 0.089 mg/kg for malathion. Detection of an organophosphorus pesticide such as malathion in fresh milk collected from 15 different markets showed no contamination in all analyzed samples.

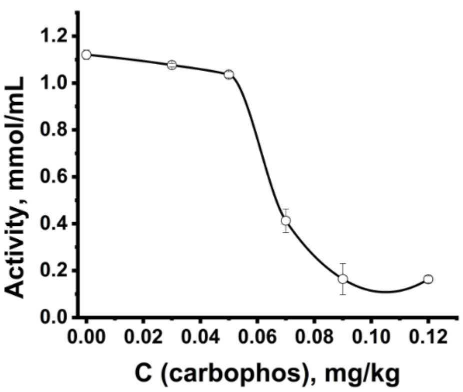

Fig. 5 The dependence of the activity of the AChE enzyme on the concentration of malathion in milk.

D. The Limit of Detection

Our work's purpose was not exact determination (by objective methods) of the finding limit of the whole range of malathion inhibitor. The plan of the study was only to validate whether the newly developed test system could provide results as good as known biosensors while using visual observation of the color change. Organophosphorus pesticide malathion was used for this purpose due to this pesticide in the agricultural treatment of crops. The specified detection limit corresponded to the concentration of malathion. The control sample was yellow. The value of the detection limit was 0.089 mg/kg.

IV. Conclusion

In this work, we have developed and optimized the immobilization of acetylcholinesterase on a glass rod. The dipstick-type AChE inhibitor test-system showed great performance for detecting pesticide malathion in milk with a detection limit of 0.089 mg/kg, in 5 min incubation time. The naked eye can detect the pesticide residue because of the color change due to an indicator's pH drop. A new biosensor is an alternative tool for convenient and straightforward screening of pesticide residue in biological samples with continuous monitoring, low cost, reliable and not required highly trained personnel. Thus, the prepared test systems with the immobilized acetylcholinesterase enzyme subsequently used to formulate the enzymatic reaction in the analyzed solution (milk) to determine organophosphorus pesticides and demonstrated the potential application of the glass stick device for the screening of OP residues in actual samples. The resulting test system is promising for the creation of express tests to determine various toxicants in biological objects. Membranes and screen-printed electrodes immobilized by enzymes are conducive to cheap material used and indulge with fast and reliable measurements. Enzyme entrapment in alginate polymer crosslinked by $CaCl_2$ allowed stable sensors for three months at 4^0C with the enzyme activity >75%. Focusing on developing the color change-based diagnostic platforms is useful in locations where resources are scarce.

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