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Photometric and fluorometric alkaline phosphatase assays using the simplest enzyme substrates

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ABSTRACT

In this contribution a highly cost-effective flow analysis system for determination of alkaline phosphatase (ALP) activity is presented. This fully-mechanized bioanalytical system is based on economic solenoid micropumps and microvalves (powered and actuated by Arduino microcontroller) and extremely cheap dedicated optoelectronic flow-through detectors allowing absorbance and fluorescence measurements. The detection schemes for ALP assaying realized in this system are based on orthophosphate determination. For the detection of these ions formed in the course of enzymatic conversion a molybdate method requiring only common and inexpensive chemicals is utilized. Thus, for the enzymatic assays the simplest not-chromogenic/not-fluorogenic ALP substrates can be applied. Such approach results in the use of low-cost reagents for ALP assays, whereas the mechanization of assay causes low consumption of reagents as well as samples. In the course of reported investigations six ALP substrates were examined and the most promising results have been obtained for the inorganic compound – monofluorophosphate (MFP). The obtained linear ranges of absorbance and fluorescence measurements are 100-600 $U \cdot L^{-1}$ and 30-30/30-100 $U \cdot L^{-1}$, with sensitivities of 0.7 $mV \cdot L^{-1} \cdot U^{-1}$ and 2.3/1.0 $mV \cdot L^{-1} \cdot U^{-1}$, respectively. The calculated limits of detection are 5.1 $U \cdot L^{-1}$ (photometry) and 0.9 $U \cdot L^{-1}$ (fluorometry). The throughputs of the developed system are 13 and 12 samples/h for photometric and fluorimetric detections, respectively. To demonstrate the practical utility of the developed bioanalytical system the ALP assays in complex matrix samples have been carried out. The results of ALP activity determination in serum samples are well-correlated with those obtained using reference method recommended for routine clinical analysis.