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Short Commentary Open Access

L-lactate Biosensor Based on LOx/SiO_2@ZrONPs/CHIT/Au

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L-Lactate is a metabolite formed from pyruvate in muscles, liver and kidney under oxygen deficiency. L-lactate is an analyte of great interest, due to its critical role in sports medicine, heart diseases, food processing industry and overall metabolic function. The normal range of lactate in blood is 0.5 mM-2.5 mM, but in lacto-acidosis patients, the level of lactate in blood increases up to 12 mM-25 mM, due to regular intensive exercise and boundless work. The increased level of L-lactate in blood plasma indicates the oxygenation state of tissues, severe heart or lung diseases, a severe infection with sepsis, blood disorders and hypervolemia shock. The constant build-up of L-lactate in the body, results in excessively low pH, hence causes a condition called lactic-acidosis. It is a subtype of metabolic acidosis, where excessive lactic acid is generated due to a problem with the body's metabolism. The diabetes also may lead to disturbed lactate metabolism. Hence determination of L-lactate in blood is very important for diagnosis and medical management of a number of tissue and muscle related diseases. Pundir et.al. [1] have reviewed various types of lactate biosensors. The principle of LOx based biosensors is as follow:

L-lactate+Lox → Pyruvate+Lox red

Lox red+ O_2 → LOxox+ H_2O_2

H_2O_2 → O_2+$2H^+$+$2e^-$ (Current in mA proportional to lactate concentration)

Electrochemical reactions involved in LOx based lactate biosensor.

The paper by Dagar and Pundir [2] describes the fabrication of an improved aerometric L-lactate biosensor by immobilizing covalently, L-lactate oxidase (LOx) onto zirconia coated silica nanoparticles/chitosan (SiO_2@ZrONPs/CHIT) nano composite modified Au electrode. SiO_2@ZrONPs is a dynamic catalyst and a electron mediator, which enhanced the transfer of electrons i.e. the current response, which led to increase the sensitivity of biosensor. Since SiO_2@ZrONPs provides an environment for the amplified electro-catalytic effect, the biosensor was able to generate current at a low working potential. The nano composite of SiO_2@ZrONPs and CHIT contributes a collaborative response on electocatalytic oxidation of H_2O_2, which offered an excellent performance of the biosensor and $-NH_2$ groups, provided hydrophilic environment and base for covalent immobilization of LOx. The cyclic voltammetry of SiO_2@ZrONPs/CHIT/Au displayed higher currents than CHIT/Au, due to the larger efficient surface area of SiO_2@ZrONPs/CHIT/Au electrode than that of CHIT/Au. The SiO_2@ZrONPs/CHIT/Au composite film could contribute a conducting path through the composite matrix for accelerated kinetics. The continuation of SiO_2@ZrONPs and CHIT provided a favourable potential aperture and electro catalytic behaviour for the electron transfer of H_2O_2 to the electrode. Hence, the SiO_2@ZrONPs acting as electron transfer mediator, assist in improving the response of biosensor and thus increases its sensitivity. Thus an innovative approach of immobilizing lactate oxidase (LOx) onto SiO_2@ZrONPs/CHIT/Au electrode is expected to improve electrochemical measurement of lactate level in biological materials. Further covalent immobilization of LOx onto SiO_2@ZrONPs/CHIT/Au electrode could increase its stability.

A nanocomposite of SiO_2@ZrONPs was synthesized by adding drop-wise 4.0 ml of NH_4OH into a mixture of 2.0 ml tetra ethyl ortho silicate and 20.0 ml ethanol followed by stirring for 8 h and centrifugation at 5000 g for 5 min to precipitate SiO_2NPs. A suspension (0.2 ml) of commercial ZrONPs was added 10.0 ml aqueous suspension of SiO_2NPs followed by addition of NH4OH until SiO_2@ZrONPs were formed A mixture of SiO_2@ZrONPs suspension in DW and 0.2% CHIT in 1 M KCl was electrodeposited onto cleaned Au electrode in a potentiostat. Then LOx was immobilized onto glutaraldehyde activated SiO_2@ZrONPs/CHIT/AuE, which acted as working electrode. An amperometric L-lactate biosensor was constructed by connecting this working electrode with a reference electrode (Ag/AgCl) and a counter electrode (Pt wire) through potentiostat. The biosensor was tested in by dipping the three electrode system into 25 ml of 0.1 M sodium phosphate buffer, pH 7.5 containing 10 mM lactic acid. The working conditions of biosensor were optimized by measuring its current response (mA) at varying pH, incubation temperature, time and substrate (Lactic acid) concentration. The biosensor was applied to measure L-lactic acid concentration in plasma of apparently healthy and lacto-acidosis patients. It was evaluated by calculating its detection limit (LOD), % recovery of added lactic acid into plasma, precision and correlation with the standard method. The storage stability of working electrode at 4^0 degree celcius was studied.

The biosensor exhibited a quicker response (3 s), a lower LOD (0.2 nM) and wider working range (0.1 µM-4000 µM) with a higher sensivity ,better reproducibility and higher storage stability (210 days) compared to earlier biosensors. The recovery of added lactic acid in plasma at a concentration of 5 mM was found 99% and within and between coefficient of variation (CV) were <2.0% and 3.0% respectively. The lactate level in plasma from apparently healthy persons and lacto-acidosis patients, as measured by present biosensor showed a good correlation (R2=0.99) with those by standard method. The laboratory imitation of this biosensor could be miniaturized by chip designing or preparing a micro-fluidic two electrode system on paper to design a portable/commercial device for direct use by the patient.

References

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