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# Determination of Peroxidase-Like Activity of Magnetic Particles: Basic Platforms for Peroxidase Biosensors

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Magnetic particles (MPs) are a current mean suitable for separation, biosensors construction, nanomedicine etc. They can be easily covered with a biomolecule and used for analytical purposes. Many enzymological assays have to be combined with use peroxidase. In this point of view, glucose assay by glucose oxidase can be exampled. In the present work, we focused our attention on the determination whether MPs based on iron oxide have pseudo-peroxidase activity allowing use them directly without linking to peroxidase. Hydrogen peroxide ($H_2O_2$) as the very common substrate of peroxidase was used for this measurement. Michaelis-Menten kinetics was performed for demonstration of catalytic activity of MPs by measuring various concentrations of $H_2O_2$ and square wave voltammetry (SWV), cyclic voltammetry (CV) and spectrophotometry were performed for the determination of pseudo-peroxidase activity. The used MPs had significant pseudo-peroxidase activity and Michaelis-Menten like behavior was observed and kinetics constants were determined. Michaelis-Menten constant ($K_m$) was equal to 156.6 - 199.1 mmol/l (values are slightly different for the used analytical methods). Sensitivity of electrochemical and optical methods was also compared resulting in limits of detection found to be 0.42 mmol/l, 0.36 mmol /l and 13.1 mmol /l for SWV, CV and spectrophotometry respectively. In a conclusion, iron oxide MPs have high pseudo-peroxidase activity resulting in opportunity to use them for the determination of hydrogen peroxide. If an oxidase applied as a biorecognition element, there is no need to co-immobilize peroxidase. $K_m$ values were also determined for HRP using spectrophotometry (25.1 mmol/l), SWV (167. mmol/l) and CV (163.2 mmol/l).

**Keywords:** magnetic particles; pseudo-peroxidase activity; hydrogen peroxide; horseradish peroxidase; voltammetry; spectrophotometry

## 1. INTRODUCTION

Peroxidases are group of enzymes containing phorphyrin in their structure and catalyzing redox reactions. They belong to one of the most significant enzymes in bioanalytical chemistry with broad

possibility of application in the both detection and as supporting enzymes [1-4]. Horseradish peroxidase (HRP) presents the most commonly used type of peroxidase in construction of biosensors because of its good availability and low price [5]. HRP is glycosylated metalloenzyme containing heme as prosthetic group in its active site and it is well known from many assays, where redox reactions are taking place [1, 5]. HRP catalyzes both oxidation of substrates (such as phenols and aromatic amines) and reduction of substrates ($H_2O_2$, organic hydroperoxides etc.) [2]. It is enzyme most widely linked to construction of voltammetric biosensor, but it is also performed in spectrophotometric assays like immunoassays, $H_2O_2$ and glucose colorimetric assays [2, 6].

$H_2O_2$ is a substance highly used in food, pharmaceutical, clinical, environmental and other industries. Because of $H_2O_2$ broad applicability and lack of simple methods, accurate analysis is needed. Moreover, it is the substance creating during many enzymatically catalyzed reactions and it is the product of cell metabolism [7, 8]. Concentration of $H_2O_2$ is crucial parameter giving edge between harmless and harmful impact on the body. According to the Scientific Committee on Consumer Products, tooth bleaching products containing more than 0.1 % of $H_2O_2$ may cause potential health problems and $H_2O_2$ in concentration higher than 6 % can cause severe health damage. Only non-food products (toys, textiles, clothing, personal care products, domestic products etc.) containing less than 0.1 % of $H_2O_2$ are allowed to be sold on the open market in European Union [8]. Concentration of $H_2O_2$ in products is the most commonly controlled by enzymatic methods, namely methods using enzyme HRP [7]. However, severe drawbacks like low reproducibility, low stability of the enzyme and relatively high price are limits for the enzymatic assays [1, 5].

Although enzymes are highly sensitive, substrate specific and quite effective, their lifetime is just about few weeks. Furthermore, immobilization or harsh environment conditions can reduce their lifetime due to unfolding of their structure followed by stability and activity loss [9, 10]. Physico-chemical conditions may influence pH optimum, thermal stability, kinetics and long-term stability of both enzyme and biosensor [9]. Moreover, use of enzymes in sensor´s construction is limited by demands given on price that is quite high in for enzymes isolated from natural samples or cell lines in recombinant technologies [11]. Difficulty to be prepared in considerable quantity is another disadvantage of HRP [12].

Research has focused on particles and substances with pseudo-enzymatic and it is highly desired in the current time [10]. The particles are known as enzyme mimetics or mimics, artificial enzymes, or enzyme-like compounds [10, 12, 13, 14]. Ligand anchoring supramolecular complexes, micelles, vesicles, nanogels and nanoparticles appear to be appropriate artificial enzymes [15, 16, 17]. Nowadays, MPs, coated MPs, copper nanoclusters, CuS nanoparticles, $CuInS_2$ nanocrystals or polyoxometalates are potent enough to replace HRP in constructed colorimetric biosensors depending on peroxidase activity for determination of glucose or $H_2O_2$ [10, 12, 13, 14, 15, 18, 19, 20]. On the other hand, electrochemical biosensors depending on peroxidase activity for detection of $H_2O_2$, phenols, DNA or dopamine are still based on activity of natural HRP [1, 2, 5, 21].

In our manuscript, we have studied peroxidase-like activity of MPs using $H_2O_2$ as substrate and o-phenylenediamine as chromogen when spectrophotometry was performed. Our intention was to prove suitability of MPs replacing HRP in novel constructions of both optical and electrochemical biosensors and to prepare basic platform suitable for construction of peroxide biosensor after further

optimization. Colorimetric and voltammetric assays were performed to compare of sensitivity and kinetics of optical and electrochemical methods. Another purpose of our study was to determine $K_m$ value of HRP and to compare kinetics of MPs and HRP as catalysts.

## 2. EXPERIMENTAL PART

### *2.1. Chemicals*

All chemicals were obtained from commercial sources in at least analytical grade and used without further purification. Peroxidase from horseradish type VI (250-330 U/mg of solid), o-phenylenediamine dihydrochloride (o-PD, 0.5 mg/ml) and iron oxide magnetic micro particles carboxy functionalized (20 mg/ml) were purchased from Sigma Aldrich, Saint Louis, Missouri, USA. $H_2O_2$ (30 %) and sodium acetate trihydrate were bought from Penta (Prague, Czech Republic). Demineralized water was prepared by reverse osmosis process using device from Aqua Osmotic, Tisnov, Czech Republic.

### *2.2. Apparatus*

The spectrophometric measurements were performed at a room temperature by ELISA spectrophotometer Sunrise (Tecan Salzburg, Austria). Electrochemical measurements (CV, SWV) were taken by Electrochemical Sensor Interface PalmSens (PalmSens, Utrecht, The Netherlands). Measurements were done on three-electrode screen printed sensors composed of carbon working electrode (circle shaped with 1 mm diameter), platinum counter electrode (circle around working and reference electrodes) and silver reference electrode (circle over the working electrode) covered with silver chloride. The electrodes were obtained from BVT Technologies (Brno, Czech Republic).

### *2.3. Colorimetric assay*

The colorimetric proof of MPs pseudo-peroxidase activity was determined by measuring absorbance of concentration series at 450 nm using 96 well microplates and the aforementioned spectrophotometer. $H_2O_2$ was diluted in the concentration range from 0.25 % to 30.0 % (0.25, 0.5, 1, 2, 4, 6, 9, 12, 16, 20, 25 and 30 %) for measuring of calibration curve. 180 µl of o-PD solution (0.5 mg of o-PD powder dissolved in 1 ml of 50 mmol/l sodium acetate buffer, pH 5.5), 20 µl of $H_2O_2$ (final concentration of $H_2O_2$ in measured suspension was 10 times lower than in original solution) and 8 µl of MPs were added into well of microplate. MPs were magnetically separated using ferromagnetic iron plate and absorbance was measured in time 0 minutes and 10 minutes. Average absorbance per minute for each concentration was calculated for creation of concentration curve.

The colorimetric determination of $K_m$ value for HRP was performed by similar spectrophotometric measurement like MPs pseudo-peroxidase activity. The assay was adopted in the following way: the ratio of reaction component in solution in well was 190 µl of o-PD solution (0.1 mg

of o-PD powder dissolved in 1 ml of 50 mmol/l sodium acetate buffer, pH 5.5), 10 µl of $H_2O_2$ (final concentration of $H_2O_2$ in measured suspension was 20 times lower than in original solution) and 2 µl of HRP (1 mg of powder HRP dissolved in 1 ml of $H_2O$).

All the assays were performed in pentaplicate (n = 5) under standard ambient temperature and pressure (SATP) conditions.

### *2.4. Voltammetric assay*

The voltammetric determination of pseudo-peroxidase activity of MPs was performed using square wave and cyclic voltammetry. The electrochemical device was operated through PS Lite software. $H_2O_2$ in concentration range from 0.25 % to 12.0 % (0.25, 0.5, 1, 2, 4, 6, 9 and 12 %) was used for creation of calibration curve. Concentration series of $H_2O_2$ were analyzed in different applied potentials (from 0.0 to 1.1 V) and the best concentration curve was chosen. CV measurement was performed in increasing potential from 0.0 to 1.1 V in one cycle with potential step 0.01 V, and scan rate 0.05 V/s. SWV was performed also in increasing potential from 0.0 to 1.1 V with potential step 0.004, amplitude of potential 0.01 V, and frequency 1 Hz. 10 µl of $H_2O_2$ was dropped onto electrode surface with 50 mmol/l sodium acetate buffer (pH 5.5) poured in 1:1 ratio. Reaction was started by addition of 1 µl of MPs and current was recorded. Average current for each concentration and chosen voltage was used for creation of signal to concentration of substrate dependence. Electrochemical determination of $K_m$ value of HRP was done under same conditions like assay based on MPs as catalyst. HRP solution used for the measurement was prepared by dissolving of 1 mg of HRP in 1 ml of $H_2O$.

Additionally to the aforementioned experiment, voltammetry of $H_2O_2$ without catalyst was performed in order to judge what reaction was initiated by catalyst and what was spontaneous. For this type of measurement, 1 µl of $H_2O$ replaced MPs as catalyst in reaction mixture. Electrochemical activity of $H_2O_2$ was measured in concentration range from 0.25 % to 12.0 % (0.25, 0.5, 1, 2, 4, 6, 9 and 12 %) of $H_2O_2$. Reaction and measuring conditions were used the same as for measuring of pseudo-peroxidase activity of MPs. Resulting current of each concentration was subtracted from current of corresponding concentration of reaction catalyzed by MPs and concentration curve was constructed.

The assays were performed in pentaplicate under SATP conditions.

### *2.5. Comparison of catalytic activity of MPs, HRP and $H_2O_2$ itself*

Solution containing 1.5 % of $H_2O_2$ was subsequently measured by CV, SWV and spectrophotometry. MPs, HRP and $H_2O$ (non-catalyzed reaction) were used as catalyst. Catalytic activities of each catalyst were measured under the same reaction conditions described above in chapter Colorimetric assay and Voltammetric assay. Colorimetric measurements were performed with o-PD and $H_2O_2$ in ration 10:1 for MPs and $H_2O$ as catalysts and 20:1 for HRP as catalyst.

Voltammetric determination of $H_2O_2$ was measured using $H_2O_2$ of 3 % concentration diluted 2-times in sodium acetate buffer and 1 µl of catalyst was added into this solution.

### *2.6. Data processing*

Non-linear curve fitting using Michaelis-Menten equation was chosen for saturation curves construction. The achieved experimental data were processed in Origin 9.1 (OriginLab Corporation, Northampton, MA, USA). Signal vs. noise equal to three criterion (*S/N=3*) was used for limit of detection calculation.

## 3. RESULTS

### *3.1. Colorimetric Assay*

Previous articles proved catalytic activity of magnetic particles enabling oxidation of chromogen in the presence of $H_2O_2$ to colored compound measurable by spectrophotometer [11, 18]. In our study, o-PD was used as a chromogen. Oxidation of colorless o-PD to yellow-orange solution of 2,3-diaminophenazine, which can be measured at 450 nm, is shown in Figure 1. Absorbance of solution arises with increasing concentration of $H_2O_2$ in a concentration response manner which is depicted in Figure 2. The final calibration curve shows normal distribution with correlation coefficient equal to 0.998. Limit of detection was determined be 13.1 mmol/l. Michaelis-Menten kinetics was studied to prove enzymatic activity of MPs and $K_m$ value was found to be 199 mmol/l of $H_2O_2$. Concentration curve of reaction catalyzed by HRP is shown in Figure 3. Calibration curve replies Michaelis-Menten behavior with correlation coefficient 0.992 and with $K_m$ value equal to 25.1 mmol/l.

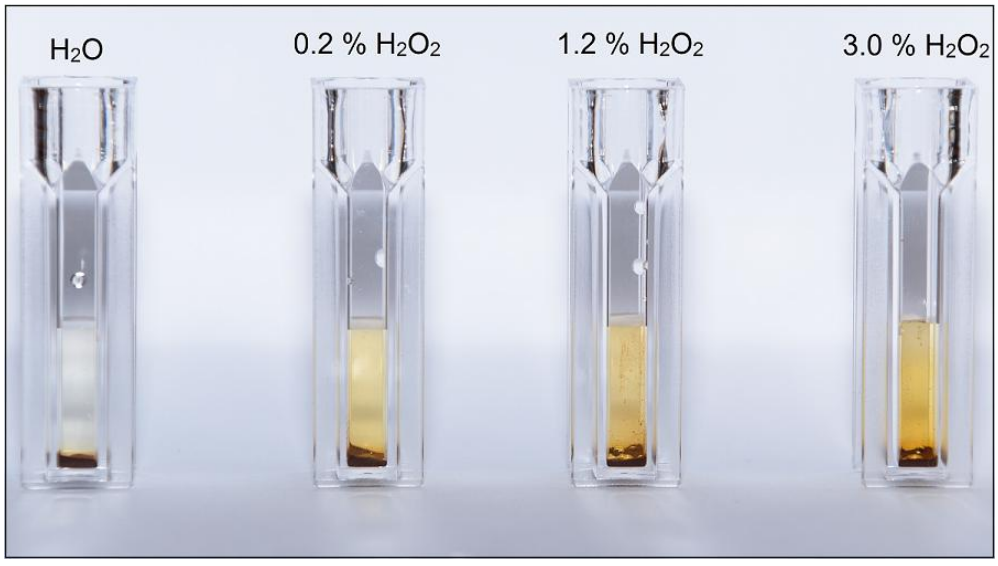


**Figure 1.** Photography of color reactions of o-PD with water and with three concentrations of $H_2O_2$ in solution (0.2 %, 1.2 % and 3.0 %) catalyzed by MPs in time 10 minutes.

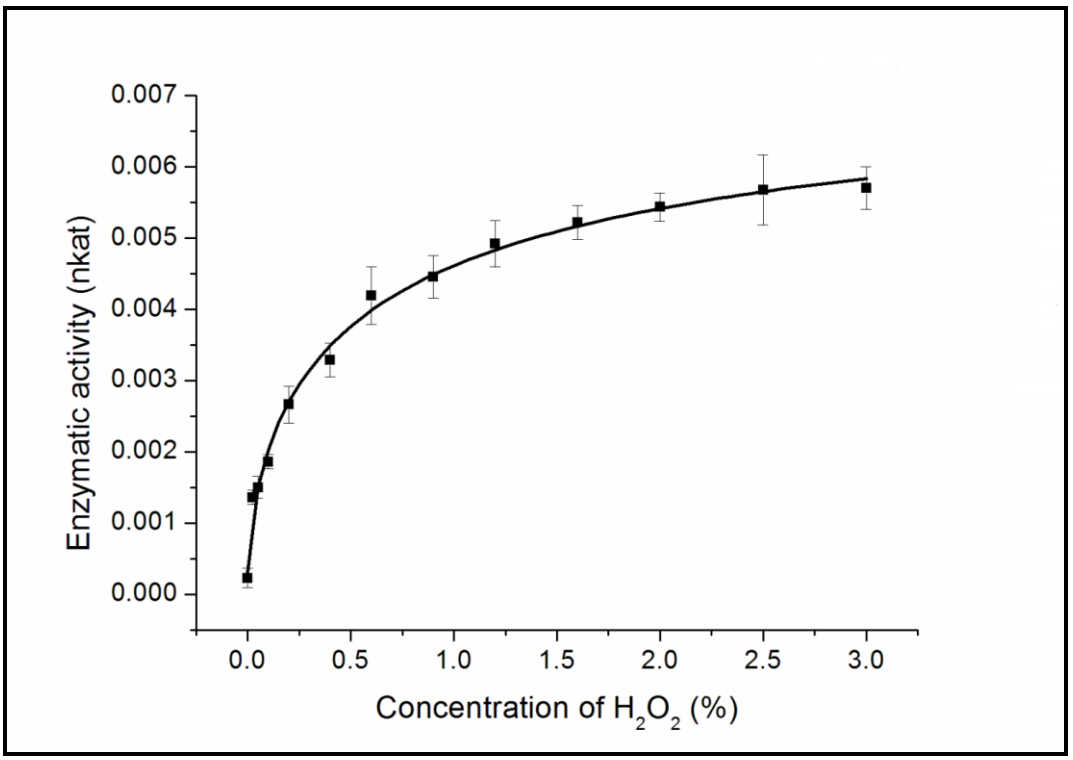


**Figure 2.** B: Concentration-dependent curve of spectrophotometric detection of $H_2O_2$ at 450 nm catalyzed by 160 μg of MPs. Error bars indicate standard deviation for n = 5.

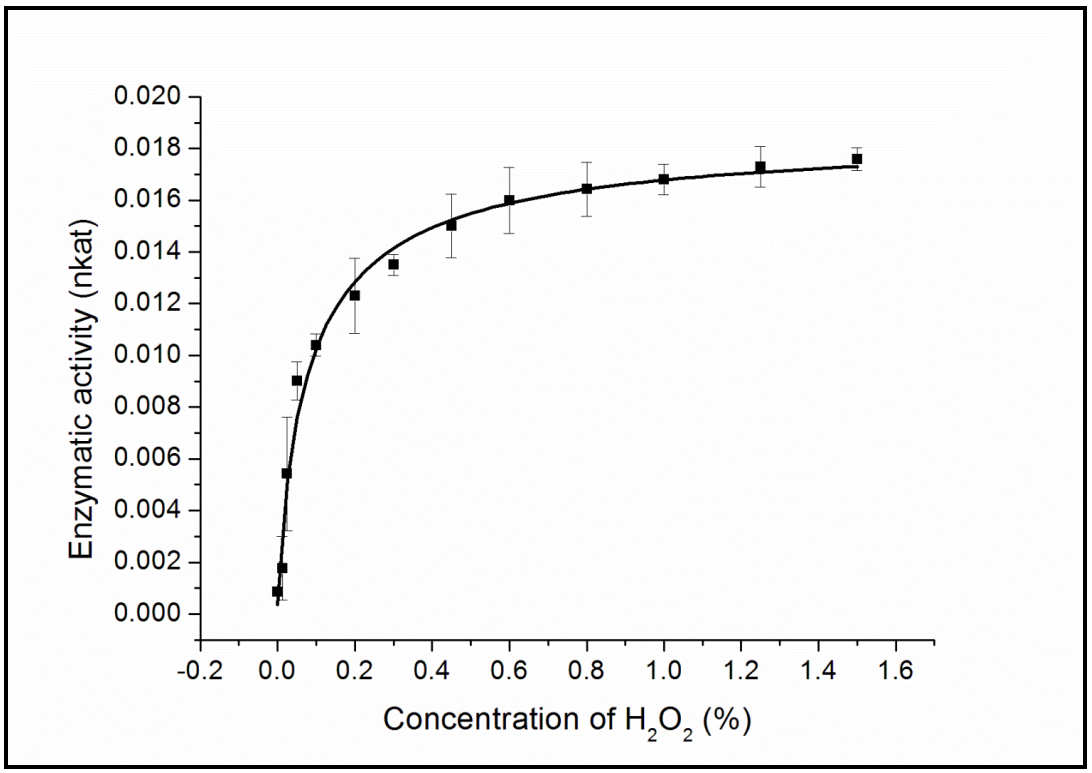


**Figure 3.** Concentration-related curve of spectrophotometric determination of $H_2O_2$ at 450 nm with HRP as catalyst. Error bars indicate standard deviation for n = 5.

*3.2. Electrochemical assays*

Electrochemical devices are often used in peroxidase biosensors, but unlike the spectrophotometric ones, HRP has not been replaced by MPs in their construction yet. In our study peroxidase-like activity of MPs was assumed also in electrochemical measurements. CV was chosen as one of two electrochemical methods to prove activity of MPs and its ability to make pseudo-peroxidase activity. Response of current depending on increasing concentration of $H_2O_2$ is shown in Figure 4. The potential 1.09 V was chosen for construction of concentration curve because of the best correlation coefficient (R=0.999). Detection limit of concentration curve was found to be 0.36 mmol/l. Michaelis-Menten kinetics was measured due to demonstration of enzymatic activity of MPs and $K_m$ value was equal to 156.6 mmol/l. Difference between current of reaction catalyzed by MPs and current of non-catalyzed reaction at potential 1.09 V is shown in Figure 5. Difference was calculated for each concentration and concentration-dependent curve of this difference was constructed. Correlation coefficient was calculated to be 0.987 and $K_m$ value of this reaction was determined to be 92.4 mmol/l. This curve proves variation between catalyzed and non-catalyzed reaction and it also proves the electrocatalytical activity of MPs.

Current of each concentration of $H_2O_2$ was measured by insertion of increasing potentials and final voltammetric curves are shown in Figure 6.

Michaelis-Menten kinetics of HRP was also measured by CV and it is shown in Figure 7. Curve responses the Michaelis-Menten dependence with correlation coefficient 0.999 and $K_m$ value was calculated to be 163 mmol/l.

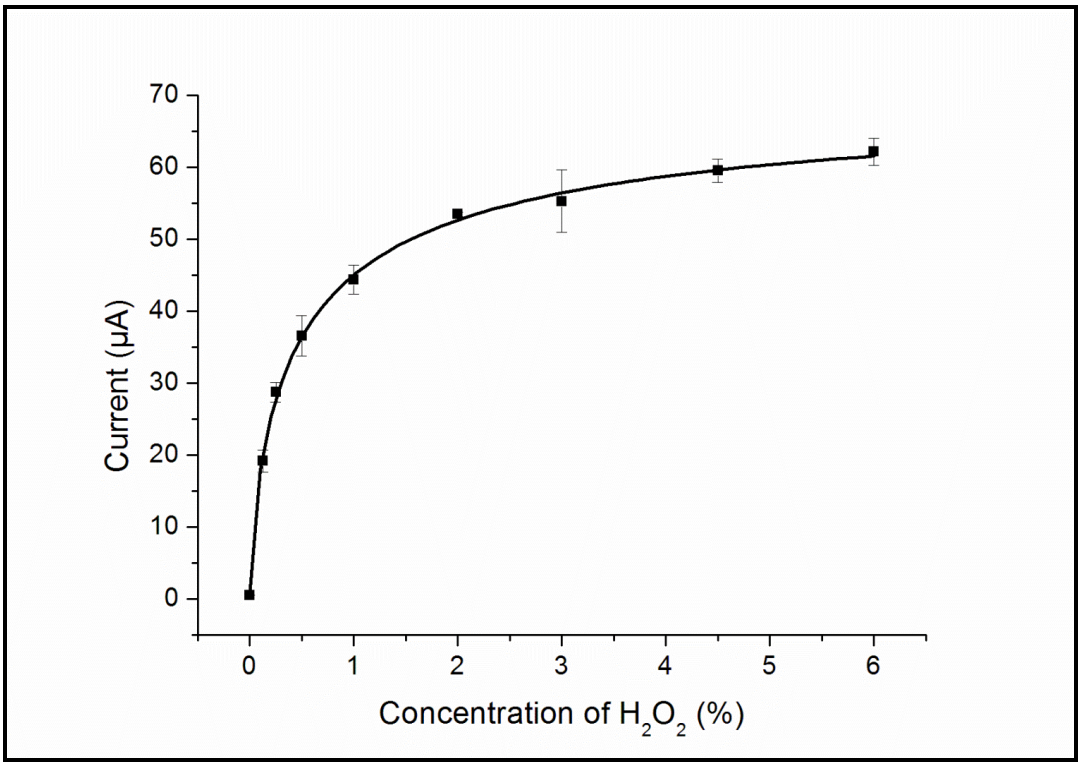


**Figure 4.** Dose-dependent curve for determination of $H_2O_2$ by CV at potential 1.09 V using 20 µg of MPs as catalyst. Error bars indicate standard deviation for n = 5.

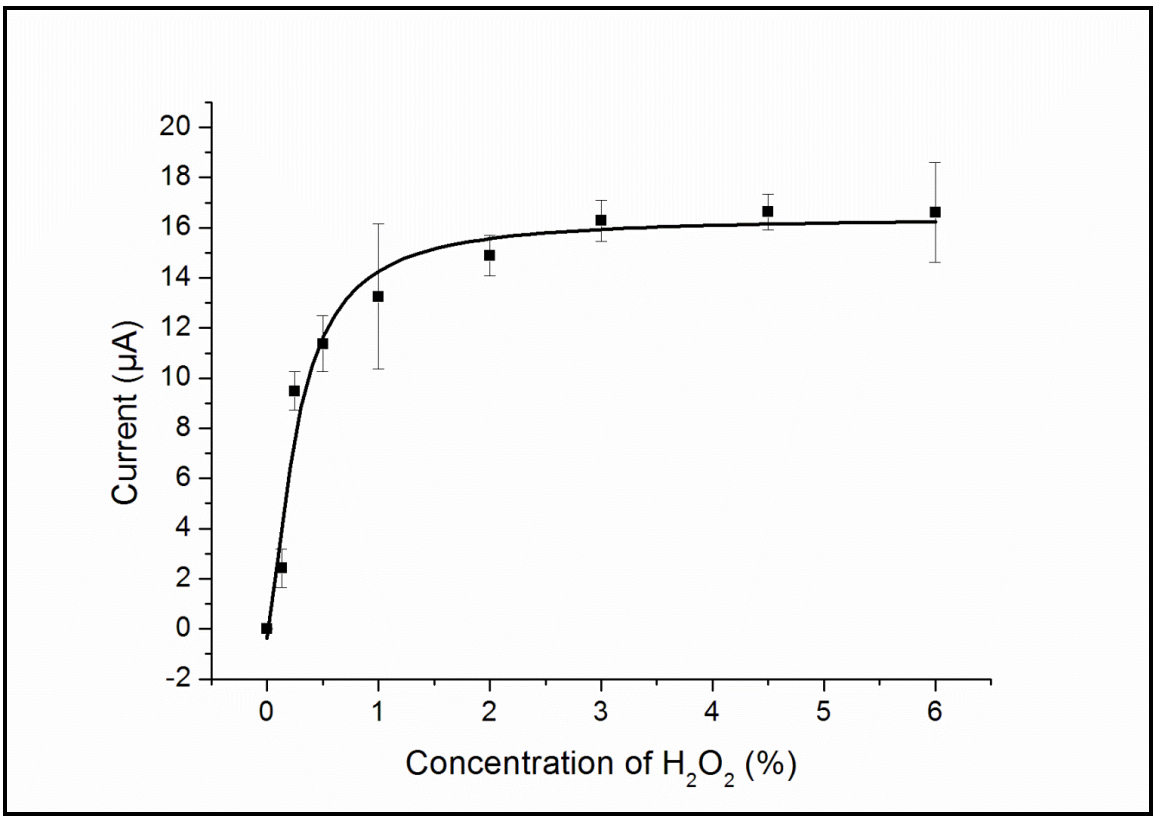


**Figure 5.** Curve of difference of currents between catalyzed and non-catalyzed reaction measured by CV using potential 1.09 V. Error bars indicate standard deviation for n = 5.

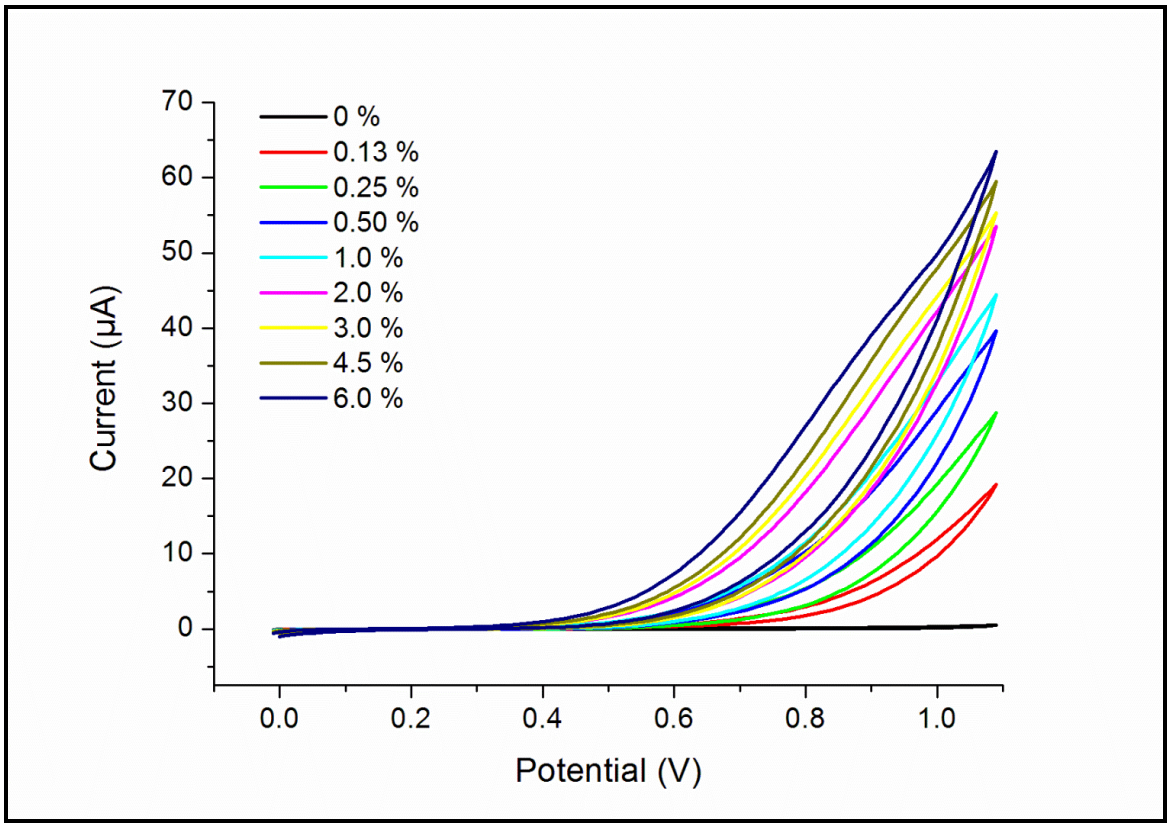


**Figure 6.** Graph of measured curves of each concentration of $H_2O_2$ using CV.

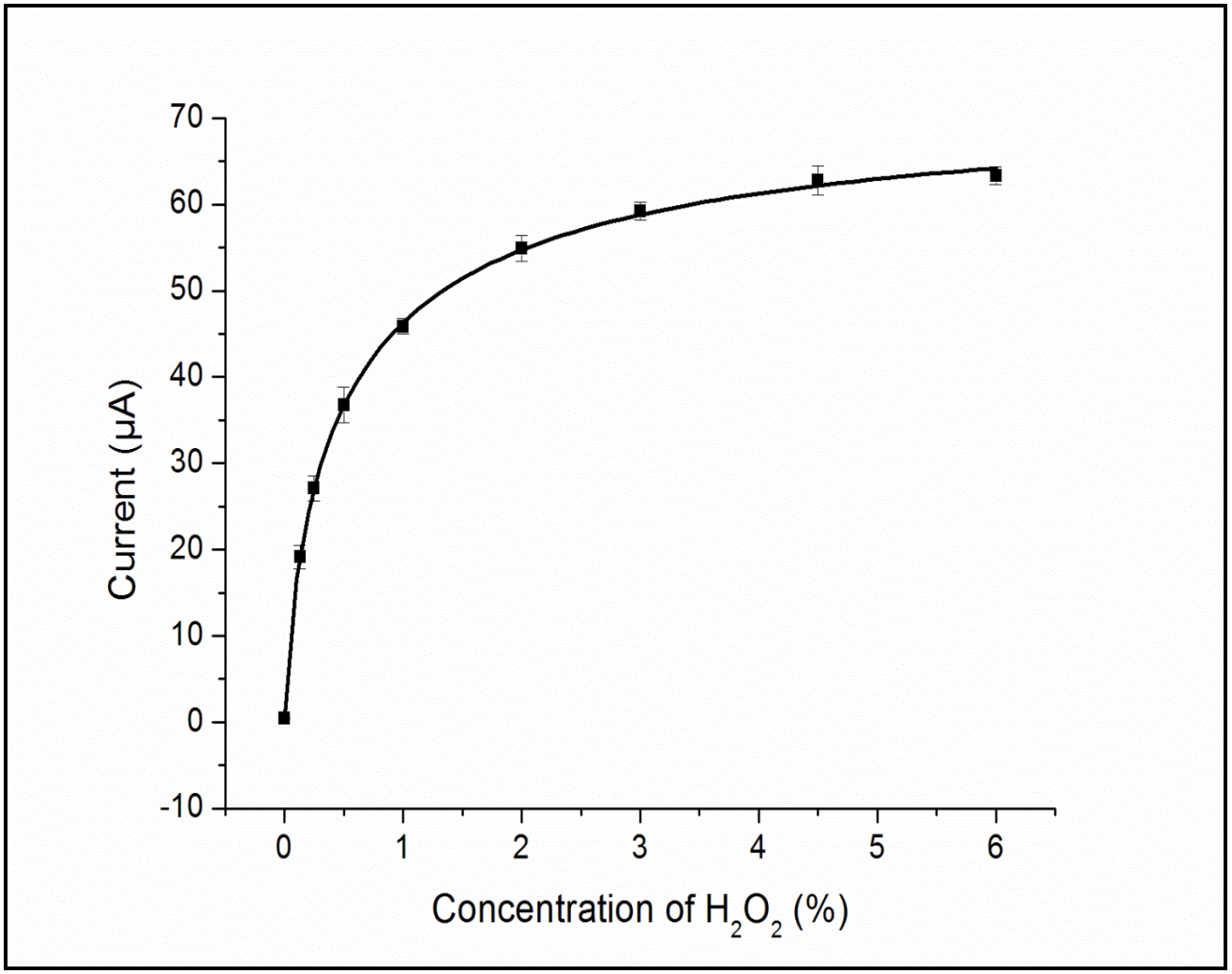


**Figure 7.** Calibration curve based on increasing concentration of $H_2O_2$ using HRP as catalyst and measured by CV using potential 1.09 V. Error bars indicate standard deviation for n = 5.

Results of SWV are shown from the Figure 8 to Figure 11. Figure 8 demonstrates concentration curve at potential 0.7 V. Current depends on increasing concentration of $H_2O_2$ and calibration curve fits this dependence with correlation coefficient 0.998. Detection limit for $H_2O_2$ was equal to 0.42 mmol/l and $K_m$ value indicating enzymatic activity of MPs was calculated to be 183 mmol/l. Concentration curve based on the difference between currents of catalyzed and currents of non-catalyzed reactions in potential 0.7 V is shown in Figure 9. $K_m$ value of this curve with correlation coefficient 0.997 was determined to be 67.9 mmol/l. In Figure 10, there are shown voltammetric curves of measurements of particular concentration of $H_2O_2$ under growing potential. Figure 11 shows concentration-dependent curve of $H_2O_2$ reacted with HRP as catalyst. Points of curve was interposed by Michaelis-Menten equation with correlation coefficient 0.993 and $K_m$ value was set to be 167 mmol/.

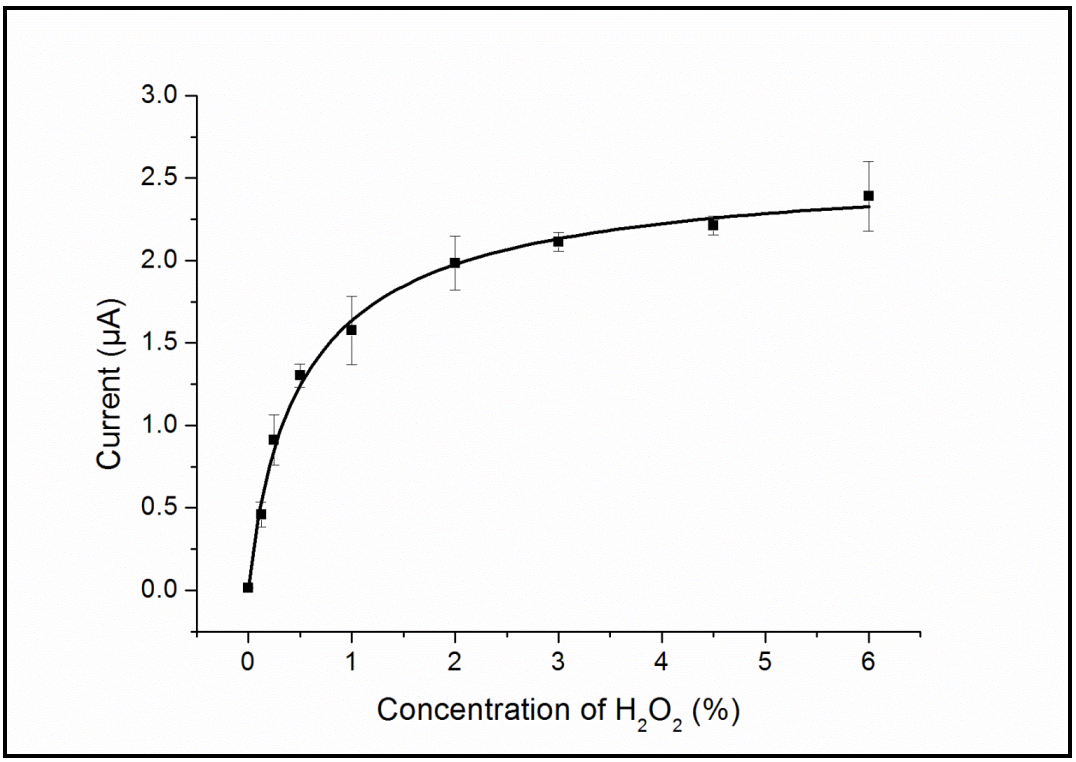


**Figure 8.** Concentration-responding curve for assessment of $H_2O_2$ by SWV at potential 0.7 V using 20 μg of MPs as catalyst. Error bars indicate standard deviation for n = 5.

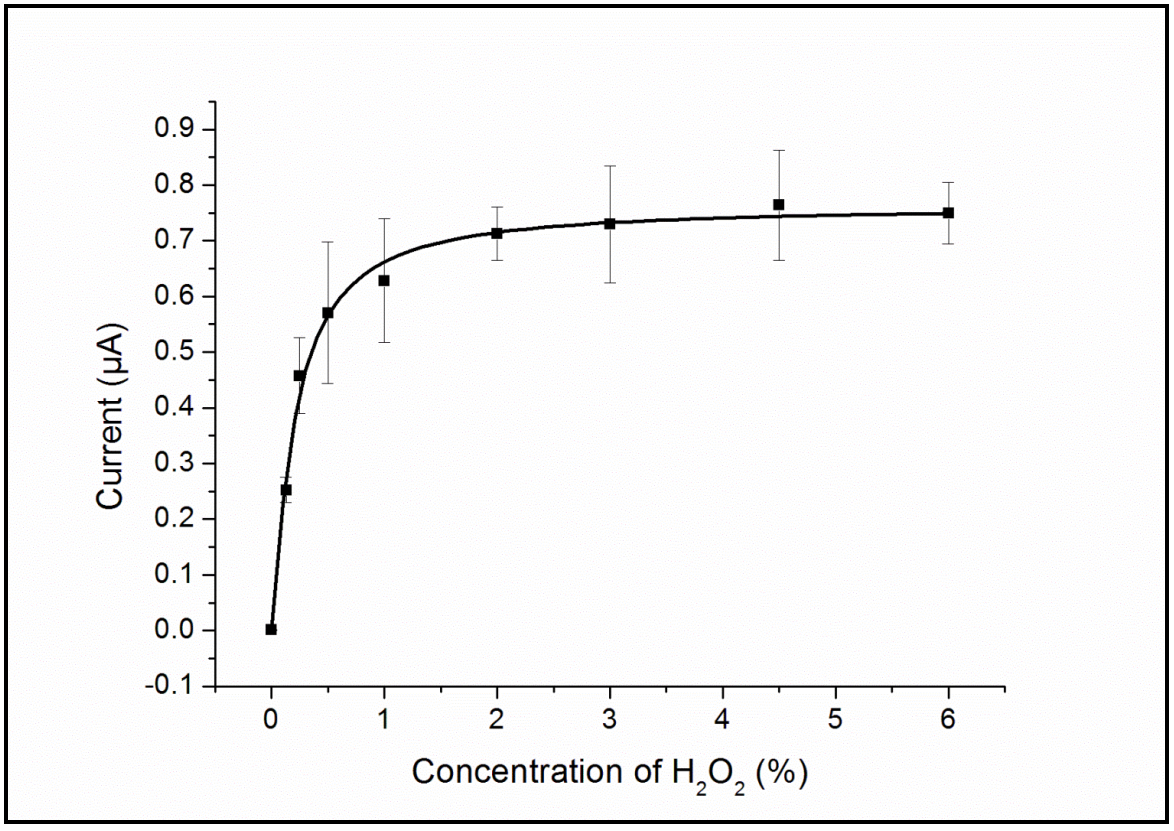


**Figure 9.** Curve construction based on difference between currents of catalyzed and non-catalyzed reactions at potential 0.7 V. Error bars indicate standard deviation for n = 5.

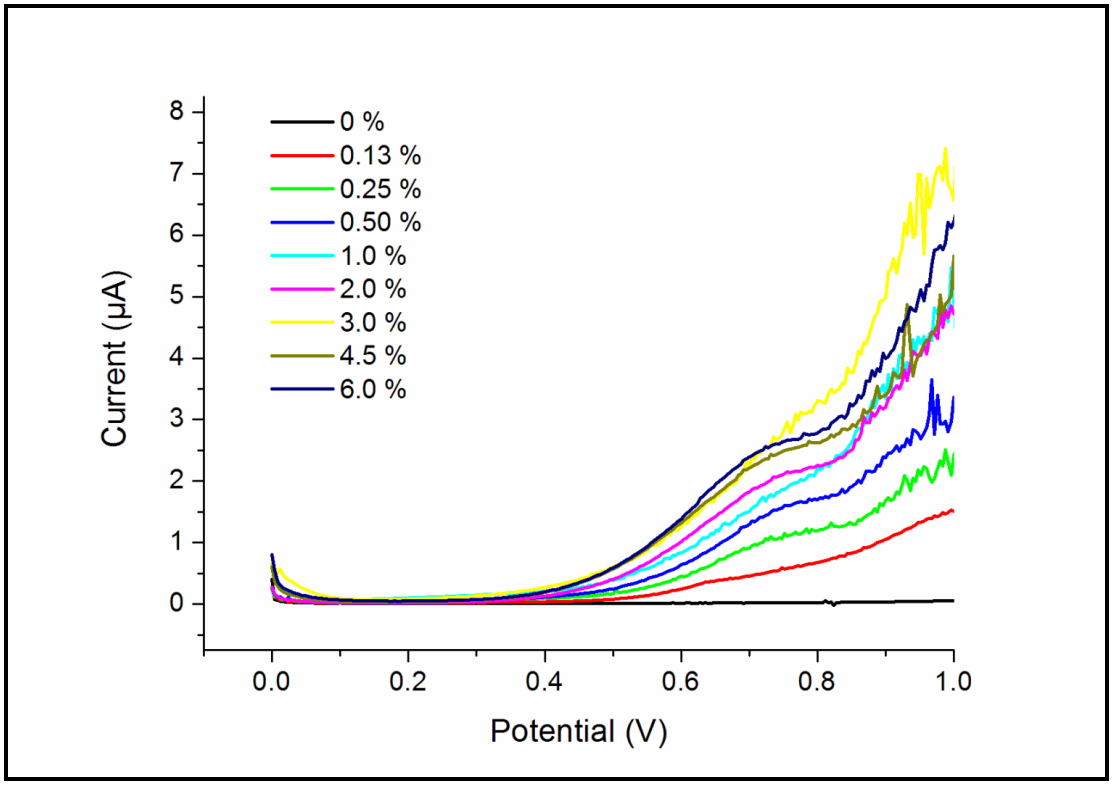


**Figure 10.** Square wave voltammograms for increasing concentrations of $H_2O_2$.

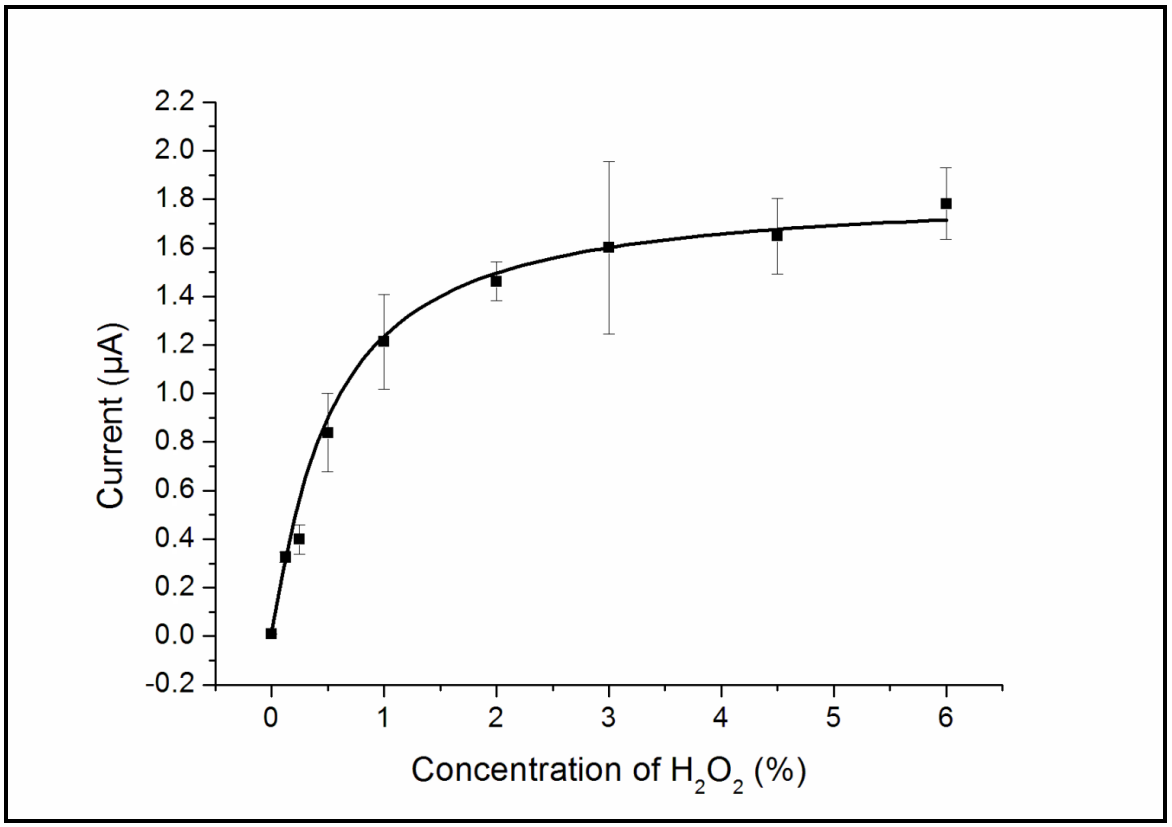


**Figure 11.**Dose-depending curve on increasing concentration of $H_2O_2$ after catalysis of HRP and SWV measurement at 0.7 V. Error bars indicate standard deviation for n = 5.

### *3.3. Comparison of catalytic activity of MPs, HRP and $H_2O_2$ itself*

Catalytic activity of MPs, HRP and $H_2O_2$ itself was compared by spectrophotometry, CV and SWV. Response of devices on HRP activity was set as 100 % for every method. Response of devices on activity of MPs and $H_2O$ (use for simulation of reaction without catalyst, where spontaneous transformation of $H_2O_2$ to measured product is observed) are expressed as percentage of activity of HRP (Figure 12). Using spectrophotometry, HRP has the best catalytic activity, MPs have also catalytic activity, but not such distinct like HRP has. Spontaneous reaction of $H_2O_2$ with chromogen without catalysis of enzyme or MPs is minimal and it replies to spontaneous light-depending oxidation of chromogen o-PD to 2,3-diaminophenazine.

Electrocatalytical activity of $H_2O_2$ is quite high because $H_2O_2$ is electroactive substance and it is spontaneously reduced to oxygen and hydrogen producing measurable electrons as it is shown in Equation 1 [3].

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \quad (1)$$

On the other hand, reaction catalyzed by HRP shows significantly ($p>0.05$) higher response of device than non-catalyzed reaction and MPs show significantly ($p>0.05$) better catalytic activity than HRP using both voltammetric methods.

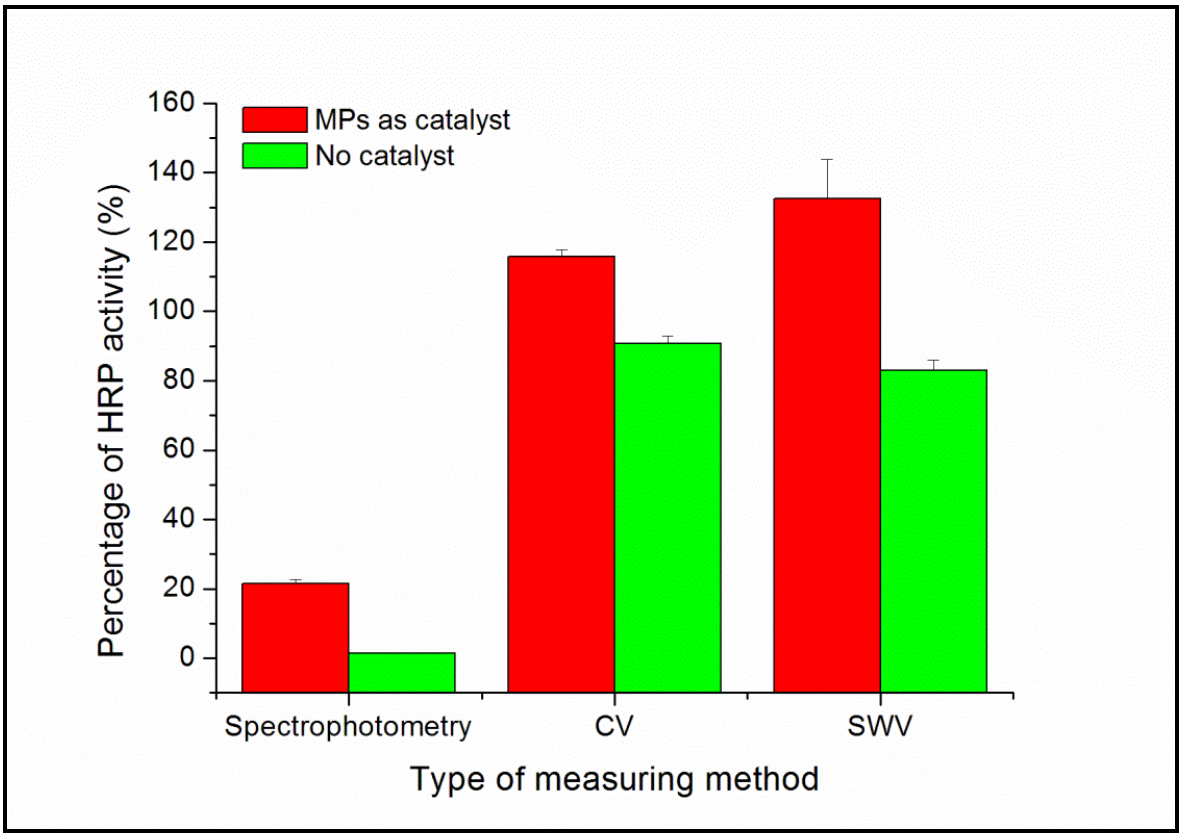


**Figure 12.** Comparison of devices response on reaction catalyzed by MPs and on non-catalyzed reaction toward devices response on reaction catalyzed by HRP presenting 100 % of catalytic activity of catalysts. Measuring was performed using spectrophotometry, CV and SWV. Error bars indicate standard deviation for n = 5.

## 4. DISCUSSION

$K_m$ value is a parameter characterizing catalyst and affinity of catalyst to analyzed substrate, that means the lower $K_m$ value the stronger affinity of catalyst to substrate. $K_m$ value of MPs was calculated to be 199 mmol/l, 183 mmol/l and 157 mmol/l for spectrophotometry, SWV and CV assay, respectively. They are quite high values of $K_m$ comparing to $K_m$ values of HRP measured before and it indicates lower affinity of MPs to analyte than the HRP has. Chen and coauthors (2008) [22] measured $K_m$ value of HRP from *Armoracia rusticana* of 2.33 mmol/l and Karasyova and coauthors (2003) [23] measured $K_m$ value of HRP from the same source to be even lower, 0.38 mmol/l [22, 23]. On the other hand, modification of MPs surface may decrease value of $K_m$. Liu and coauthors (2014) [24] compared $K_m$ values of MPs modified by casein and bare MPs. While bare MPs show $K_m$ value 154 mmol/l, casein-MPs exerted $K_m$ value 4.75 mmol/l, the value much closer to native HRP [24]. Also comparison in study of Dutta and coauthors (2013) [25] was concluded by a statement that there is significant difference between modified and bare MPs. The modified MPs by Prussian Blue resulting in $K_m$ value 1.22 mmol/l [25].

In our study, $K_m$ value of HRP was also determined. $K_m$ value of HRP measured by spectrophotometry (25.1 mmol/l) was calculated to be much lower than $K_m$ value of MPs (199 mmol/l) but it didn´t reach such low as $K_m$ values mentioned above in study of Karasyova and coauthors (2013) [23]. It was probably caused by conditions of measuring, which were optimized for measurement of $H_2O_2$ catalyzed by MPs. Our intention was to preserve the same measurement conditions for measuring $K_m$ value of both MPs and HRP for comparison of measured results. According Karasyova and coauthors (2013) [23], pH optimum of peroxidase is equal to 6 and they used phosphate-citrate buffer, so our lower pH (5.5) and used buffer (sodium acetate) are not optimal for determination of $K_m$ value of HRP. Furthermore, in study of Feng and coauthor (2008) [26], different types of HRP and substrates were used and $K_m$ values ranger from 1.48 to 18.8 mmol/l according used type of enzyme and chromogen. Electrochemically determined $K_m$ values of HRP were calculated to be 163 and 167 for CV and SWV respectively in our study. The values correspond with $K_m$ values of MPs, that means affinity of HRP and MPs are very similar. Chen and coauthors (2008) [22] measured $K_m$ value of HRP by amperometry of 2.33 mmol/l. Difference can be caused by modification of electrode by Chen and coauthors (2008) [22] which may improve behavior of biosensors.

Comparison of $K_m$ values and measuring conditions are summarized in Table 1 located below.

Although our study was aimed to demonstration of pseudo-peroxidase activity of MPs, detection limits of all methods were also revealed. Hydrogen peroxide can be determined from biological samples, food, personal non-food products and environmental samples. Peroxidase biosensors are also constructed for measuring of other peroxidase substrates, such as dopamine, alcohols and amino phenols. Because of very low concentrations of determined substances in samples, the possibly lowest limit of detection is required. According Yuen and Benzie (2003) [27] concentration of $H_2O_2$ in urine ranged from 0.84 to 5.71 μmol/l and according Bleau and coauthors (1998) [28] concentration of hydrogen peroxide in aqueous humor ranged from 25 to 60 μmol/l [27, 28]. Levels of $H_2O_2$ in water sample were measured by Sunil and Narayana (2008) [29] and they ranged in values of μmol/l [29]. Ambade and coauthor (2009) [30] measured level of dopamine in

blood and it ranged from 0 to 0.59 nmol/l [30]. Xiao and Becker (1994) [31] determined concentration of extracellular striatal dopamine to be 4.5 nmol/l [31]. Our limits of detection (13.1 mmol/l for spectrophotometry, 0.42 mmol/l for SWV and 0.36 mmol/l for CV) are not sufficiently low for determination substrates of peroxidase from this type of samples. On the other hand, concentration of $H_2O_2$ in non-food products may reach 0.1 % (32.6 mmol/l) in compliance with regulations of European Union and Scientific Committee on Consumer Products [8]. Our method is suitable for control of hydrogen peroxide in the products regulated by European Union.

**Table 1.** Summary of $K_m$ values of HRP, MPs and modified MPs.

| Catalyst | Detection device | Substrate | $K_m$ value (mmol/l) | Reference |
|---|---|---|---|---|
| MPs | spectrophotometry | $H_2O_2$ and o-PD | 199 | This study |
| MPs | SWV | $H_2O_2$ | 183 | This study |
| MPs | CV | $H_2O_2$ | 157 | This study |
| MPs | spectrophotometry | $H_2O_2$ and 3,3′,5,5′-tetramethylbenzidine | 154 | [24] |
| HRP | spectrophotometry | $H_2O_2$ and o-PD | 25.1 | This study |
| HRP | SWV | $H_2O_2$ | 167 | This study |
| HRP | CV | $H_2O_2$ | 163 | This study |
| HRP | amperometry | $H_2O_2$ | 2.33 | [22] |
| HRP | spectrophotometry | $H_2O_2$ and o-PD | 0.380 | [23] |
| native or reconstituted HRPs | spectrophotometry | $H_2O_2$ and p-hydroxybenzoic acid/phenol/4-aminophenol | 1.48-18.8 | [26] |
| Prussian Blue-MPs | spectrophotometry | $H_2O_2$ and 3,5-di-tert-butylcatechol | 1.22 | [25] |
| casein-MPs | spectrophotometry | $H_2O_2$ and 3,3′,5,5′-tetramethylbenzidine | 4.75 | [24] |

## 5. CONCLUSION

In summary, the pseudo peroxidase activity of MPs was demonstrated in all three measuring systems with sufficient sensitivity and low false response. Behavior of measured concentration curves replies Michaelis-Menten kinetics so values of $K_m$ can be set. MPs as mimic peroxidase was used before in spectrophotometric assays, but in this study was also proved their enzymatic activity in electrochemical assays. Even though MPs show lower affinity to substrate than HRP, possible modification of MPs is promising way to rearrange measuring methods for construction of mimic peroxide biosensor using MPs. Limits of detections were also determined for all three measurements. The performed methods are not sensitive enough for determine $H_2O_2$ or other peroxidase substrates from biological samples, but they are sufficiently low for control measurement of $H_2O_2$ content in non-food products. Sensitivity of the methods can be further increased by modification of MPs.

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