Characterization of Mn-nanoparticles decorated organo-functionalized SiO_2–Al_2O_3 mixed-oxide as a novel electrochemical sensor: application for voltammetric determination of captopril

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Abstract

In this work a new sensor in nano size containing Mn–nanoparticles decorated organo-functionalized nanosized SiO_2–Al_2O_3 mixed-oxide support was developed as a new electrocatalysis for oxidation of organic compounds such as captopril. SiO_2–Al_2O_3 mixed–oxide were functionalized with Schiff base ligand and thereatfer, in the next step, Mn–nanoparticles were prepared over the organo–functionalized SiO_2–Al_2O_3 mixed–oxide. The synthesized materials were characterized with different methods such as FT–IR spectroscopy, UV–Vis, CHN elemental analysis, TEM, ICP–OES, cyclic voltammetry, and electrochemical impedance spectroscopy. Under the optimum conditions (pH 7.0) in cyclic voltammetry, the oxidation of the captopril was occurred at a potential about 650 mV at a surface of the modified electrode. Linear sweep voltammetry exhibited two wide linear dynamic ranges of 0.30 – 5.0 and 5.0 – 340 μmol L^{-1} captopril. The detection limit was found to be 0.095 μmol L^{-1} captopril. The kinetic parameters such as electron transfer coefficient and catalytic reaction rate constant were also determined. Finally, the modified electrode used as a novel electrochemical sensor for the determination of captopril in real samples such as pharmaceutical, patient and safe human urine.

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1. Introduction

Nanoparticles of a variety of shapes, sizes and compositions are changing nowadays the bioanalytical measurement.[1] Preparation and investigation of novel nano materials is important in martial science. There are various nano-materials including carbon nanotubes, nano-wires and nano-particles those are important for scientific works. The nanostructures with large specific surface area could provide an important and feasible platform for catalysis,[2] separation,[3] sensing,[4–9] and fuel cells.[10] Derivatization of inorganic solids with organ functional groups was widely studied and successfully applied in many divergent areas of research. This synthetic route is still the subject of considerable interest due to the numerous possibilities of designing new materials, especially by exploiting the versatility of sol–gel chemistry. It is essential to look for a new method with high sensitivity, simplicity and efficiency for the detection of captopril.

Electrochemical techniques using modified electrodes can be considered for the determination of pharmaceutical compounds as a strong alternative to the other instrumental methods. The chemically modified electrodes (CMEs) are very interesting tools for the analysis of many substances at trace level, using very sensitive electroanalytical techniques. An important point in CMEs utilization in speciation work is to choose the most convenient modifier for each analyte, because the sensitivity and selectivity of the electroanalytical response depend on the characteristics of the modifier.

Captopril, 1-(3-mercapto-2-methyl-1-oxopropyl)-L-proline, is a synthetic dipeptide, an active inhibitor of the angiotensin-converting enzyme (ACE) used in clinical practice to treat for

hypertension and heart failure and in a combined therapy of myocardial infarction.[11] Additionally, captopril is the only inhibitor of ACE bearing a thiol group; therefore, it can take up free radicals in living systems and exhibit antioxidant properties.[12–14] Also, captopril is metabolized in liver (it is oxidized into the corresponding disulfide) and is mainly excreted with the urine (40–60%) of the excreted drug remain unchanged in the urine.[15] Therefore, the determination of captopril is important from a physiological point of view as well as for the purposes of quality control. Several methods have already been reported for the determination of captopril in pharmaceutical formulations and clinical samples, including high performance liquid chromatography,[16–22] gas chromatography,[23,24] photometry,[25] spectrophotometry,[26–31] fluorimetry,[32,33] radiochemical,[34] FT–Raman spectroscopy,[35] capillary electrophoresis,[36–38] chemiluminescence [39–42] and electrochemical methods.[43–46]

Here, we have demonstrated the electrocatalytic potential of Mn–nanoparticles decorated organo-functionalized nanosized SiO_2–Al_2O_3 mixed-oxide as a mediator in catalytic determination of captopril. This versatile and mild method for immobilization of functional Mn complex on the surface of a mixed-oxide support allows for unprecedented functionalization densities while retaining Mn ions nanoparticles activity and preventing Mn complex leaching from the mixed-oxide support into the solution. Moreover, the suitability of the modified electrode is discussed by cyclic voltammetry, chronoamperometry, differential pulse voltammetry, and electrochemical impedance spectroscopy. Finally, the ability of this methodology for the determination of captopril in pharmaceutical and illness urines has been discussed.

2. Experimental

2.1. Materials

All reagents were purchased from Merck or Fluka and were used without further purification, except that solvents were treated according to the standard method. Phosphate buffer solution (PBS) with different pH values were used for the study of the influence of pH.

A 1.0×10^{-3} mol L^{-1} captopril solution was prepared daily by dissolving 0.0224 g captopril (97%) in 100 mL water. The solution was kept at 4 °C and in dark. More dilute solutions were prepared by serial dilution with water.

High viscose paraffin (d = 0.88 kg L^{-1}) from Merck was used as the pasting liquid for the preparation of carbon paste electrode. Spectrally pure graphite powder (particle size <50 μm) from Merck was used as the substrate for the preparation of the carbon paste electrode.

Captopril tablets (Darou Pakhsh Company, Iran, labeled 25 and 50 mg captopril per tablet) was purchased from Red Cross drug store in Isfahan.

2.2. Characterization

Diffuse reflectance spectra were recorded using a JASCO V–550 UV–Vis spectrophotometer. Fourier transform IR spectra were recorded using a JASCO FT–IR (680 plus) spectrometer using KBr pellets. The vibrational transition frequencies are reported in wave numbers (cm^{-1}). Elemental analysis was performed by a CHNO–Rapid Heraeus elemental analyzer (Wellesley MA).

Chemical analyses are carried out by inductively coupled plasma optical emission spectroscopy (ICP–OES) using a Shimadzu ARL 34000 instrument (spectroflamed; typically, 30 mg sample was dissolved in 500 μL 40% HF solution, 4 mL 1:4 H_2O:H_2SO_4 solution and 45 mL

H_2O). Nitrogen (99.999%) adsorption experiments have been performed at –196 °C using a volumetric apparatus (Quantachrome NOVA automated gas sorption analyzer). Before the adsorption experiments, the sample was out gassed at 120 °C for 16 h. The specific surface areas are calculated from the BET method.[47]

Transmission electron microscopy (TEM) was carried out on the powder samples with a TecnaiF30-TEM operating at an accelerating voltage of 300 kV. In addition, energy dispersive X–ray analysis was conducted on each sample. Electrochemical measurements were carried out with Micro–Autolab, potentiostat/galvanostat instrument (µ3–AUT70751), connected to a three–electrode cell. Conventional three electrodes cells were used for the all experiments. The modified and unmodified electrodes used as a working electrode, platinum wire as an auxiliary electrode, and an Ag/AgCl/KCl electrode as a reference electrode were used.

Electrochemical impedance measurements were carried out in a conventional three-electrode cell, powered by an electrochemical system comprising the Autolab (AUT83593) at a frequency range of 0.1 Hz to 10000 Hz. The AC voltage amplitude was 5 mV.

2.3. Preparation of the organometallic functionalized nanosized SiO_2–Al_2O_3 mixed-oxide

SiO_2–Al_2O_3 (1:1) was used as a support. The support was prepared by the sol–gel method according to the literature.[48] The support is denoted as SiO_2–Al_2O_3 (1:1). SiO_2–Al_2O_3 (1:1)-supported 2-aminoethyl-3-aminopropyl (scheme 1)[50] was prepared by refluxing 5.2 g SiO_2–Al_2O_3 (1:1), that was activated at 550 °C for 6 h under air, with 4.4 mL (0.0195 mol) of 2-aminoethyl-3-aminopropyl-trimethoxysilane in dry dichloromethane (100 mL) for 24 h. The solid was filtered and washed off with methanol, dichloromethane and dried at 100 °C under vacuum for 6 h. The functionalized SiO_2–Al_2O_3 (1:1) mixed oxide that was prepared with the spacer identified

hereafter by Si–Al–*pr*–NH–*et*–NH_2. Then, methyl-2-pyridylketone was added to a suspended solution of Si/Al–*pr*–NH–*et*–NH_2 in dry methanol. The mixture was refluxed for 24 h to prepare a Schiff base (*vide* scheme 1)[50] on the surface of the mixed-oxide (a bi-dentate ligand). Sensor containing Mn(III)–Schiff base complex was obtained by the procedure previously considered and was fully characterized.[49,50] **"Here Scheme 1"**

2.4. Preparation of the modified electrode

10.0 mg of Mn–nanoparticles immobilized on the organo-functionalized SiO_2–Al_2O_3 mixed–oxide support hand mixed with 80.0 mg of graphite powder in a mortar and pestle. Using a syringe, 0.880 g paraffin was added to the mixture and mixed well for 40 min until a uniformly-wetted paste was obtained. The paste was then packed into a glass tube. Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper. The unmodified carbon paste electrode (CPE) was prepared in the same way without adding Mn–nanoparticles immobilized on the surface of the organo-functionalized SiO_2–Al_2O_3 mixed-oxide support to the mixture to be used for comparison purposes.

2.5. Preparation of real samples

For preparation of tablets solutions, ten tablets of captopril labeled with amount of 25 and 50 mg per tablet, were completely ground and homogenized. 100 mg of the powders was accurately weighed and dissolved in 100 mL water with ultrasonicating. After mixing completely, the mixture was filtered with an ordinary filter paper. Then, the filtered solution was transferred into

a 1000-mL volumetric flask and the solution was diluted to the mark with water. 1.0 mL of the solution plus 9.0 mL of the buffer (pH 7.0) was used for the analysis using standard addition method.

Urine samples were taken from humans and were used for measurements after its centrifuged (3000 rpm, 25 ^{o}C) and diluted two–times with PBS without any further pretreatment. Standard addition method was used for the determination of the captopril contents.

3. Results and discussion

3.1. Characterization of SAPEM

The structures of the obtained organometallic–modified Si–Al mixed oxide was confirmed by elemental analysis, BET (N_2 adsorption–desorption technique), FT–IR spectroscopy, UV–Vis–spectrophotometry, TEM, ICP–OES, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Nitrogen sorption measurements of the modified Si/Al mixed oxide confirm the presence of the Mn–complex attached to the modified Si/Al mixed oxide (Table 1). The considerable decrease in the specific surface area (S_{BET}) clearly indicates functionalization of the surface of the mixed oxide with the Mn–complex. The nitrogen and Mn contents of the organometallic–modified Si/Al mixed oxide (SAPEM) were determined by elemental analysis and ICP, respectively. Consequently, the content of the immobilized SAPEM was computed. The results are listed in Table 1. From these data could calculate that the aluminosilicate support bearing 4.1 mmol g^{-1} of the Schiff base and 3.0 mmol g^{-1} of Mn ions. In fact, during the condensation and immobilization, the dosage of methyl-2-pyridylketone was excessive to minimize the amount of the untreated residual linker on the support. A change in the color of the resulted powders can also be visualized during the reactions (scheme 1)[50].

Here Table 1"

3.1. IR Spectroscopy

Figure 1 shows the IR spectra of different mixed oxide. The band at around 1050 cm^{-1} is due to the asymmetric stretching vibration of $(Si/Al)O_4$ units of Si/Al mixed oxide. The bands at 2851–2921cm^{-1} are assigned to the stretching mode of $–CH_2$ groups. From the presence of these bands it can be inferred that the Si/Al mixed oxide was modified by amine spacer group successfully. The N–H deformation peak at 1540–1560 cm^{-1} confirms the successful functionalization of the Si/Al mixed oxide with 2–AE–3–APTMS. In agreement with previously reported data,[51] C=N (Schiff base) absorptions appear in the 1635 cm^{-1} region, the vibration of C=N in pyridine groups are in the 1571–1569 cm^{-1} range (Fig. 1).[52] The peaks in 3070–3060 cm^{-1} range are attributed to the C–H stretching vibrations of phenyl group. The peaks at 1436 cm^{-1} can be assigned to C=C stretching vibration of pyridine groups. The FT–IR spectra of Schiff base clearly show the C–H vibrations of Py groups at 3070–3060 cm^{-1}, further confirming the existence of pyridine groups on the Si/Al mixed oxide after immobilization of the ligand. Further evidence for this coordination mode was provided by the *v*(Mn–N) bands at ca. 412 cm^{-1}, respectively.[53] The FT–IR results demonstrate the formation of the Mn–complex, immobilized on the Si/Al mixed oxide through 2–AE–3–APTMS linker. **Here Figure 1"**

3.2. Dynamic Reflectance UV–Vis spectroscopy

The UV–Vis spectrum of Si/Al mixed oxide only had a side-band adsorption near 244 nm, while the spectra of the Si/Al–*pr*–NH–*et*–N=methyl–2–pyridylketone–Mn was dominated by strong absorptions in the 250 to 305 nm due to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the ligand (Fig. 2). Furthermore, Si/Al–*pr*–NH–*et*–N=methyl–2–pyridylketone–Mn nanosensor exhibited the

broad and weak bands around 391 nm, probably attributable to the ligand-to-metal charge transfer transitions, similar to the metallosalen compounds.[54] Several low intensity asymmetric shoulder broad bands appeared over 500 nm in the visible region at λ = 530–680 nm are attributed to the d→d transitions expected for the manganese complexes with a square pyramidal geometry, ($d_{xz} \rightarrow d_{x^2-y^2}$), ($d_{yz}$, $d_{xy} \rightarrow d_{x^2-y^2}$) and ($d_{z^2} \rightarrow d_{x^2-y^2}$) which were similar to related metal (salen) compounds described in literatures.[55] DR–UV–Vis and FT–IR spectra reveal that Si/Al–*pr*–NH–*et*–N=methyl–2–pyridylketone–Mn nanosensor is synthesized upon coordination of Mn ions to the organo-functional groups. The charge transfer bands, the stretching frequency at 1635 cm^{-1} (Fig. 1) and the elemental analyses results (Table 1) clearly confirm that the Schiff base ligands and C=N groups are not affected or destroyed through immobilization on the functionalized Si/Al mixed oxide. **"Here Figure 2"**

3.3. TEM study

TEM micrograph of the modified mixed–oxide (Fig. 3) shows the involvement of the Mn complex confined onto the organo-functionalized nanosized SiO_2–Al_2O_3 mixed-oxide (Mn ions probably did not accumulate and dispersed as Mn nanoparticles) are well dispersed**.**

"Here Figure 3"

3.4. Electrochemistry of the mediator

As the Mn complex is insoluble in aqueous solutions, it can be easily incorporated into a carbon paste without concern for its leaching from the electrode surface. This fabrication process yields a stable chemically modified electrode. The electrochemical behavior of Mn^{3+}–modified CPE was investigated using cyclic voltammetry at different scan rates in a 0.1 mol L^{-1} phosphate buffer, pH 7.0 (Fig. 4, inset). As can be seen, the cyclic voltammogram exhibits an anodic peak at the

forward scan of the potential related to the oxidation of the Mn^{3+} to Mn^{4+}. In the reverse scan of the potential, a cathodic peak appears related to the reduction of the Mn^{4+} to Mn^{3+}. The plot of the anodic peak current was linearly dependent on $\upsilon^{1/2}$ with a correlation coefficient of 0.9945 at all scan rates (Fig. 4). This behavior indicates that the nature of the redox process is diffusion controlled. **"Here Figure 4"**

3.5. Catalytic role of the organo-functionalized nanosized SiO_2–Al_2O_3 mixed-oxide for determination of captopril

Captopril is an oxidisable compound and can be detected by electrochemical methods based on anodic oxidation. The use of chemically modified electrodes greatly increases the selectivity and sensitivity toward this compound.[43,45,46] As can be seen in Fig. 5, a significant enhancement in the anodic peak current of captopril at MCPE was achieved at a potential close to the formal potential of Mn^{3+}/Mn^{4+} redox couple along with a decrease in the cathodic current (Fig. 5a). On the other hand, captopril oxidation (without the mediator) does not take place at the surface of the electrode up to +1.00 V (Fig 5c). Figure 4b shows the cyclic voltammograms of the mediator at the surface of GCE in PBS (pH 7.0). In addition, cyclic voltammograms of the organo-functionalized SiO_2–Al_2O_3 mixed–oxide (without Mn) in the absence and presence of 600 μmol L^{-1} captopril were taken as shown in Figs. 5d and 5e, respectively. These figures confirms that Mn–nanoparticles as Mn^{3+}/Mn^{4+} redox couple acts as a main role on the electrocatalytic oxidation of captopril. Therefore, this novel mediator is a suitable for electrocatalytic oxidation of captopril (diagram 1). **"Here Figure 5 & Diagram 1"**

The influence of scan rate on the electrocatalytic oxidation of 400 μmol L^{-1} captopril at MCPE was investigated using cyclic voltammetry (Fig. 6, inset). The results showed that the oxidation peak potential shifts with increasing scan rates towards a more positive potential,

confirming the kinetic limitation of the electrochemical reaction. In addition, a plot of the peaks height (I_p) *vs.* the square root of the scan rate ($v^{1/2}$) in the range of 8 – 30 mV s^{-1} (Fig. 6) was found to be linear, suggesting that at sufficient overpotential the process is diffusion rather than surface controlled. **"Here Figure 6"**

The effect of sample solution pH on the electro-oxidation of captopril at the surface of the modified electrode was studied at different pH values (4.0 to 8.0). The results showed that (Fig. 7) the maximum electrocatalytic current was obtained at pH 7.0 and then it's decreased. At lower pH values, protonation of the –SH group on captopril cause affect its oxidation potential. Therefore, pH 7.0 was chosen for further study. **"Here Figure 7"**

In order to get the information about the rate determining step, a Tafel plot was developed for MCPE in presence of captopril using the data derived from the raising part of the current–voltage curve (Fig. 8). The slope of the Tafel plot is equal to $n(1-\alpha)F/2.3RT$ and equals to 5.5238 V $decade^{-1}$. We can calculate $n\alpha = 0.67$. If assuming $n = 1$, then $\alpha = 0.67$. **"Here Figure 8"**

Chronoamperometry (Fig. 9) can also be employed to evaluate the catalytic rate constant, k, by setting the working electrode potential at 0.30 V (in the first potential step) and 0.70 V (in the second potential step) for the oxidation of captopril at the MCPE according to the method of Galus:[56]

$$I_C/I_L = \gamma^{1/2}[\pi^{1/2}\mathrm{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \qquad (1)$$

where I_C is the catalytic current of captopril at the surface of MCPE, I_L the limited current in the absence of captopril, and $\gamma = kC_bt$ (C_b is the bulk concentration of captopril) is the argument of the error function. In cases where γ exceeds 2, the error function is almost equal to 1 and, therefore, the above equation can be reduced to:

$$I_C/I_L = \pi^{1/2}\,\gamma^{1/2} = \pi^{1/2}\,(kC_bt)^{1/2} \qquad (3)$$

where t is the time elapsed (s). Equation (3) can be used to calculate the rate constant of the catalytic process, k. Based on the slope of I_C/I_L *vs.* $t^{1/2}$ plot (Fig. 9, inset A), k can be obtained for a given captopril concentration. From the values of the slopes, an average value obtained for k as 3.05×10^3 mol^{-1} L s^{-1}. Also, the experimental plots of I *vs.* $t^{-1/2}$ with the best fits for different concentrations of captopril were employed (Fig. 9, inset B). Using these plot and Cottrell equation we calculated a diffusion coefficient of 3.62×10^{-6} cm^2 s^{-1} for captopril.

"Here Figure 9"

3.6. Electrochemical impedance spectroscopy studies

Electrochemical impedance spectroscopy was also employed to investigate the oxidation of captopril at the surface of MCPE. Figure 10 presents Nyquist diagrams of the imaginary impedance (Z_{im}) *vs.* the real impedance (Z_{re}) of the EIS obtained at the modified electrode recorded at 0.60 V dc–offset in the absence (curve a) and in the presence of 500 µmol L^{-1} captopril (curve b) in 0.1 mol L^{-1} PBS (pH 7.0), respectively. In the absence of captopril, the Nyquist diagram comprises a depressed semicircle at high frequencies which may be related to the combination of charge transfer resistance of mediator electrooxidation and the double-layer capacitance, followed by a straight line with a slope of nearly 45°. The latter is due to the occurrence of mass transport process via diffusion. **"Here Figure 10"**

The equivalent circuit compatible with the Nyquist diagram recorded in the absence and presence of captopril is depicted in Fig. 10 (inset). In this circuit, R_s, Q, and R_{ct} represent solution resistance, a constant phase element corresponding to the double-layer capacitance, and the charge transfer resistance associated with the oxidation of low-valence mediator species. W is a finite-length Warburg short-circuit term coupled to R_{ct}, which accounts for the Nernstian diffusion. In the presence of captopril, the diameter of the semicircle decreases, confirming the

electrocatalytic capability of the mentioned electrocatalyst for captopril oxidation. This is due to the instant chemical reaction of captopril with the high-valence mediator species. The catalytic reaction of oxidation of captopril that occurred via the participation of mediator species virtually caused an increase in the surface concentration of low valence species of electrocatalyst, and the charge transfer resistance declined, depending on the concentration of captopril in the solution.

3.7. Stability and reproducibility

The repeatability and stability of MCPE was investigated by cyclic voltammetry measurements of 10.0 μmol L^{-1} captopril. The relative standard deviation (*RSD%*) for ten successive assays was 1.2%. When using five different electrodes, the *RSD%* for five measurements was 1.5%. When the electrode was stored in our libratory, the modified electrode retains 98% of its initial response after a week and 96% after 35 days. These results indicate that MCPE has good stability and reproducibility, and could be used for captopril.

4. Calibration plot and limit of detection

Linear sweep voltammetry was used to determine the concentration of captopril. The results showed two linear segments with different slopes for captopril concentration; namely, for 0.3 – 5.0 μmol L^{-1}, the regression equation was $I_p(\mu A) = (1.2720\pm0.0850)C_{Captopril} + (5.1010\pm0.2120)$ ($r^2 = 0.9882$, n = 5), while for 5.0 – 340 μmol L^{-1} captopril, the regression equation was $I_p(\mu A) = (0.3700\pm0.0120)C_{Captopril} + (11.4580\pm0.3130)$ ($r^2 = 0.9913$, n = 7), where $C_{Captopril}$ is μmol L^{-1} concentration of captopril.

The detection limit was determined at 0.095 μmol L^{-1} captopril according to the definition of $Y_{LOD} = Y_B + 3\sigma$.[57]

5. Interference studies

The influence of various substances as compounds potentially interfering with the determination of captopril were studied under the optimum conditions with 10.0 μmol L^{-1} captopril at pH 7.0. The potentially interfering substances were chosen from the group of substances commonly found with captopril in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5% for the determination of captopril. After the experiments, we found that neither 1000–fold of glucose, sucrose, lactose, fructose and nor 200–fold of alanine, phenyalanine, glycine, lucine, methionine, valine and nor 800–fold of Ca^{2+}, Mg^{2+}, Al^{3+}, NH_4^+, and F^- affected the selectivity. Nor did saturation of starch solution and 150–fold of urea interfere with the determination of captopril. Only ascorbic acid typically shows some interference, it minimized by using ascorbic oxidize enzyme which exhibits a high selectivity to oxidation of ascorbic acid.

5. Real sample analysis

In order to evaluate the applicability of the proposed sensor for the determination of captopril in real samples, we have examined the ability of the electrochemical sensor for the determination of captopril in tablet and urine samples using standard addition method (Table 2). The samples were also analyzed by a standard method including potentiometric titration with potassium iodate.[58] For more investigation, we analyzed captopril in patient human urine and healthy men and women urine that had used captopril. However, repelling of captopril was happening from 1–3 h after consumption of the tablet and from 2.5 h this repelling is maximum.[43] The results obtained for these samples by the proposed method were compared with the standard method statistically,[43] using Student's t–test (for the accuracy), and variance ratio, F–test, (for the

precision) at 95% confidence levels. The results are given in Table 3. This is very interesting that the amount of captopril in patient's urines were maximum at 2.5 h and those values were more than of healthy men and women urine in similar condition (in this work). This fact shows that some part of the drug absorbed in patient body and cannot excreted into urine. Those results demonstrated the ability of MCPE for voltammetric determination of captopril in real samples. A typical LSV for the determination of captopril in a urine sample (Table 3, row no. 7) is shown in Fig. 11. **"Here Tables 2 & 3 and Fig. 11"**

6. Conclusion

In this study, we described an application of the Mn-nanoparticles immobilized on the surface of the organo functionalized nanosized SiO_2–Al_2O_3 mixed-oxide support as a nano mediator in electrocatalytic determination of captopril in aqueous buffer solution (pH 7.0). The results showed that the Mn nanoparticles mediator could catalysis the oxidation of captopril at a pH range 7.0. The kinetic parameters such as electron transfer coefficient and the catalytic reaction rate constant were also determined. The proposed method was also used as a selective, simple and precise new sensor for voltammetric determination of captopril in real samples such as pharmaceutical and urine. In addition, because there was no leaching out of the electroactive material from the electrode, a single electrode surface can be used for multiple analytical determinations over several weeks. Finally, the present work illustrates the interest of SiO_2–Al_2O_3 mixed-oxide, chemically modified with the Schiff base ligand covalently attached to the backbone (SiO_2–Al_2O_3 mixed-oxide), for designing a new highly selective electrode modifier liable to be applied to chemical sensing.

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Table's caption

Table 1 Chemical composition and physicochemical properties of the organometallic functionalized SiO_2/Al_2O_3 mixed oxide.

Table 2 Determination of captopril in tablet and urine sample (n=3).

Table 3 Concentration values obtained from the proposed and the reference method for captopril analysis in urine sample using the proposed method under the optimum conditions (n=3).

Table 1 Chemical composition and physicochemical properties of the organometallic functionalized SiO_2/Al_2O_3 mixed oxide.

Catalyst	Elemental analyses (wt%)[a]		Organic functional group (mmol/g mixed oxide)[b]	Immobilized Mn–Schiff–base complex (mmol/g mixed oxide)[c]	Structural parameters[d]		
	N	Mn			Surface area (m^2/g)	Pore volume (cm^3/g)	Pore diameter (A^o)
SiO_2/Al_2O_3 mixed oxide	–	–	–	–	243	0.028	20
Si/Al-*pr*-NH-*et*-N=methyl-2-pyridylketone	6.2	–	4.4	–	131	0.014	16
Si/Al-*pr*-NH-*et*-N=methyl-2-pyridylketone-Mn	5.7	3.0	4.1	0.56	114	0.014	16

[a] Nitrogen was estimated from the elemental analyses. Mn content determined from ICP analysis.
[b] Determined from the N-contents.
[c] Determined from the Mn-content.
[d] The pore size calculated using the BJH method.

Table 2 Determination of captopril in tablet and urine samples (n=3).

Sample	Captopril added ($\mu mol\ L^{-1}$)	Expected value ($\mu mol\ L^{-1}$)	Captopril founded ($\mu mol\ L^{-1}$)	Standard Method ($\mu mol\ L^{-1}$)
Tablet[a]	––	20.0	20.23±0.30	20.95±1.10
	10.0	30.0	29.58±0.61	30.61±0.85
	10.0	40.0	39.83±0.44	39.54±0.83
Tablet[b]	––	50.0	50.33±0.41	50.68±0.91
	10.0	60.0	60.21±0.38	59.42±1.02
	20.0	80.0	80.12±0.25	80.56±0.75
Urine	––	15.0	15.23±0.59	15.64±0.74
	10.0	25.0	24.65±0.51	25.45±0.65
	10.0	35.0	35.43±0.48	35.57±0.75

[a] 50 mg tablet, Darou Pakhsh Company, Iran
[b] 25 mg tablet, Darou Pakhsh Company, Iran

Table 3 Concentration values obtained from the proposed and the reference method for captopril analysis of urine sample using the proposed method under optimum conditions (n=3).

Sample	Proposed method (μmol L^{-1})	Standard method (μmol L^{-1})	F_{ex}	F_{tab}	t_{ex}	$t_{tab(95\%)}$
Urine[a]	3.24±0.35	3.55±0.85	6.5	19	2.4	3.8
Urine[b]	4.45±0.53	4.75±1.02	7.9	19	2.9	3.8
Urine[c]	5.50±0.85	5.95±1.12	8.5	19	3.3	3.8
Urine[d]	4.85±0.55	5.01±0.84	7.5	19	2.8	3.8
Urine[e]	4.93±0.44	4.85±0.65	6.8	19	2.6	3.8
Urine[f]	10.32±0.45	10.85±0.96	7.1	19	2.7	3.8
Urine[g]	9.55±0.32	9.28±0.55	5.5	19	1.9	3.8

±Shows the standard deviation.

a Sampling was made after 1.0 h from a man who had heart problem and used captopril.

b Sampling was made after 2.0 h from a man who had heart problem and used captopril.

c Sampling was made after 2.5 h from a man who had heart problem and used captopril.

d Sampling was made after 2.5 h from a woman had heart problem and used captopril.

e Sampling was made after 3.0 h from a man who had heart problem and used captopril.

f Sampling was made after 2.5 h from a woman who is safe and used captopril.

g Sampling was made after 2.5 h from a man who is safe and used captopril.

Legends for the figures, schemes, and diagram:

Fig. 1 FT–IR spectra the organometallic functionalized SiO_2/Al_2O_3 mixed oxide in the region 4000–400 cm^{-1}. Legends: a) Si/Al–*pr*–NH–*et*–NH_2; b) Si/Al–*pr*–NH–*et*–N=methyl–2–pyridylketone; c) Si/Al–*pr*–NH–*et*–N=methyl–2–pyridylketone–Mn.

Fig. 2 UV–Vis diffuse reflectance spectra of the organo-functionalized SiO_2–Al_2O_3 mixed-oxide and immobilized manganase nanomediator. a): SiO_2/Al_2O_3 mixed oxide; b) Si/Al-*pr*-NH-*et*-N=methyl-2-pyridylketone; c) Si/Al-*pr*-NH-*et*-N=methyl-2-pyridylketone–Mn (red line).

Fig. 3 TEM micrograph of Si/Al–*pr*–NH–*et*–N=methyl–2–pyridylketone–Mn modified electrode..

Fig. 4 Plot of I versus $\nu^{1/2}$ for the oxidation of MCPE. Inset: The cyclic voltammograms at various scan rates of: (1) 5.0; (2) 10.0; (3) 30.0; (4) 50.0; (5) 70.0, (6) 85.0 and (7) 95.0 mV s^{-1} in 0.1 mol L^{-1} PBS (pH 7.0).

Fig. 5 Cyclic voltammograms of MCPE in 0.1 mol L^{-1} PBS (pH 7.0) at a scan rate of 25 mV s^{-1} a) In the presence of 600 μmol L^{-1} captopril; b) in the absence of captopril. c) is as (a) at CPE without the mediator; d) Cyclic voltammogram of CPE with SiO_2, Al_2O_3 and the other attached ligands (without Mn) in the presence of 600 μmol L^{-1} captopril; and e) Cyclic voltammogram of CPE with SiO_2, Al_2O_3 and the other attached ligands (without Mn) in the absence of captopril.

Fig. 6 Plot of I versus $\nu^{1/2}$ for the oxidation of captopril at MCPE. Insert A) Cyclic voltammograms of 400 μmol L^{-1} captopril at various scan rates; 1) 8.0; 2) 10.0; 3) 17.0; 4) 25 and 5) 30 mV s^{-1} in 0.1 mol L^{-1} PBS (pH 7.0).

Fig. 7 Current–pH curve for electro-oxidation of 600 μmol L^{-1} captopril at MCPE with a scan rate of 25 mV s^{-1}.

Fig. 8 Tafel plot for MCPE in 0.1 mol L^{-1} PBS (pH 7.0) with a scan rate of 17.0 mV s^{-1} in the presence of 200 μmol L^{-1} captopril.

Fig. 9 Chronoamperograms obtained at MCPE a): in the blank solution; b) in the presence of b) 100, c) 300 d) and 400 μmol L^{-1} captopril at pH 7.0. Inset A: Dependence of I_c/I_L on the $t^{1/2}$ derived from the chronoamperograms data. Inset B: Plot of I *vs.* $t^{-1/2}$ for the data from the chronoamperograms.

Fig. 10 Nyquist diagrams of MCPE a) in the absence, and b) in the presence of 500.0 μmol L^{-1} captopril.

Fig. 11 Linear sweep voltammograms of MCPE in a solution containing 5.0 mL of the buffer (pH 7.0) and 5.0 ml of a urine sample for row No. 7 from Table 3. Captopril added as a) 0.0; b) 0.9; c) 2.3; d) 3.0; and e) 15.0 μmol L^{-1}.

Scheme 1 Immobilization procedure of the organometallic functionalized SiO_2–Al_2O_3 mixed-oxide.

Diagram 1 Catalytic mechanism for the oxidation of captopril at the surface of the modified electrode.