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# Nonenzymatic coated screen-printed electrode for electrochemical determination of acetylcholine

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## Abstract

In the present study, a screen-printed electrode based-sensor with electrochemical detection was developed for rapid and sensitive determination of acetylcholine. At first, the screen-printed carbon electrode was modified by using a magnetic core shell and then was used for voltammetric determination of acetylcholine in ampoule, serum and urine samples. The electrochemical behaviour of acetylcholine at the modified electrode was investigated by cyclic voltammetry. The modified electrode displayed a decrease in the overpotential (ca. 130 mV) and an obvious increase in the peak current compared to the non-modified screen-printed electrode. The results indicated that modified screen-printed electrode enhanced electrocatalytic activity towards the oxidation of acetylcholine. Under optimized conditions, the limit of detection from the experiment of acetylcholine determination was 0.02  $\mu\text{M}$  with acetylcholine concentration in range of 0.1–500.0  $\mu\text{M}$ . The reproducibility of the measurements was tested by recording the responses for 50.0  $\mu\text{M}$  acetylcholine with four different developed sensors prepared on the same manner and the same day. A relative standard deviation value of 4.3% was obtained. Finally, a recovery test is done as a part of accuracy evaluation of the system.

**Keywords:** Acetylcholine determination, Magnetic core shell nanoparticles, Screen-printed carbon electrode, Voltammetry

## Introduction

The nervous system plays an important role in the human body and control body functions by regulating and coordinating their various activities, which is mainly carried out in the form of electrochemical signaling by neurotransmitters [1].

Acetylcholine (ACh) is an important neurotransmitter in both peripheral and central nervous systems. ACh have vital roles in the brain. ACh is implicated in many human behaviors, such as arousal and attention, and plays a key role in memory formation and learning. Decreases in the level of ACh cause various neurological disorders, including Parkinson's disease, Alzheimer's and dementia, and schizophrenia. On the other hand,

increases in the level of ACh result in a decreased heart rate and increased production of saliva [2–6]. Hence, the quantitative determination of ACh is very important in biological sciences and clinical analysis.

Various methods had been reported for ACh detection which includes matrix-assisted laser desorption ionization time-of-flight mass spectrometry [7], high performance liquid chromatography coupled to post-column chemiluminescence detection [8], gas chromatography mass spectrometry [9], capillary zone electrophoresis [10], potentiometry [11], colorimetric detection [12], optical detection [13], chemiluminescence [14, 15], photoelectrochemistry [16] and enzymatic electrochemical biosensors [17]. These methods are complicated, time-consuming and require expensive equipment that is not practical for widespread application.

Over the past decade, development of electrochemical non-enzymatic ACh biosensors has risen at considerable rate for ACh detection. The advantages include simple

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design, cost effectiveness, good stability and effective enzyme-like catalysis against temperature and pH [18].

Nanomaterials, because of their unique properties, have been extensively developed. Nanoparticles can act as conduction centers facilitating the transfer of electrons and provide great catalytic surface areas [19–23]. Among them, nanosized metal particle modified electrodes have emerged as a promising alternative for the electroanalysis of organic and inorganic compounds [24–28]. Metal nanoparticles have some distinct advantages such as higher mass transport, lower influence of the solution resistance, low detection limit, and better signal-to noise ratio over the conventional macroelectrodes [29–31].

The development of screen-printed electrodes (SPEs) has become a major revolution in the construction of electrochemical sensors/biosensors [32]. The SPEs have been designed especially for miniaturization of electrochemical analytical systems [33]. SPEs are highly-versatile, easy to use, cost-effective analytical tools, also suitable to miniaturization [34]. Furthermore, a SPE avoids the cleaning process, unlike conventional electrodes such as a GCE [35].

In the present work, we synthesized magnetic core-shell manganese ferrite nanoparticles (MCSNP) [36] and screen printed carbon electrodes were modified with MCSNP. To the best of our knowledge, no study has been reported so far on the determination of acetylcholine by using MCSNP/SPE.

## Experimental

### Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302 N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System software. Screen printed carbon electrodes were purchased from Drop Sens Co. A Metrohm 710 pH meter was used for pH measurements.

Acetylcholine chloride and all the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0–9.0. Magnetic core-shell manganese ferrite nanoparticles were synthesized in our laboratory as reported previously [36].

### Preparation of the electrode

The bare screen-printed electrode was coated with MCSNP as follows. A stock solution of MCSNP in 1 mL aqueous solution was prepared by dispersing 1 mg MCSNP with ultrasonication for 1 h, and a 4  $\mu$ L aliquot of the MCSNP/H<sub>2</sub>O suspension solution was casted on

the carbon working electrodes, waiting until the solvent was evaporated in room temperature.

### Preparation of serum, ampule and urine samples

The sample of the ACh ampule was prepared by the appropriate dilution with 0.1 M PBS solution (pH 7.0) and directly used for determination of ACh. Finally, a suitable volume of the resultant solutions were transfer to electrochemical cell and the resulting solution was used for the analysis of ACh.

Human serum samples were obtained from a Hospital in Kerman. The only pretreatment was ten-fold dilution of serum sample with buffer solution.

Urine samples were stored in a refrigerator immediately after collection. Twenty milliliters of the sample was centrifuged for 10 min at 3000 rpm. The supernatant was filtered out using a 0.45  $\mu$ m filter. Then, different volume of the solution was transferred into a 50 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine sample was spiked with different amounts of ACh. The ACh contents were analyzed by the proposed method using the standard addition method in order to prevent any matrix effect.

The samples were spiked with different amounts of ACh and contents were analyzed by using the standard addition method in order to prevent any matrix effect. The amount of unknown ACh in the ACh ampule can be detected by extrapolating the plot.

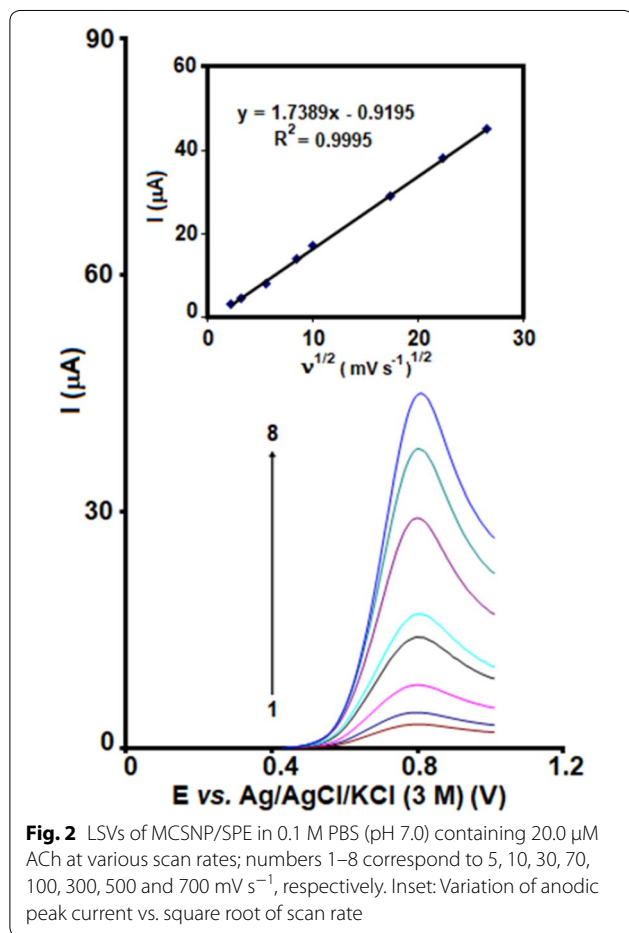
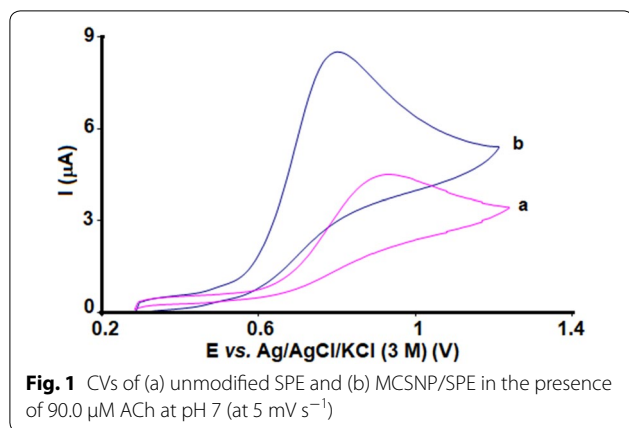
## Results and discussion

### Electrochemical behavior of acetylcholine at the surface MCSNP/SPE

The electrochemical behavior of ACh is dependent on the pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the best results for electrooxidation of ACh. Thus the electrochemical behaviors of ACh were studied in 0.1 M PBS in different pH values (2.0–9.0) at the surface of MCSNP/SPE by voltammetry. It was found that the electro-oxidation of ACh at the surface of MCSNP/SPE was more favored under neutral conditions than in acidic or basic medium. Thus, the pH 7.0 was chosen as the optimum pH for electro-oxidation of ACh at the surface of MCSNP/SPE.

Figure 1 depicts the CV responses for the electro-oxidation of 90.0  $\mu$ M ACh at an unmodified SPE (curve a) and MCSNP/SPE (curve b). The peak potential due to the oxidation of ACh occurs at 800 mV, which is about 130 mV more negative than that of unmodified SPE.

Also, MCSNP/SPE shows much higher anodic peak current for the oxidation of ACh compared to unmodified SPE, indicating that the modification of unmodified



SPE with MCSNP has significantly improved the performance of the electrode toward ACh oxidation.

**Effect of scan rate**

The effect of potential scan rates on the oxidation current of ACh (Fig. 2) have been studied. The results showed

that increasing in the potential scan rate induced an increase in the peak current. In addition, the oxidation processes are diffusion controlled as deduced from the linear dependence of the anodic peak current ( $I_p$ ) on the square root of the potential scan rate ( $v^{1/2}$ ).

Tafel plot was drawn from data of the rising part of the current voltage curve recorded at a scan rate of 5 mVs<sup>-1</sup> for ACh (Fig. 3). This part of voltammogram, known as Tafel region, is affected by electron transfer kinetics between substrate (ACh) and MCSNP/SPE. Tafel slope of 0.1298 V was obtained which agree well with the involvement of one electron in the rate determining step of the electrode process [37] assuming charge transfer coefficients,  $\alpha = 0.55$  for ACh.

**Chronoamperometric measurements**

Chronoamperometric measurement of ACh at MCSNP/SPE was carried out by setting the working electrode potential at 1000 mV vs. Ag/AgCl/KCl (3.0 M) for the various concentrations of acetylcholine (Fig. 4) and in PBS (pH 7.0). For electroactive materials (ACh) with a diffusion coefficient of  $D$ , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation: [37]

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \tag{1}$$

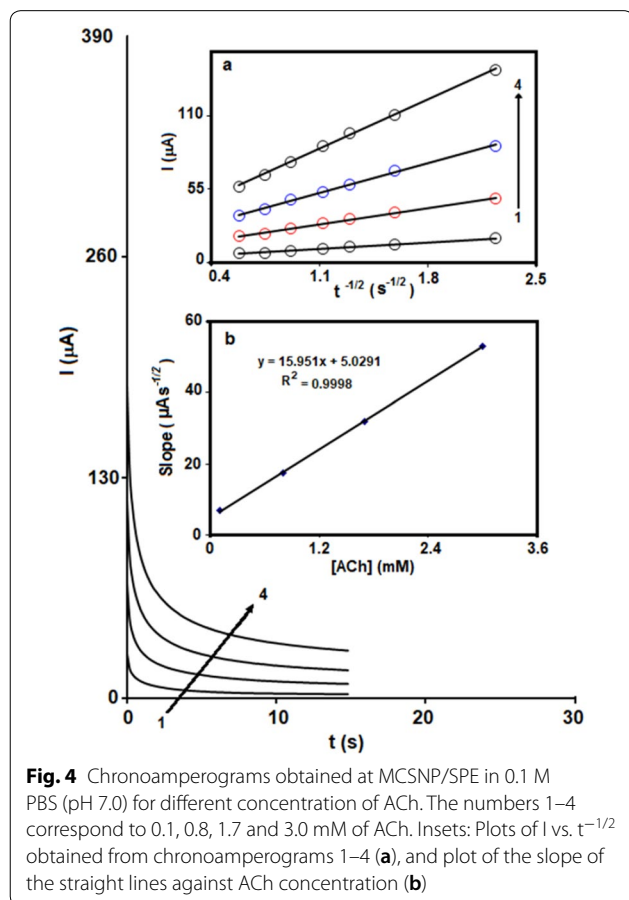
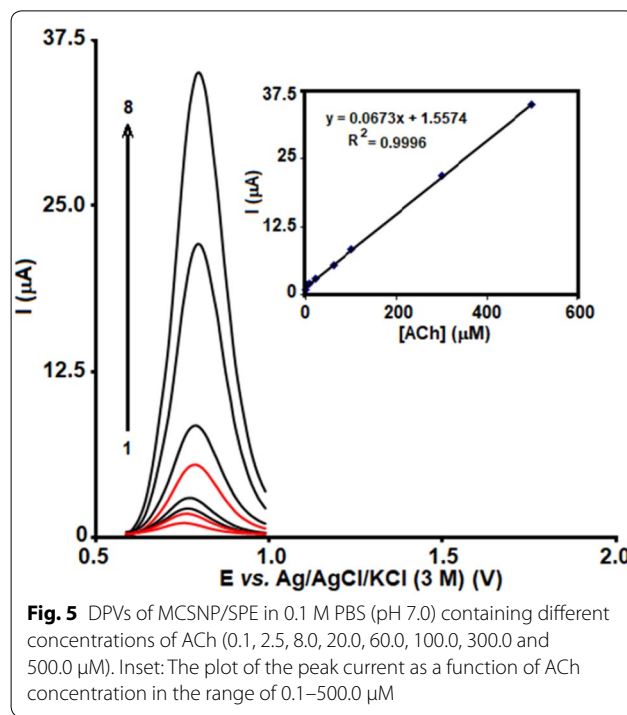
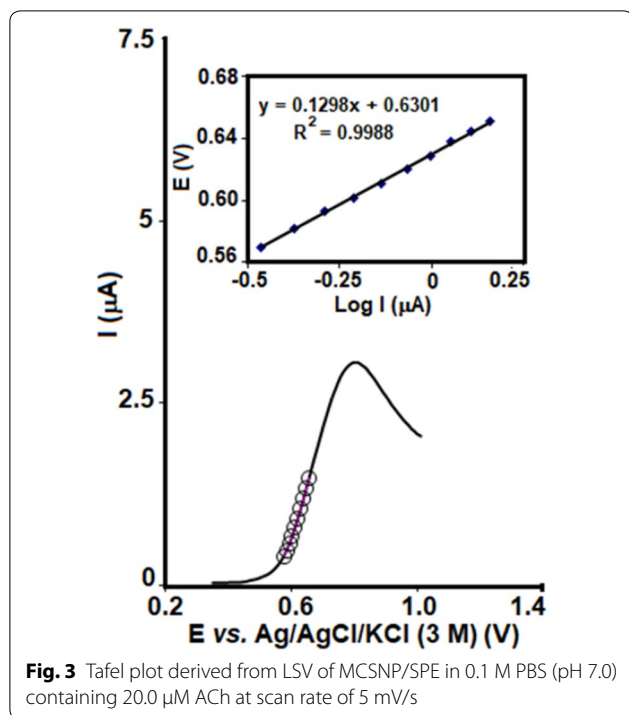
where  $D$  and  $C_b$  are the diffusion coefficient (cm<sup>2</sup> s<sup>-1</sup>) and the bulk concentration (mol cm<sup>-3</sup>), respectively. Experimental plots of  $I$  vs.  $t^{-1/2}$  were employed, with the best fits for different concentrations of ACh (Fig. 4a). The slope of the resulting straight lines were then plotted vs. ACh (Fig. 4b) concentrations. From the resulting slope and Cottrell equation the mean value of the  $D$  was found to be  $8.7 \times 10^{-5}$  cm<sup>2</sup>/s for ACh.

**Calibration plots and limit of detection**

The electro-oxidation peak current of ACh at the surface of the MCSNP/SPE can be used for determination of ACh in solution. Since, DPV has the advantage of an increase in sensitivity and better characteristics for analytical applications, therefore, DPV experiments were performed using MCSNP/SPE in 0.1 M PBS containing various concentrations of ACh (Fig. 5). The results show the electrocatalytic peak currents of ACh oxidation at the surface of MCSNP/SPE was linearly dependent on the ACh concentrations, over the range of  $1.0 \times 10^{-7}$ – $5.0 \times 10^{-4}$  M (with a correlation coefficient of 0.9996) and the detection limit ( $3\sigma$ ) was obtained  $2.0 \times 10^{-8}$  M.

**Interference studies**

The influence of various substances as compounds potentially interfering with the determination of ACh was studied under optimum conditions. The potentially



interfering substances were chosen from the group of substances commonly found with ACh 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  $\pm 5\%$  in the determination of ACh. According to the results, L-lysine, glucose, NADH, acetaminophen, uric acid, L-asparagine, L-serine, L-threonine, L-proline, L-histidine, L-glycine, L-phenylalanine, lactose, saccharose, fructose, benzoic acid, methanol, ethanol, urea, caffeine, dopamine, epinephrine, norepinephrine, serotonin, ascorbic acid, isoproterenol, levodopa, carbidopa,  $Mg^{2+}$ ,  $Al^{3+}$ ,  $NH_4^+$ ,  $Fe^{+2}$ ,  $Fe^{+3}$ ,  $F^-$ ,  $SO_4^{2-}$  and  $S^{2-}$  did not show interference in the determination of ACh (equal molar). The results were shown in Table 1.

**The repeatability and stability of the MCSNP/SPE**

The long-term stability of the MCSNP/SPE was tested over a 3 week period that stored in atmosphere at room temperature. Then, CVs were recorded. The results were showed that the peak potential for ACh oxidation was unchanged and the current signals showed less than 2.6% decrease relative to the initial response. The antifouling properties of the MCSNP/SPE toward ACh oxidation and its oxidation product was investigated by recording the CVs of the MCSNP/SPE before and after use in the presence of ACh. CVs were recorded in the presence of ACh after having cycled the potential 15 times at a scan rate of 50 mV/s. The results were showed that the peak potentials

**Table 1 Effect of interference in the determination of 50.0  $\mu$ M ACh (equal molar)**

Interference	Current in the absence of interference ( $\mu$ A)	Current in the presence of interference ( $\mu$ A)
L-Lysine	4.98	5.07
Glucose, L-asparagine, saccharose, isoproterenol	4.98	5.18
NADH	4.98	4.82
Acetaminophen	4.98	5.04
Uric acid	4.98	4.98
L-Serine, dopamine	4.98	5.09
L-Threonine	4.98	4.92
L-Proline, benzoic acid	4.98	4.81
L-Histidine	4.98	4.90
L-Glycine, Fe <sup>+2</sup>	4.98	5.13
L-Phenylalanine, Al <sup>3+</sup>	4.98	4.91
Lactose, norepinephrine	4.98	5.22
Fructose, ascorbic acid	4.98	4.75
Methanol, serotonin, Fe <sup>+3</sup>	4.98	5.20
Ethanol	4.98	5.01
Urea	4.98	5.12
Caffeine	4.98	4.96
Epinephrine	4.98	4.89
Levodopa	4.98	5.16
Carbidopa, Mg <sup>2+</sup>	4.98	4.80
NH <sub>4</sub> <sup>+</sup>	4.98	5.05
F <sup>-</sup>	4.98	4.76
SO <sub>4</sub> <sup>2-</sup>	4.98	4.95
S <sup>2-</sup>	4.98	5.15

were unchanged and the currents decreased by less than 2.3%. Therefore, on the surface of the MCSNP/SPE, not only does the sensitivity increase, but the fouling effect of the analyte and its oxidation product also decreases.

#### ACh ampule, urine and serum analysis

Finally, MCSNP/SPE was applied for determination of ACh in ACh ampule, serum and urine samples. For this purpose, the determination of ACh in these samples were carried out by using standard addition method to prevent any matrix effects. The results are shown in Table 2. Also, the recovery of ACh from samples spiked with known amounts of ACh was studied. The results were showed that, the added ACh was quantitatively recovered from these samples. These results demonstrate the applicability of the MCSNP/SPE for determination of ACh in the ampule, serum and urine samples. Also, the reproducibility of the method was demonstrated by the mean RSD.

**Table 2 The application of MCSNP/SPE for determination of ACh in ampoule, serum and urine samples (n = 5)**

Sample	Spiked ( $\mu$ M)	Found ( $\mu$ M)	Recovery (%)	RSD (%)
ACh ampoule	0.0	34.0	–	2.6
	10.0	43.7	97.0	3.0
	20.0	54.4	102.0	2.5
	40.0	73.0	97.5	3.1
Urine	0	Not detect	–	3.2
	10.0	10.2	102.0	2.6
	20.0	20.3	101.5	3.1
	40.0	40.9	102.2	2.9
Serum	0	Not detect	–	4.1
	10.0	9.7	97.0	3.8
	20.0	20.6	103.0	4.2
	40.0	41.5	103.8	3.7

The amounts of ACh in ampule was found to be 50.07 mg/mL. It was found that there is no significant difference between the results obtained by the MCSNP/SPE and the nominal value on the ampule label (50.00 mg/mL). The t-test was applied to both sets of results and showed that there was no significant difference at the 95% confidence level.

#### Conclusions

In this work, employing magnetic core shell nanoparticles as modifier in modification of SPEs, a novel sensor has been developed that provides a sensitive method for the determination of ACh. The proposed protocol demonstrated herein a novel, simple, portable, inexpensive and easy-to-use fabrication method for the measurement of ACh concentration in ampoule, serum and urine samples with good analytical performance. Due to the unique properties of magnetic core shell nanoparticles, the sensor exhibited remarkable electrochemical activity toward the oxidation of ACh. Under optimized conditions, DPV exhibited linear dynamic ranges from 0.1 to 500  $\mu$ M with detection limit of 20.0 nM.

#### Authors' contributions

SZM and HB conceived experiments and discussed results. ST and HB designed assays and performed experiments. SZM and HB analysed data. SZM wrote the manuscript with input from ST and HB. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

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