



Research Article

Utilizing Nanoparticle Catalyzed TMB/H₂O₂ System for Determination of Aspirin in Exhaled Breath Condensate

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Abstract

Background: According to the poison center data for most countries, more than thousands of people's exposure to aspirin or salicylate-containing products. So, this work aimed was to offer a rapid colorimetric method for monitoring aspirin concentration in exhaled breath condensate (EBC).

Methods: A method based on a redox reaction catalyzed by nanoparticles was validated for the analysis of aspirin. 3,3',5,5'-Tetramethyl benzidine /H₂O₂ and sodium dodecyl sulfate modified silver nanoparticles were used as the redox reagents and catalyst, respectively.

Results: Detection mechanism of aspirin using this system is based on the inhibitory effect of aspirin on the signal intensity of the colorimetric systems. Since the decrement in signal intensity was proportional to aspirin level, a colorimetric method was proposed for its quantification in EBC samples. This method shows a linear relationship with aspirin concentration in the range of 10–250 mg.L⁻¹ with a relative standard deviation of < 3.5%.

Conclusion: This method has great potential for aspirin determination due to some features such as high reliability, and fast response time.

Introduction

Aspirin is an analgesic, and antipyretic drug that has anti-inflammatory effects by inhibiting the cyclooxygenase and preventing the synthesis of prostaglandins. It also inhibits the production of thromboxane A₂ in platelets by inhibiting cyclooxygenase and preventing platelet aggregation.¹ Aspirin is immediately absorbed from the gastrointestinal tract in the forms of aspirin and salicylate, and its maximum serum level typically achieved in 1 hour.² According to the poison center data for most countries, more than thousands of people's exposure to aspirin or salicylate-containing products.²⁻⁴ Although the overall death in aspirin/salicylate poisoning is not high, such figures can be very deceptive as severe poisoning leads to metabolic acidosis, convulsions, coma, renal failure, hyperpyrexia, and pulmonary edema.⁵ Death happens in 5% of individuals who exposures such a severe poisoning and is related to cardiac arrest or multiple complications after severe brain damage.⁶ In severe poisoning with

aspirin/salicylate, delay in diagnosis leads to a 15% mortality in comparison to a much lower rate in patients in whom early diagnosis and initiation of treatment were performed.⁷

Literature study shows that there were various methods for the determination of aspirin such as UV spectrophotometry,^{8,9} HPTLC,¹⁰ GC,¹¹ fluorimetry,¹² voltammetry,¹³ and HPLC-UV¹⁴ individually and in combined dosage form with other drugs. The chromatographic methods have some drawbacks such as time consuming, utilizing expensive and hazardous solvents and needing a skilled operator and the normally spectroscopic methods suffer from a lack of enough sensitivity and selectivity. So, the proposition and validation of a reliable technique for the determination of drug' levels in the biological samples have always attracted the attention of clinicians. Besides two factors, selectivity and sensitivity, should not be ignored in clinical applications. In the last decades, growing attention has been attracted to the use of

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nanostructures for drugs determination.¹⁵ Nanostructures with particular optical properties due to their size can have significant advantages for biosensing¹⁶ and can be used as a sensor, adsorbent, catalyst, substrate, etc.¹⁷

Selection of sample type for drug determination is considered as an important issue in clinical studies. Recently, exhaled breath condensate (EBC) was introduced as a possible alternative sample type for drug monitoring and pharmacokinetic/pharmacodynamics studies of drugs.¹⁸ EBC is a low-protein and highly diluted aqueous matrix containing volatile and non-volatile compounds which reflects the biomarker and/or drug concentration in the lungs and the blood.¹⁹ EBC possesses a few numbers of interfering compounds compared with other biological fluids such as plasma, blood, urine, saliva and sputum.²⁰ The published studies confirmed that biomarkers, drugs or metabolites can be detected in EBC samples.²¹⁻²³ In the current work, to continue our research on drug analysis in the exhaled breath condensate (EBC) samples as a non-invasive biological fluid, sodium dodecyl sulfate modified silver nanoparticles (SDS-capped Ag NPs) were synthesized and used as an enzyme mimetic for a catalytic reaction of 3,3',5,5'-tetramethyl benzidine (TMB) with H₂O₂. The mentioned system was used as a colorimetric probe for the determination of aspirin concentration in the EBC samples. Optimization procedure was performed by one-at-a-time method and after validation of the method, it was used for aspirin analysis in the real samples.

Materials and Methods

Reagents and solutions

The deionized water (Shahid Ghazi Pharmaceutical Co., Iran), silver nitrate (AgNO₃, Merck, Germany), sodium borohydride (NaBH₄, Sigma Aldrich, USA), SDS (Carlo erba Reactifs, Italy), TMB (Merck, Germany), hydrogen peroxide (H₂O₂, Merck, Germany), and sodium dihydrogen phosphate (NaH₂PO₄, Scharlau, Spain) were applied in the current work. Aspirin was obtained from Temad Pharmaceutical Co. Tehran, Iran. A 1000 mg.L⁻¹ aspirin solution was prepared freshly every day by dissolving a defined amount in methanol.

Apparatus

For measuring the absorbance of the solutions, a double-beam UV-Vis spectrophotometer model UV-1800 (Shimadzu, Japan, www.shimadzu.com) with 1.0 cm quartz cells was employed. A digital pH-meter model 744 (Metrohm Ltd., Switzerland, www.metrohm.com) and an electronic analytical balance model AB204-S (Mettler Toledo, Switzerland, www.mt.com) were used in this work. To investigate the properties of the nanoparticles, a transmission electron microscopy (TEM) model CM30 (Philips, The Netherlands), field emission scanning electron microscope (SEM) model MIRA 3 (TESCAN, Czech Republic) and energy dispersive X-ray (EDX) analyzer connected to the same FE-SEM device were employed. Powder X-ray diffraction (XRD) measurement was used

to crystallinity of the SDS-modified NPs in the scanning angle (2θ) range of 5-70° by using a D500 (Bruker AXS, Germany) device. FT-IR spectra were recorded with a Bruker-Tensor 270 spectrometer (Bruker, Germany) in the wavelength range of 4000 – 400 cm⁻¹.

Preparation of SDS-capped Ag NPs

SDS-capped Ag NPs were prepared by following a chemical reduction method given in the literature.²⁴ After stirring 50 mL deionized water composed of 0.0038 g NaBH₄ and 0.0085 g SDS for 30 min at room temperature, 0.0017 g AgNO₃ was dissolved in 50 mL deionized water and added droply into the prepared solution. The resulting mixture was stirred for 1 h in a dark place at room temperature. The as-prepared NPs were kept in a dark place at 4 °C.

Sample preparation

EBC samples employed for the current study were collected by a lab-made system.²⁵ EBC samples for the optimization and validation of the proposed method were obtained from a healthy volunteer. EBC samples were directly used without any preparation step. EBC samples employed in the real sample analysis were obtained from six patients taking aspirin treatment. Sample donors signed a consent form approved by the ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1399.322).

General procedure

Aspirin analysis was performed in a 2 mL micro-tube by a batch method. Briefly, 20 μ L of 0.1 mol.L⁻¹ phosphate buffer (pH 5.0), and 20 μ L of 3.0 mmol.L⁻¹ TMB, and 20 μ L of H₂O₂ (7.5% v/v) were added into 0.25 mL of EBC spiked with a proper amount of standard aspirin solution in the range of 10.0 to 250.0 mg.L⁻¹. Then, 12.5 μ L Ag NPs were added to the mentioned mixture and its volume was adjusted to 0.5 mL with deionized water and the signal intensity was read at $\lambda = 680$ nm as an analytical signal.

Results and Discussion

Characterization of SDS-capped Ag NPs

The size and shape of the NPs were confirmed by TEM and SEM analysis. Figures 1a and b present that NPs have a spherical morphology with a mean dimension of <20 nm. XRD pattern was measured to identify the crystallinity of the modified Ag NPs. The XRD analysis (Figure 1c) shows three characteristic peaks at 38.3°, 44.5° and 64.8° related to (111), (200) and (220) planes, respectively. By comparing JCPDS (file no: 89-3722), the typical pattern of as-prepared NPs was an FCC structure.²⁶

For a surface study of the as-prepared NPs, FT-IR was measured and shown in Figure 1d. The peaks at 2922 and 2852 cm⁻¹ were related to the C-H bonds of SDS and the peaks at 989–1075 cm⁻¹ and 824 cm⁻¹ were subscribed to symmetric and antisymmetric stretching bonds of the SO₄ part of SDS and the bending vibration of the S-O-C part of SDS showing that Ag NPs has been well functionalized with SDS.²⁷ Furthermore, the preparation of the modified

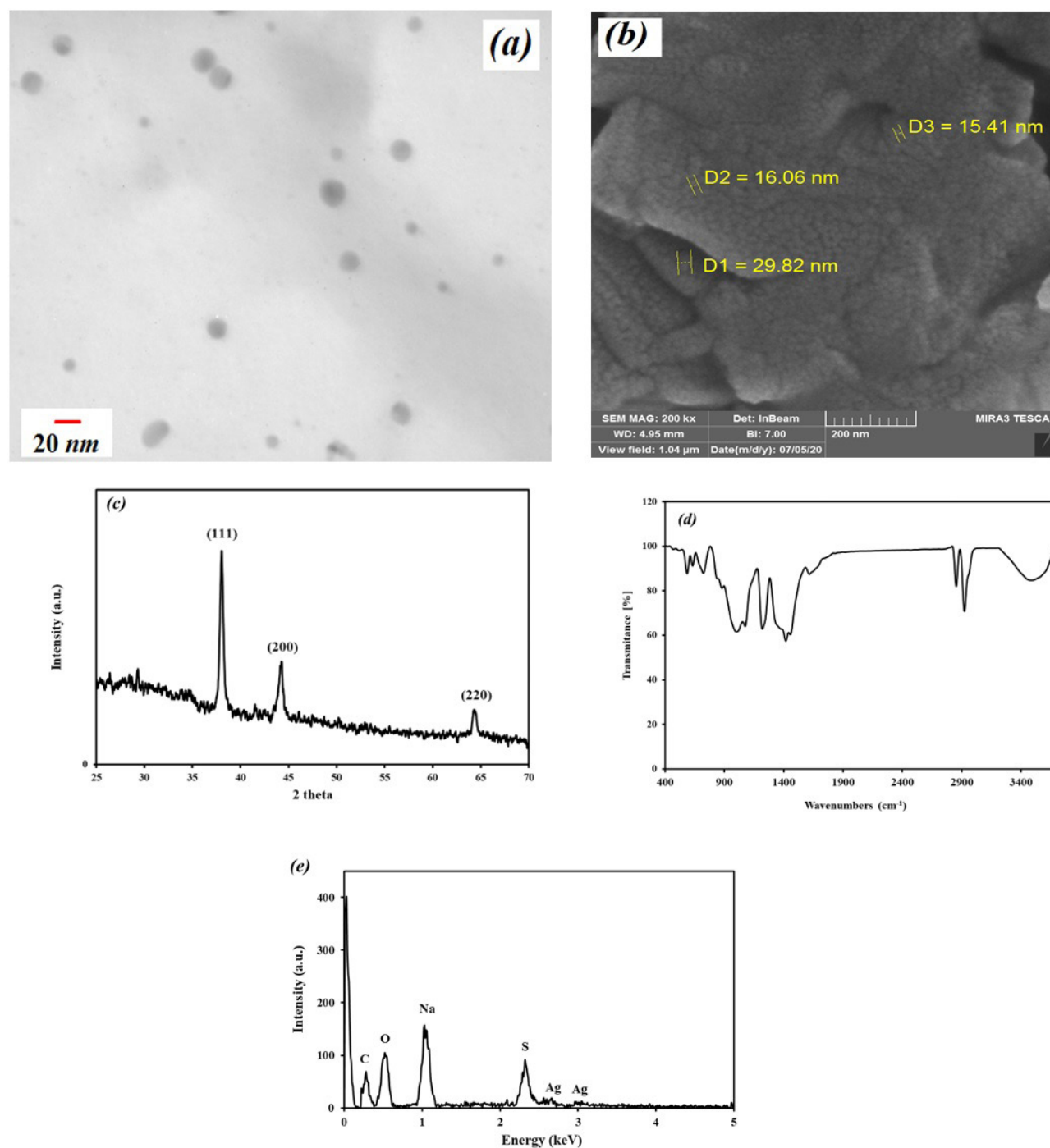


Figure 1. (a) TEM image, (b) SEM image (c) XRD patterns; (d) FT-IR spectrum; and (e) EDX of the SDS- capped Ag NPs.

NPs was also confirmed by EDX analysis. The distribution of silver, sodium, oxygen, sulfur and carbon elements was clearly observed in Figure 1e.

Detection mechanism discussion

TMB itself shows no absorbance or special color in the visible region; however, in the presence of peroxidase or peroxidase mimetic materials, TMB can react with H_2O_2 to produce a colored product. The employed peroxidase mimetic material in the current study was SDS-capped Ag NPs. An efficient peroxidase mimetic degrades H_2O_2

to OH radicals which can induce the conversion of non-colored TMB to blue-colored oxidized TMB. Figure 2 shows absorbance spectra under different conditions.

The Ag NPs catalyzed TMB- H_2O_2 system was used for the determination of aspirin in EBC samples. We suggested that aspirin with a standard electrode potential (E°) of -0.5 V involve in competition with TMB ($E^\circ=0.741$ V) to react with H_2O_2 ($E^\circ=1.763$ V). As aspirin has a low oxidation potential compared to TMB, it oxidized firstly and can inhibit TMB oxidation in proportional to its concentration (Figure 3). This inhibition was observed as a decrease in the

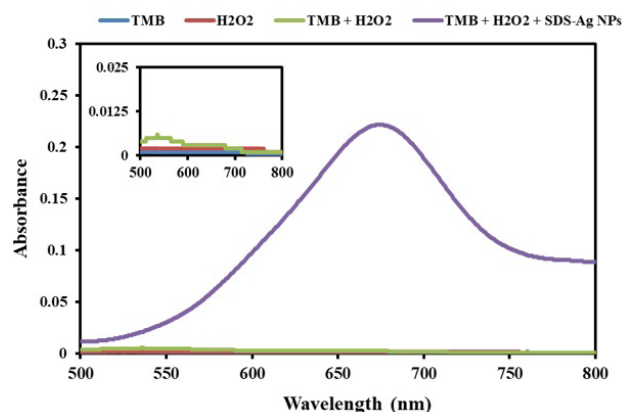


Figure 2. The absorbance spectra of (a) TMB (b) SDS capped Ag NPs (c) H_2O_2 (d) TMB- H_2O_2 and (e) TMB- H_2O_2 in the presence of SDS capped Ag NPs.

response signal. As a decrease in signal was proportional to analyte concentration; it can be developed as a new method for aspirin determination in the biological samples.

The important point was that aspirin was a very unstable compound and hydrolysis to salicylic acid in water. In order to investigate the effect of salicylic acid on the system's response, we compared the response for 50 mg.L^{-1} aspirin, 50 mg.L^{-1} salicylic acid and a mixture of 25 mg.L^{-1} aspirin and 25 mg.L^{-1} salicylic acid. As can be seen in Figure 4 all of them show a similar response indicating that our method can be determined the whole concentration of aspirin and its degradation product.

Optimization of reaction conditions

For achieving the maximum response in the determination of aspirin, the impact of important parameters such as pH, reagent concentrations and reaction time were studied.

Herein, the analytical response was the difference between the absorbance of the mixture in the presence and absence of aspirin. 50 mg.L^{-1} of aspirin was selected for optimization investigations.

Each reported data was the mean of three replicated data. At first, the effect of pH on aspirin determination was investigated. It was studied in the range of 3.0 – 10.0 and the results (Figure 5a) show the maximum response was obtained in the acidic media (pH = 5.0). It can be related to the fact that the oxidation of TMB was mediated under acidic conditions as was confirmed in the literature.²⁸ The effect of the concentrations of all used reagents on the response was also investigated. The concentration of TMB

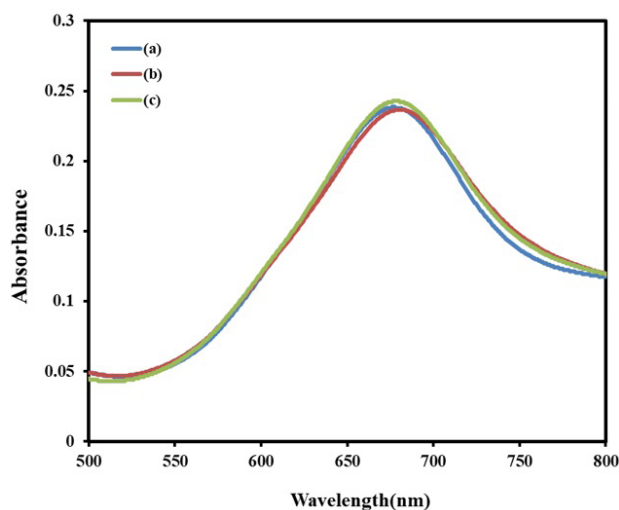


Figure 4. Comparison the response of TMB- H_2O_2 system catalyzed by Ag NPs for a mixture of 25 mg.L^{-1} aspirin and 25 mg.L^{-1} salicylic acid (a), 50 mg.L^{-1} salicylic acid (b) and 50 mg.L^{-1} aspirin (c).

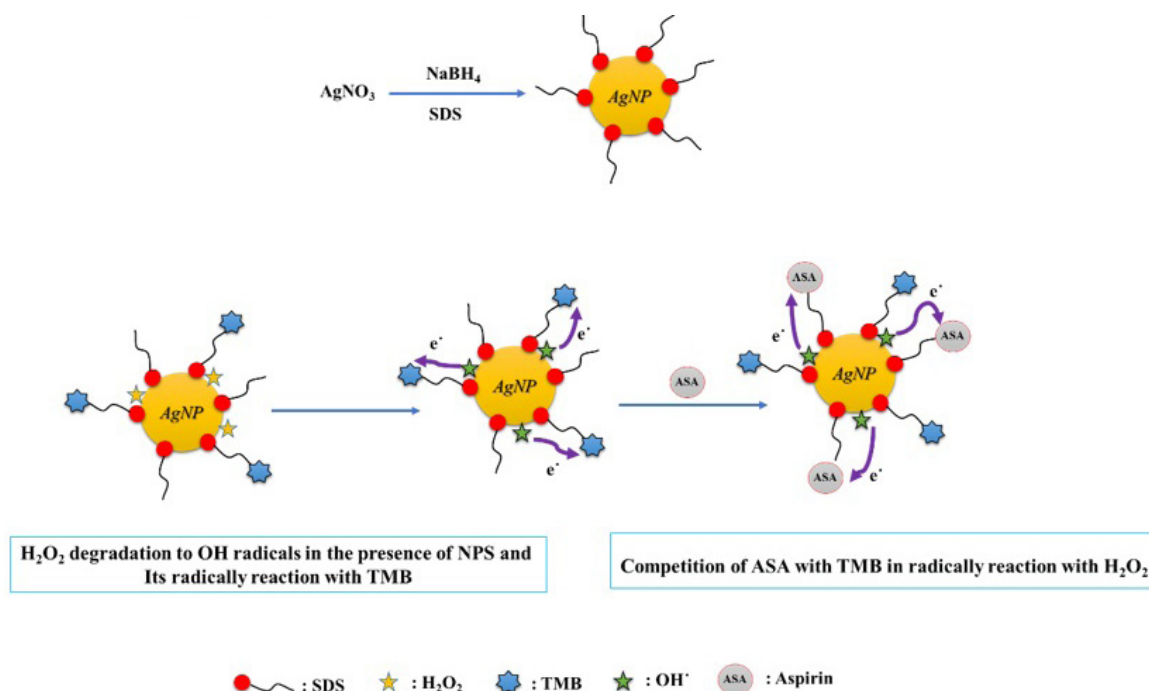


Figure 3. Schematic representation of response of TMB- H_2O_2 system catalyzed by Ag NPs system to aspirin.

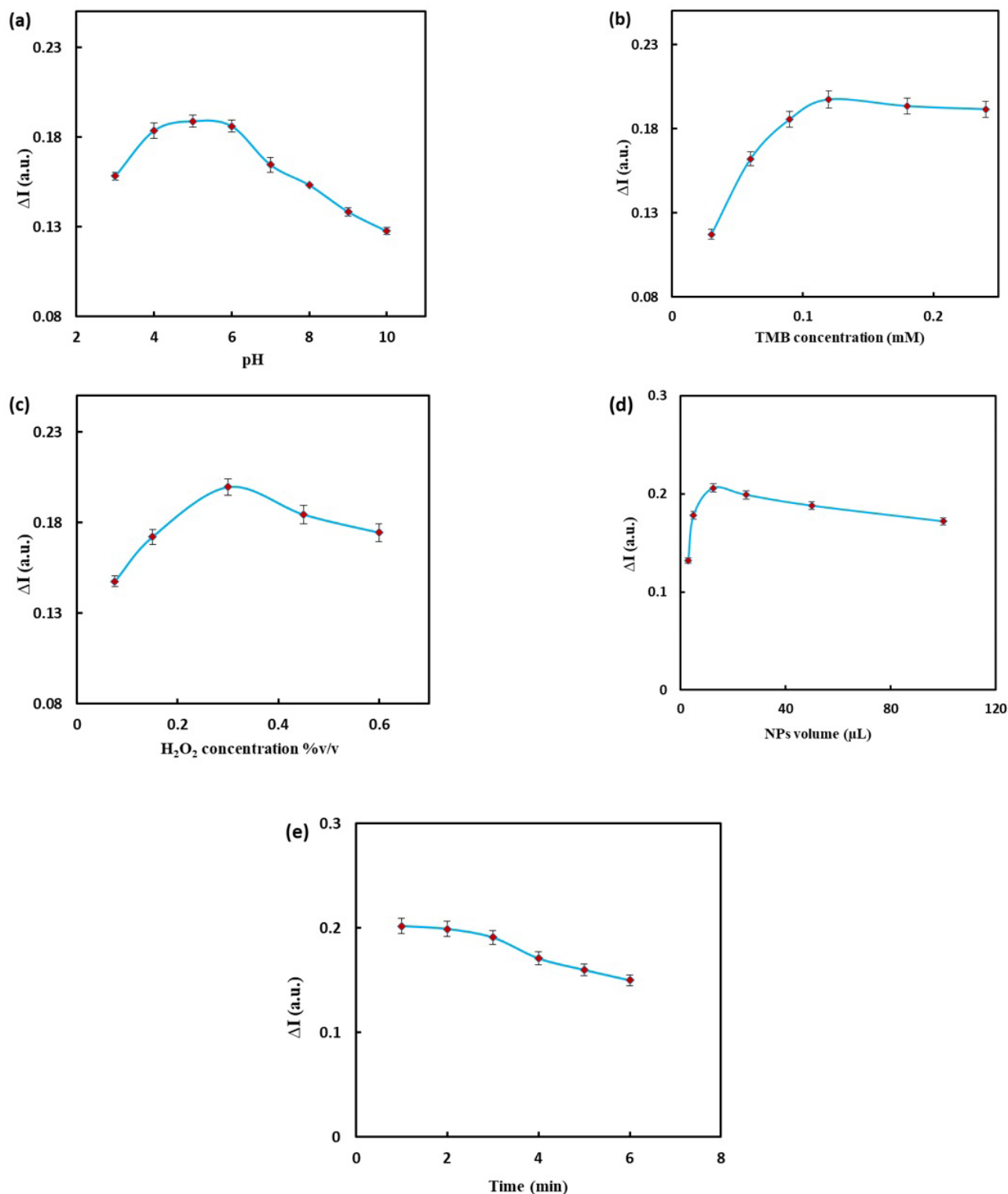


Figure 5. Effect of pH (a), concentration of TMB (b), H_2O_2 (c), SDS capped Ag NPs (d) and time (e) on response of the developed probe.

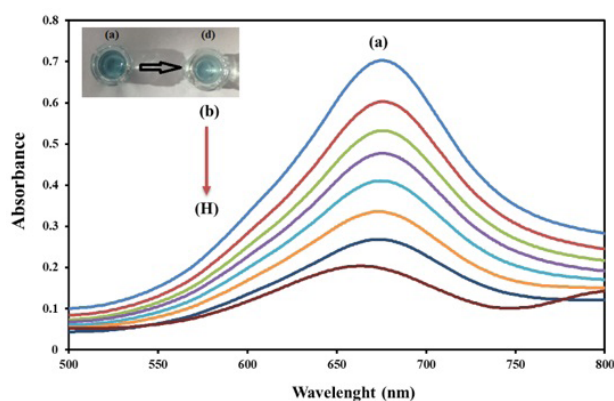
as a substrate in the catalytic reaction was studied in the range of 0.03 – 0.24 $mmol.L^{-1}$ and Figure 5b shows that the system response was increased with TMB concentration increase and reaches a plateau at 0.12 $mmol.L^{-1}$ which can be related to its excess amount so that low concentration of aspirin cannot change the color of the solution. H_2O_2 concentration as an oxidant in this reaction was

investigated in the range of 0.075 – 0.45 %v/v. The best results were achieved at H_2O_2 concentration of 0.30 % v/v (Figure 5c) and more than this concentration, a slight decrease was observed in system response. It means that in the high concentration of H_2O_2 , there was enough concentration of H_2O_2 to oxidation of both aspirin and TMB so that no competition in the oxidation procedure

occurs in the solution. The effect of SDS-capped Ag NPs volume as other reagents was studied in the range of 3.0 – 100 μL . Figure 5d shows that the response increase with increasing the nanoparticles volume and after 12.5 μL , it decreases with a gentle slope. It means that this volume was enough for catalysis of the reported concentration of TMB and H_2O_2 . Finally, the incubation time for the reaction system was optimized and the results (Figure 5e) show that the response was relatively constant for up to 3 min and after that, the color of the blank and sample solution vanishes and the response was decreased.

Analytical figures of merit

Under optimized conditions, a linear relationship with $R^2 = 0.9962$ was obtained by plotting aspirin concentration against ΔI (absorbance intensity in the absence of aspirin – absorbance intensity in the presence of aspirin) in the range of 10–250 $\text{mg}\cdot\text{L}^{-1}$ (Figure 6). The regression equation was $\Delta I = 0.0021 C_{\text{ASA}} + 0.0939$. The limit of detection (LOD), expressed as $3 S_b/m$ (where S_b is the standard deviation of the blank and m is the slope of the calibration curve), for the developed technique was 4.1 $\text{mg}\cdot\text{L}^{-1}$. For the precision study, a solution with a known amount of aspirin (100 $\text{ng}\cdot\text{mL}^{-1}$) was measured on the same day and on different days. The intra-day and inter-day relative standard deviations (%RSD) for five replications were 1.0 % and 3.5 %, respectively. Furthermore, the impact of nanoparticles given from different synthesis batches on the signal intensity was investigated and a relatively an acceptable batch-to-batch reproducibility with %RSD=5.2% ($n = 3$) was reported. Most of the reported methods in the literature for aspirin determination were the chromatographic method and provide a LOD < $\text{ng}\cdot\text{mL}^{-1}$ in the biological samples. However, the main purpose of the current work was the determination of aspirin concentration in cases of aspirin/salicylate poisoning. The toxic level of the aspirin in the plasma samples was reported to be 300–350 $\text{mg}\cdot\text{L}^{-1}$ 29 which was very higher than our calibration range demonstrating that the developed method has a good capability for poisoning monitoring.



Interferences study

To check the selectivity of the proposed method, the interferences of the commonly used drugs including over-the-counter pharmaceuticals on the signal of the sensor for the determination of aspirin were measured and the results were given in Table 1. The tolerance limit was the maximum concentration of the interfering that obtain a relative error (RE) less than 5% for the response signal. A particular concentration of aspirin (50 $\text{mg}\cdot\text{L}^{-1}$) was selected for the interference study. As can be seen, the high interferences were related to naproxen, celecoxib, and clonazepam with a tolerance limit of 50 which means their tolerating concentration was should be 250 $\text{mg}\cdot\text{L}^{-1}$ to affect the response signal. This concentration was very higher than their therapeutic concentration in the biological samples. These results demonstrate that the developed method has adequate selectivity for aspirin analysis.

Real sample analysis

Although the proposed method has been developed with the aim of determination of aspirin concentration in EBC of poisoned individuals with aspirin/salicylate, we did not have access such cases. So, in order to study the applicability of the validated method on real samples, the technique was employed to measure aspirin in EBC of six volunteers receiving aspirin in the therapeutic dosages. The results were summarized in Table 2. To study the accuracy of the method, the recovery calculation was also done by spiking the studied EBC samples with a known and constant amount of aspirin. A recovery of between

Table 1. Tolerance limits of some over-the-counter and co-administrated drugs in the determination of aspirin by the developed method.

Interfering substance	Tolerance limit
Cetirizine	1000
Nicotinamide, Alprazolam	500
Chlordiazepoxide, Oxazepam	400
Diazepam, Ibuprofen	200
Pantoprazole, Diltiazem, Acetaminophen	100
Naproxen, Celecoxib, Clonazepam	50

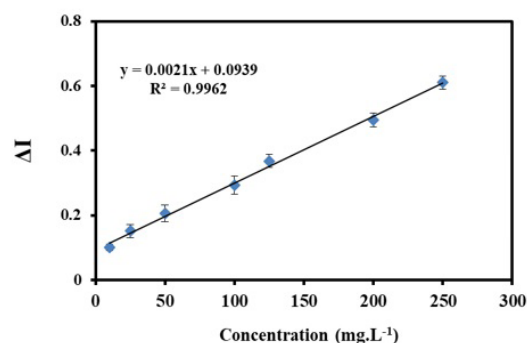


Figure 6. Absorbance spectra of TMB- H_2O_2 system catalyzed by Ag NPs in the absence and presence of aspirin in the concentration range of 10–250 $\text{mg}\cdot\text{L}^{-1}$ (left) and calibration plot (right). Conditions: pH 5.0, [TMB]= 0.12 $\text{mmol}\cdot\text{L}^{-1}$, [H_2O_2]= 0.3 % v/v, and SDS capped Ag NPs volume= 12.5 μL .

Table 2. The determined aspirin concentration in EBC of patients receiving it in the therapeutic range and recovery experiments.

No.	Gender	Age (year)	Co-administered drugs	Added (mg.L ⁻¹)	Found (mg.L ⁻¹)	Recovery (%) ^a
1	Female	60	–	– 25.0	– 24.8 ± 1.2	– 99.2
2	Male	61	Metoprolol, Amlodipine, Duloxetine, Pregabalin, Hydrochloroquine, Carbamazepine	– 25.0	– 23.2 ± 1.8	– 92.8
3	Male	65	Ranitidine, Amlodipine, Losartan, Trifluoperazine, Gabapentin, Clonazepam	– 25.0	– 23.9 ± 0.9	– 95.2
4	Male	39	Phenobarbital, Naproxen, Carbamazepine	– 25.0	– 24.9 ± 1.1	– 99.6
5	Male	20	Omega3, Penicillin V, Calcium, Vit D3, Vit K, Selenium	– 25.0	– 24.1 ± 0.8	– 96.4
6	Male	20	Penicillin V, Calcium, Vitamin D3, Vitamin K, Defripiron	– 25.0	– 24.4 ± 1.4	– 97.6

^aRecovery (%)=[(Found–Base)/Added] × 100. “Base” and “Found” refer to the amount of the analyte in samples before and after spiking, respectively.

92.8% and 99.8% was observed for spiked samples which confirms the method's accuracy and selectivity for aspirin analysis in the EBC of patients that were taking different co-administered drugs.

Conclusion

A colorimetric method based on nanoparticles catalyzed redox reaction was validated for aspirin determination in EBC samples. The novelty of this study was the aspirin analysis in EBC sample as a newly introduced biological sample. The proposed method shows good applicability for aspirin analysis due to its high reliability and fast response time. The validated method was successfully used for the quantification of the spiked values of aspirin in the EBC samples taken from the patients.

Ethical Issues

EBC sample donors signed a consent form which was approved by the Ethics Committee of Tabriz University of Medical Sciences with code IR.TBZMED.VCR.REC.1399.322.

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Author Contributions

Samaneh Mohammadzadeh Abachi: Homa Rezaei: Investigation. Maryam Khoubnasabjafari: Investigation. Vahid Jouyban-Gharamaleki: Methodology. Elaheh Rahimpour: Conceptualization, Methodology. Abolghasem Jouyban: Conceptualization, Supervision.

Conflict of Interest

The authors declare that they have no competing interests.

References

- Vane J. Aspirin and other anti-inflammatory drugs. *Thorax*. 2000;55:S3-S9. doi:10.1136/thorax.55.suppl_2.S3
- Chyka PA, Erdman AR, Christianson G, Wax PM, Booze LL, Manoguerra AS, et al. Salicylate poisoning: an evidence-based consensus guideline for out-of-hospital management. *Clin Toxicol*. 2007;4(2):95-131. doi:10.1080/15563650600907140
- Krause DS, Wolf BA, Shaw LM. Acute aspirin overdose: mechanisms of toxicity. *Ther Drug Monit*. 1992;14:441-51. doi:10.1097/00007691-199212000-00001
- Seche J, de Tornay R. Experiences in clinical problem solving. Nursing decisions. Emergency care of an aspirin overdose patient. *RN*. 1978;41(10):83-7.
- Chapman B, Proudfoot A. Adult salicylate poisoning: deaths and outcome in patients with high plasma salicylate concentrations. *QJM*. 1989;72(2):699-707. doi:10.1093/oxfordjournals.qjmed.a068363
- McGuigan MA. A two-year review of salicylate deaths in Ontario. *Arch Intern Med*. 1987;147(3):510-2. doi:10.1001/archinte.1987.00370030114022
- Thisted B, Krantz T, Ström J, Sørensen MB. Acute salicylate self-poisoning in 177 consecutive patients treated in ICU. *Acta Anaesthesiol Scand*. 1987;31(4):312-6. doi:10.1111/j.1399-6576.1987.tb02574.x
- Mishra P, Dolly A. Simultaneous determination of clopidogrel and aspirin in pharmaceutical dosage forms. *Indian J Pharm Sci*. 2006;68(3):365-8. doi:10.4103/0250-474X.26682
- Kokot Z, Burda K. Simultaneous determination of salicylic acid and acetylsalicylic acid in aspirin delayed-release tablet formulations by second-derivative UV spectrophotometry. *J Pharm Biomed Anal*. 1998;18(4-5):871-5. doi:10.1016/S0731-7085(98)00225-8
- Damle MC, Sinha PK, Bothra KG. A validated stability indicating HPTLC method for determination of aspirin

- and clopidogrel bisulphate in combined dosage form. *Eurasian J Anal Chem.* 2009;4(2):152-60.
11. Nikelly J. Gas Chromatographic Determination of acetylsalicylic acid. *Anal Chem.* 1964;36(12):2248-50. doi:10.1021/ac60218a008
 12. Karim MM, Jeon CW, Lee HS, Alam SM, Lee SH, Choi JH, et al. Simultaneous determination of acetylsalicylic acid and caffeine in pharmaceutical formulation by first derivative synchronous fluorimetric method. *J Fluoresc.* 2006;16(5):713-21. doi:10.1007/s10895-006-0115-7
 13. Setiarso P, Inggriani F. Synthesis of graphene oxide-nanozeolite composite electrode for aspirin analysis by cyclic voltammetry. *Asian J Chem.* 2020;32(10):2541-4.
 14. Pisal P, Nigade G, Kale A, Pawar S. Development and validation of stability indicating RP-HPLC method for simultaneous determination of aspirin, rosuvastatin, clopidogrel in bulk and pharmaceutical dosage form. *Int J Pharm Pharm Sci.* 2018;10(10):50-6.
 15. Wang Y, Yan B, Chen L. SERS tags: novel optical nanoprobe for bioanalysis. *Chem Rev.* 2013;113(3):1391-1428. doi:10.1021/cr300120g
 16. Merkoçi A. *Biosensing using nanomaterials.* New York: John Wiley & Sons; 2009.
 17. Sharma VP, Sharma U, Chattopadhyay M, Shukla V. Advance applications of nanomaterials: a review. *Mater Today Proc.* 2018;5(2):6376-80. doi:10.1016/j.matpr.2017.12.248
 18. Khoubnasabjafari M, Rahimpour E, Samini M, Jouyban-Gharamaleki V, Chen L, Chen D, Chan H-K, Jouyban A. A new hypothesis to investigate bioequivalence of pharmaceutical inhalation products. *Daru.* 2019;27(1):517-24. doi:10.1007/s40199-019-00250-x
 19. Khoubnasabjafari M, Rahimpour E, Jouyban A. Exhaled breath condensate as an alternative sample for drug monitoring. *Bioanalysis.* 2018;10(2):61-4. doi:10.4155/bio-2017-0205
 20. de Paiva MJN, Menezes HC, de Lourdes Cardeal Z. Sampling and analysis of metabolomes in biological fluids. *Analyst* 2014;139(15):3683-94. doi:10.1039/C4AN00583J
 21. Rahimpour E, Khoubnasabjafari M, Jouyban-Gharamaleki V, Jouyban A. Non-volatile compounds in exhaled breath condensate: review of methodological aspects. *Anal Bioanal Chem* 2018;410(25):6411-40. doi:10.1007/s00216-018-1259-4
 22. Sauvain JJ, Hemmendinger M, Suárez G, Creze C, Hopf NB, Jouannique V, et al. Malondialdehyde and anion patterns in exhaled breath condensate among subway workers. *Part Fibre Toxicol.* 2022;19(1):16. doi:10.1186/s12989-022-00456-z
 23. Pourkarim F, Rahimpour E, Khoubnasabjafari M, Jouyban-Gharamaleki V, Farhang S, Jouyban A. A simple colorimetric method for determination of ethanol in exhaled breath condensate. *Pharm Sci.* 2021;27(2):297-301. doi:10.34172/PS.2020.40
 24. Hajizadeh S, Farhadi K, Forough M, Molaei R. Silver nanoparticles in the presence of Ca²⁺ as a selective and sensitive probe for the colorimetric detection of cysteine. *Anal Methods.* 2012;4(6):1747-52. doi:10.1039/C2AY05848K
 25. Jouyban A, Khoubnasabjafari M, Ansarin K, Jouyban-Gharamaleki V. Breath sampling setup. Iranian patent 81363, 2013.
 26. Kora AJ, Rastogi L. Enhancement of antibacterial activity of capped silver nanoparticles in combination with antibiotics, on model gram-negative and gram-positive bacteria. *Bioinorg Chem Appl.* 2013;2013:871097. doi:10.1155/2013/871097
 27. Bhaduri GA, Little R, Khomane RB, Lokhande SU, Kulkarni BD, Mendis BG, et al. Green synthesis of silver nanoparticles using sunlight. *J Photochem Photobiol.* 2013;258:1-9. doi:10.1016/j.jphotochem.2013.02.015
 28. Drozd M, Pietrzak M, Parzuchowski PG, Malinowska E. Pitfalls and capabilities of various hydrogen donors in evaluation of peroxidase-like activity of gold nanoparticles. *Anal Bioanal Chem.* 2016;408(29):8505-13. doi:10.1007/s00216-016-9976-z
 29. Schulz M, Schmoldt A. Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. *Pharmazie.* 2003;58(7):447-74.