A New Colorimetric Method to Determine the Acetylcholine Esterase Mimic Activity of V2o5 Nanoparticles

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Abstract
Acetylcholine esterase (AChE) is a primary enzyme from the family of enzymes called cholinesterase (ChE) localized in the cytoplasm, blood, and neural synapses which allowing to work on hydrolyzed acetylcholine to choline and acetic acid intracellular and extracellular, this response is important to enabling a cholinergic neuron to come back to its resting state after activation. We successfully used a simple and a new method (colorimetric) to determine the mimic activity of VPNs as acetylcholine esterase using the change in color at length wave 616 nm of the solution containing bromothymol blue as an indicator which depends on the change of pH solution result from the decomposition of acetylcholine (Ache) to choline and acetic acid. Our results found that all samples get acetylcholine esterase mimic activity, while the sample annealed at 500 oC has the highest activity (13.983 x 10-4 U. min 1) compared with other samples.
Keywords: Vanadium pentoxide, nanoparticles, acetylcholine esterase.

1. Introduction

Vanadium creates a lot of compounds with oxygen in which have different structural, optical, and chemical properties because of easily changed oxidation state [1]. Vanadium oxide found in (+2, +3, +4 and +5) oxidation states and the different V-O coordination geometries respectively form VO, V2O3, VO2, and V2O5 [2, 3]. There are many phases of V2O5, such as α-V2O5, β-V2O5, and γ-V2O5 these phases different in structures. Both α-V2O5 and γ-V2O5 found as orthorhombic [3], while β-V2O5 found in monoclinic or tetragonal [4]. α-V2O5 is the most stable among these phases and all phases converted under increase temperature to α-V2O5 [2, 5], the morphology include nano flakes (VNfk), nanowires (VNW), nanosheet (VNSH), nanoflower (VNF) [6] and nanosphere (VNSp). Bulk vanadium pentoxide (V2O5) or vanadium compounds have an extremely toxic to cells but in nanoscale V2O5 protect the cell from oxidative damage (cytoprotective antioxidant) due to be very effective in catalyzing the reduction of hydrogen peroxide and the activity is different depending on morphology in which V2O5 nanowires showed the highest activities [7]. Acetylcholine esterase (AChE) is a primary enzyme (a type-B carboxylesterase enzyme), is a tetramer composed of four equal subunits (Mwt 280 kDa). Each subunit contains one active site. Family of enzymes called cholinesterase (ChE) localized in the cytoplasm, blood, and neural synapses which allowing to work on hydrolyzed acetylcholine to choline and acetic acid intracellular and extracellular, this response is important to enabling a cholinergic neuron to come back to its resting state after activation [8]. AChE found in tissue such as muscle and nerve, also found in the red blood cell membranes. Acetylcholine esterase is excreted by the muscles and remains attached to it by collagen installed on the basal lamina [9]. There are some researches to determine enzyme mimic (catalase) activities of nanoparticles [11, 12]. Acetylcholine esterase activity can be determined using two methods: the first method based on the change in measurement of absorbance at 405 nm. The technique is portrayed in detail by Ellman, G. L., et al [10]. Examine utilizes the thiol ester acetylthiocholine rather than the oxyester acetylcholine. The second method named Michel method [11], which calculates the differences between pH solutions before and after incubation within a specific time. Both these methods have disadvantaged the first method depends on multireaction proses which gets some error, and the second method depends on the very little change in the pH-solution which is difficult measured in pH-meter apparatuses.

In this report, we focused on the use of a simple and a new method (colorimetric) to determine acetylcholine esterase mimic activity for vanadium pentoxide nanoparticles annealing at different temperatures by using differences between absorbance solutions before and after incubation within a specific time.

2. Methods

Materials and reagents

The materials were used throughout this workethanol (EtOH, Sigma-Aldrich,99.9%), nitric acid (HNO3, Alpha Chem,97%), Ammonium dihydrogen orthophosphate. Disodium hydrogen orthophosphate dihydrated, acetylcholine chloride (C7H16ClNO2, BDH,99.5%) bromothymol blue

doi.org/10.31838/hiv22.02.332 Received: 16.05.22, Revised: 17.05.22, Accepted: 09.08.22

(C27H28Br2O5S,BDH,99.7%) vanadiumpentoxide nanoparticle pawder [12]

Preparation vanadium pentoxide nanoparticle solution

The powder of VPNs [12] dissolved in DMSO to have vanadium solutions (0.1mM).

Preparation of phosphate buffer solution

The buffer solution was prepared by mixing the following solutions [13, 14]. Solution A: ammonium dihydrogen orthophosphate, (NH4H2PO4), (50 mM). This solution was prepared by dissolved 0.5750 g of NH4H2PO4 salt in 20 mL of deionized water, then the volume was complete to (100 mL). Solution B: Disodium hydrogen orthophosphate dihydrated (Na2HPO4.2H2O), (50 mM). This solution was prepared by dissolved 0.7096 g of Na2HPO4.2H2O salt in 20 mL of deionized water, then the volume was complete to (100 mL). After mixing 87 mL of solution (A) with 13 mL of solution (B). we get buffer solution has concentration 50 mM and pH = 7.6.

Preparation indicator

The stock solution prepared by dissolving 0.05 g of bromothymol blue in a small amount of ethanol, then in distal water of 50 ml. The stock solution dilutes 4 ml in 50 ml which used as a reagent.

Preparation of acetylcholine chloride solution

0.01% W/V concentration of acetylcholine chloride dissolved in deionized water.

Procedure

Determine the mimetic activity of V2O5 as acetylcholinesterase (AChE) in buffer solution by using the new spectrophotometric method (modification of Michel method) [15], which included using of bromothymol blue solution as indicator. The new method includes using two tubes one labeled as a test and the other as blank. The contents of each tube shown in the Tab. (1).

Table (1): the contains of test tube and blank

Groups	Test	Blank
Oxide	0.5	0.5
Buffer	2	2.1
B.T. B	0.2	0.2
Acetylcholine chloride	0.1	-------
Total volume (mL)	2.8	2.8

Tubes are putting in a water bath (37 oC) for 20 min. At the end of the incubation time, the tubes were removed, and the absorbance was measured immediately at length wave (λ=616 nm). Differences between the absorbance of blank (B) and absorbance of tests (T), the change of B-T in incubation time (min) show the level of cholinesterase activity of the sample [16, 17]. The rate of reaction for AChE mimetic activity of V2O5 at different temperatures (90 ˚C, 250 ˚C, 500 ˚C and 750 ˚C) was calculated by the following equation:

AChE mimetic activity=Δ absorbance /T = [B1-T1-(Bo of blank)]/T

Where, T= incubation time in minutes (20 min), B1, T1 = the absorbance of a blank and test sample respectively, Bo the absorbance of blank contains all solutions except metal oxides or its composites.

3. Results and Desiccation

The mimic activity of V2O5 nanoparticles as acetylcholine esterase for each of the nanoparticle's models at annealing degrees (90 oC and 250 oC) is close to, and this may be due to the lack of formation of the entire oxide phase and the relatively large grain size. While the mimic activity as acetylcholine esterase increases to maximum when annealing with a temperature of 500 oC, maybe relate to completion of the oxide phase and reaching the minimum nanoparticles that give more surface area to the compound. While when annealing at 750 oC, the decrease in mimic activity is due to the formation of another oxide and increase in grain size to reach to the grain size of a sample annealed at 500 oC. Our results appearance that the maximum of AChE mimetic activity gives in the sample which annealing at 500 oC. The results show the AChE mimetic activity of V2O5 at different temperatures in Fig. (1), and Tab. (2).

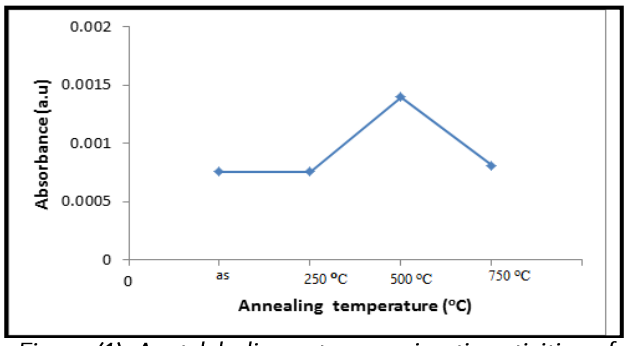

Figure (1): Acetylcholine esterase mimetic activities of V2O5 at (90 oC -750 oC) for 120 min.

Table2: Acetylcholine esterase mimetic activities of V2O5 at different temperatures (90 oC - 750 oC) for 120 min.

Vanadium Pentoxide / Annealing Temperature (oC)	Acetylcholine esterase mimetic activities (x 10-4 U. min 1)
As prepared (90)	7.654
250	7.561
500	13.983
750	8.113

4. Conclusion

Acetylcholine esterase mimic activity was measured by a new colorimetric method depends on indicator (bromothymol blue) that give different color intensity. The mimic activity as acetylcholine esterase of these samples was measured by this new method and found all of these get mimic activity, while the sample annealing at 500 oC was the highest mimic activity, maybe related to its grain size or the pure phase of V2O5 formats.

References

1. Wasmi B, Al-Amiery AA, Kadhum AAH, et al. Synthesis of vanadium pentoxide nanoparticles as catalysts for the ozonation of palm oil. Ozone:

Science & Engineering. 2016;38(1):36-41. https://doi.org/10.1080/01919512.2015.1074536
2. Farahmandjou M, Abaeiyan N. Simple synthesis of new nano-sized pore structure vanadium pantoxide (V. Bio-Inorg Hybr Nanomater. 2015;4(4):243-7.
3. Singh P, Kaur D. Influence of film thickness on texture and electrical and optical properties of room temperature deposited nanocrystalline V 2 O 5 thin films. Journal of Applied Physics. 2008;103(4):043507. https://doi.org/10.1063/1.2844438
4. Su Q, Lan W, Wang Y, et al. Structural characterization of β-V2O5 films prepared by DC reactive magnetron sputtering. Applied Surface Science. 2009;255(7):4177-9.
5. Balog P, Orosel D, Cancarevic Z, et al. V2O5 phase diagram revisited at high pressures and high temperatures. Journal of Alloys and Compounds. 2007;429(1):87-98. https://doi.org/10.1016/j.jallcom.2006.04.042
6. Karami H, Mohammadi A. Poly Vinyl Alcohol-Based Sol-Gel Synthesis of V2O5 Nanoflakes as Positive Electrodes of Li-ion Batteries. Electrochem Sci. 2015;10:7392-408.
7. Ghosh S, Roy P, Karmodak N, et al. Nanoisozymes: Crystal-Facet-Dependent Enzyme-Mimetic Activity of V2O5 Nanomaterials. Angewandte Chemie. 2018;130(17):4600-5.
8. Colovic MB, Krstic DZ, Lazarevic-Pasti TD, et al. Acetylcholinesterase inhibitors: pharmacology and toxicology. Current neuropharmacology. 2013;11(3):315-35.
9. Bittner EA, Martyn JAJ. 21 - Neuromuscular Physiology and Pharmacology. In: Hemmings HC, Egan TD, editors. Pharmacology and Physiology for Anesthesia (Second Edition). Philadelphia: Elsevier, 2019. p. 412-27. https://doi.org/10.1016/B978-0-323-48110-6.00021-1
10. Ellman GL, Courtney KD, Andres V, et al. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 1961;7(2):88-95. https://doi.org/10.1016/0006-2952(61)90145-9
11. Khalil KA, Abass KS. The Measurements of the Cholinesterase Activity of Brain and Plasma in Rabbits by Using Modified Michel and Ellman Assays. Insights in enzyme research. 2017;1:1-7.
12. Rasheed RT, Mansoor HS, Abdullah TA, et al. Synthesis, characterization of V2O5 nanoparticles and determination of catalase mimetic activity by new colorimetric method. Journal of Thermal Analysis and Calorimetry. 2021;145(2):297-307. 10.1007/s10973-020-09725-5. Available from: https://doi.org/10.1007/s10973-020-09725-5
13. Aebi H. Catalase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis (Second Edition). 2 Academic Press, 1974. p. 673-84. https://doi.org/10.1016/B978-0-12-091302-2.50032-3
14. Daswani B, Khan Y. Insights into the role of estrogens and androgens in glial tumorigenesis. J Carcinog. 2021;20:10. https://doi.org/10.4103/jcar.jcar_2_21
15. Abdullah Khalil K, Sakran Abass K. The Measurements Of The Cholinesterase Activity Of Brain And Plasma In Rabbits By Using Modified Michel And Ellman Assays. Insights in Enzyme Research. 2017;01(2:9):1-7. 10.21767/2573-4466.100009
16. Horowitz IH, Yanco EG, Landau S, et al. Whole blood cholinesterase activity in 20 species of wild birds. Journal of avian medicine. 2016;30(2):122-7.
17. Anil MA, Thayana E, Bhat JS. Judgment of pragmatic abilities: A story-based assessment in adolescents. J Nat Sci Biol Med. 2021;12(1):35-42. https://doi.org/10.4103/jnsbm.JNSBM_114_20