On-site detection of saliva-alcohol as a function of blood alcohol concentration using colorimetric biosensor based on deposited Chromium (VII) Oxide Nanoparticles on filter paper

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Abstract

Alcohol intoxication is usually associated with drowning, falls, overdoses, fires, occupational accidents, physical and sexual abusements, domestic violence and traffic accidents. Therefore, alcohol considered an important factor for the explanation of the occurrence of many types of injuries. For many purposes such as forensic, it is important to establish a detection method to ensure whether the subject or the patient have consumed alcohol at a level that would be the reason for the accidents or injuries occur. Therefore, in this work, a simple, rapid and low-cost method was developed and validated for the detection of the alcohol in saliva as a function of blood alcohol concentration (BAC). The method is based on fabricated a biosensor consisted of chromium oxide nanoparticles deposited on filter paper. The validation of the biosensor was tested on 50 participants which categories into two selected groups (1 and 2). Group 1 consisted of 20 subjects from an organized party (no alcohol), they usually consumed three to four drinks as an average per week while Group 2 consisted of 30 subjects from an organized party the local bar (alcohol group), usually consumed two to three drinks per day. The results of the present study have shown that 95% of group 1 demonstrated positive results with variable colour intensities of the BAC in comparison to the 80% only of subjects from group 2. The present study has approved that the fabricated biosensor can effectively detect 0.02% or more of BAC which can be a useful test for many purposes such as medical, forensic, research and workplace.

Keywords: Biosensor, Blood alcohol concentration, Chromium oxide nanoparticles (CrONPs), On-site alcohol detection

1.Introduction

The consumption of alcohol leads to a risk factor for morbidity and mortality which related to both unintentional and intentional injuries[1]. Globally, 16% of deaths and 13% of disabilities from injuries were estimated in 2000 to be attributed to alcohol [2]. Alcohol can coordinately affect many individual psychomotor skills such as brain-hand, foot and eye [3]. The effects of alcohol on the human body are several such as visual focus, long reaction time and delay in judgment leading to injuries from causes like falls and motor vehicle accidents[4, 5]. The individual's cognitive skill can also be affected by alcohol intoxication. Exposition of persons to alcohol can place them in a real dangerous situation, less averse to risk-taking and be more aggressive which lead to both intentional injuries as either victims or perpetrators and unintentional injuries such as burns and drowning. A large number of studies have illustrated the involvement of alcohol among nonfatal and fatal injuries. For example, in the analysis of more than 65 articles which were published between 1975 to 1995, has reported that the high percentage cases of intoxicated with alcohol were 31.5% among homicide deaths, 31.0% among non-traffic and 22.7% among suicide[2, 6]. While a moderate percentage of cases of involvement of alcohol in injuries events have suggested that alcohol is a risk factor even if the consumption of alcohol doesn't provide information about the actual risk. Several factors have contributed to increase the alcohol use from the people such as increased availability, urbanization, high-intensity mass marketing, changing in the social norms, poorly awareness and relaxation in rules of overseas trade. According to the WHO report in 2018 which has stated that the harmful using of alcohol results in approximately 3 million deaths each year which represents

5.3% of all deaths[7]. Also, the disability and the death in the early age group 20-39 years increased to 13.5% of all deaths which are caused by alcohol consumption. The harmful use of alcohol has caused lots of behavioural disorders, noncommunicable conditions and mental behaviour. In addition to causal relationships between infectious diseases such as tuberculosis, HIV/AIDS and harmful drinking[8]. The BAC (Blood alcohol concentration) considered as the most useful measurement for determining the concentration of alcohol in blood which is used for different purposes such as medical, forensic, research settings and forensic. Lots of methods, techniques and methodologies have been developed for the quantitative determination of BAC in whole blood such as electrochemical [9, 10], spectrometry [11], HNMR [12], colorimetric[13, 14] and amongst all these methods the gas chromatography is the preferable one[15-17]. However, the GC method has many disadvantages such as time-consuming, requires skills and expensive. Therefore, cheap, fast, rapid, non-invasive method is required for the quantitative determination of BAC. The BAC has been determined in urine[18, 19], breath [19, 20] and blood [21, 22]. Till now, the breath method for estimation of BAC is used the breath meter[19, 23]. Despite this method provides a rapid result but is requires calibration and person cooperation which may be sometimes very difficult in comatose or combative persons. In this paper, we aimed to develop an analytical method based on synthetic alcohol-saliva biosensor which may permit a cheap, rapid, and simple detection of ethanol content in saliva. This test provides an accurate estimate of BAC saliva. This method was validated by the serum BAC method [24]. The saliva disk consists of deposited chromium oxide nanoparticles on filter paper. The new technique is successfully applied for the detection of ethanol as a function of BAC and can be used in various purposes.

2.Materials and Methods

Reagents

All chemicals and standards were of analytical grade and all preparations were conducted using double distilled water. Potassium Dichromate, Mulberry leaves extract, filter paper and double-distilled water was used throughout the experiment. Beers, wine and liquors were purchased from the local markets.

Table 1. ***The classification of intoxication levels of BAC [25]***

Blood alcohol concentration in g/100 ml	**Level**	**Symptoms**
0.00-0.05	Sober	1- People appear clinically normal. 2- Some of them have euphoria. 3- Reaction time is slowed down two times
0.06-0.09	Light	1- Self-criticism 2- Loss of inhibitions 3- Loss of concentration and normal judgement
0.10-0.15	Moderate	1- memory problems 2- Further loss of self-criticism 3- emotional instability 4- Early ataxia, apraxia and agraphia
0.15-0.25	Strong-very strong	1- Loss of orientation 2- Apathy and emotional eruptions 3- Emotional instability 4- Partial amnesia 5- Ataxia, agraphia, apraxia
0.25-0.35	Stupor -Coma	1- Total loss of muscle coordination 2- Total loss of orientation. 3- Amnesia 4- Worsening of the abovementioned
0.35-higher	Coma-Death	1- General anaesthesia and paresis 2- Stupor followed by a comma 3- Suppression of the vital centres in the brain with cardiorespiratory collapse and death

Synthesis of chromium oxide nanoparticles

The chromium oxide nanoparticles were synthesized using the extractor of mulberry leaves which were used as a reduction agent. 20 gm of mulberry fresh leaves were mixed and boiled with 150 ml of double-distilled water in 250 ml round bottom flask at 60 C° for 1 hour. 0.7 M of potassium dichromate ($K_2Cr_2O_7$) solution was prepared by dissolving 10.3 g with 50 ml of double-distilled water. The Whatman filter paper (No 40) was used to filter the potassium dichromate solution. 10 ml of 0.7 M of potassium dichromate solution was added to the 10 ml extractor of mulberry leaves. Then the mixture solution was stirred for 15 min. After 5 minutes of stirring, the colour of the solution was changed from orange to colourless. The solution mixture was dried in two stages, first at 100 C° for 6 hours using hot air oven and second at 650 C° for 10 minutes using muffle furnace. The synthesized chromate oxide nanoparticles were then scrubbed, grinded and collected in a sealed container for further characterization.

Characterization of Chromium oxide nanoparticles (CrONPs)

The prepared nanoparticles have been characterized using AFM and UV-spectrometer Shimadzu, 1800). Several experiments of AFM were performed to image CrONPs at a range of salt concentrations (0.2M-0.8M). The results of AFM have shown that most of the CrONPs surfaces diameter have in the range of 40-70nm. Some larger of CrONPs structures >70 nm have also been observed (Fig 1). While, the prepared nanoparticles absorbance was measured in the range 190-900 nm in order to determine the wavelength. The nanoparticles have shown maximum absorbance peak at 320 nm wavelength.

a. Loading the chromium oxide nanoparticles onto the filter paper

0.5 gm prepared solid CrONPs were dissolved in the concentrated solution of sulfuric acid (15%). The prepared chromium oxide nanoparticles were impregnated into the Whatman filter paper (No 40 with paper dimension 4 length x 1 width cm) by a simple dipping process, followed drying the paper using a hairdryer. The impregnation process was repeated 5 times at least in order to ensure a full close-packed of chromium oxide nanoparticles assembly on the filter paper. The deposited filter paper is flexible, and shape and size can be adjusted based on the requirement. The loading of CrONPs onto the filter paper is easily observed by the light yellow colour which is visible to the naked eye. The light colour of the close-packed CrONPs into the filter paper is originated from the assembling and absorption of nanoparticles on the callous fibers. The desorption of nanoparticles from the filter paper has been examined by immersion in various solvents and particles have shown strong adsorption via hydrophobic interactions and van der Waals forces between the nanoparticles and the cellulose fibres. The concentrated sodium borohydride was used to wash the surface of prepared CrONPs loaded filter paper in order to facilitate access of the reactant molecules to the metal surface as shown in Fig 2.

a. Samples

In this study, a total of 50 participants with ages ranged from 25-45 years were taken apart. Twenty participants (10 male and 10 female) were chosen to form the no-alcohol group (1), while 30 participants (20 male and 10 female) were taken to form the alcohol group (2). All the participants in this study were drinkers, healthy with no medical history, volunteers, who have consumed three to four drinks per week as an average.

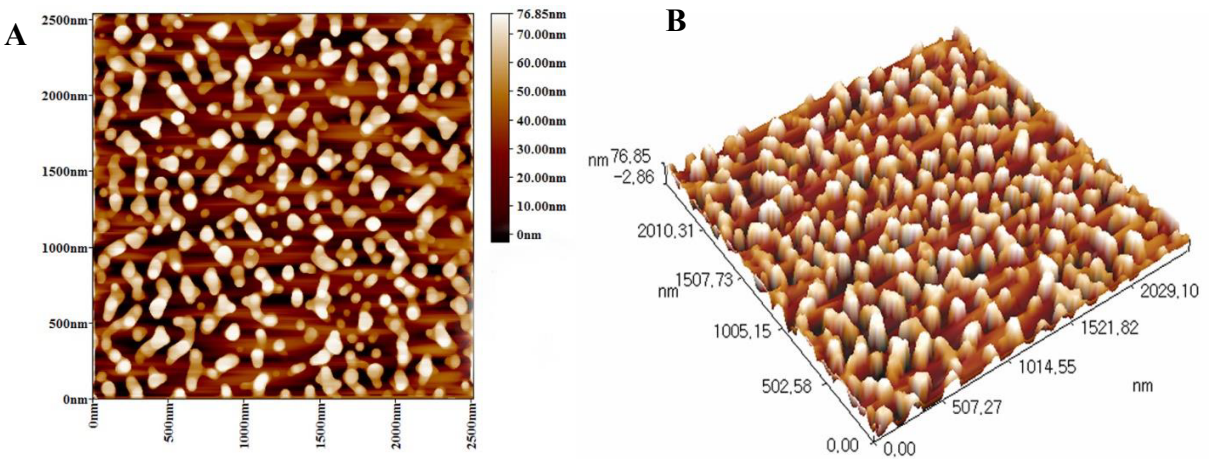

Figure 1. ***Morphological characterization of chromium oxide nanoparticles. (A) Global AFM-registration of nano-CrO, and (B) three-dimensional image of nano-CrO.***

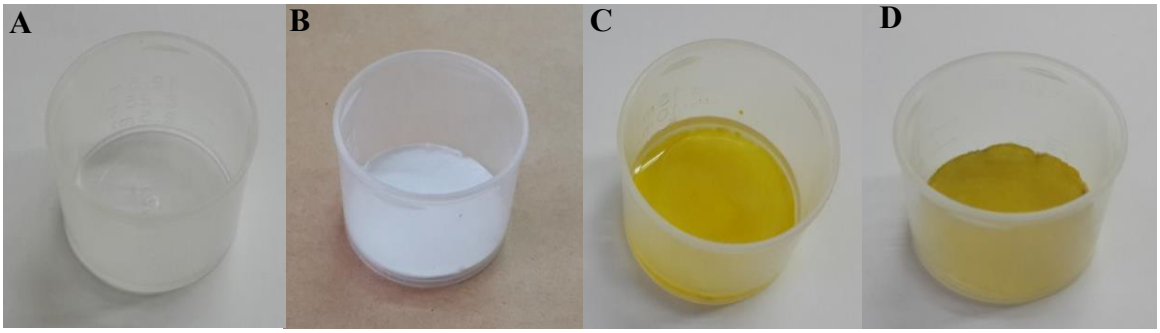

Figure 2. ***The steps of preparation of the biosensor. A is an empty cup; B is the cap with a filter paper; C is the CrO nanoparticles solution (acidic medium, 15% sulfuric acid) is added to the filter paper; D is the final shape of the biosensor.***

General Procedure

All the participants were given an instruction which included not drink any kind of drinks that contains alcohol 24 hours before the assay. While the alcohol group, their instruction included not to eat 3 hours before the assay. The alcohol assays were preformed to all participant at the same time. First, study information was given to all participants to ensure that they understood the effects of alcohol, for those with an alcohol group they were given detailed information about the alcohol dosages, assay time and types of drinks. The alcohol group participants were weighted and based on their weights, they were administered the alcohol doses at 0.8 ml/Kg of body weight. According to these doses, the blood alcohol concentrations were expected to be in the range of 0.01-0.03 g/100 ml. The participants drank 25 ml every 2 minutes for 15 minutes of standard alcohol drink which was mixed of water. Then, the alcohol assays were recorded every 0.5 hours for 3 hours. After complete the experiments, all the participants were remained in the laboratory to ensure their safety by decrease the BAC level to the safety level (Below 0.02%).

*Validation of the proposed method (***biosensor***) and Interpretation of it results*

Before using the disk in the real samples (saliva), the validity of it need to be check. Therefore, a series of alcoholic and non alcholic drinks such as whisky, vodka, wine, beer, barbican and water have been used to check whether is the prepared disk is capable to use as a detection tool for alcohol or not. The experiment has shown excellent results since all the alcholic drinks have shown positive results (positive results mean to change the colour of the disk from yellow

to greenish) as shown in Fig 3a, while the non-alcohol drinks have shown negative results (the disk remans yellow color) as shown in Fig 3 b.

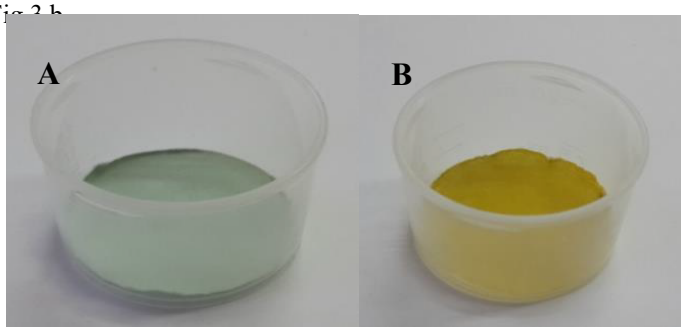

Figure 3. ***The interpretation of the results. (A) indicates of positive results; (B) indicates to the negative results***

3.Results and Discussion

As mentioned above, the development of rapid, cheap and on-site detection method of ethanol in the saliva is required. The proposed method is based on changing the colour of the composited filter paper by chromium oxide nanoparticles from colourless to the light green as a result of the following reaction:

$$2CrO_3 + 8H_2SO_4 + 3CH_3CH_2OH \quad 2Cr_2(SO_4)_3 + 3CH_3COOH + 11\ H_2O$$

Yellow Colorless Green-bluish

Therefore, in this paper, the results are considered as a positive when the colour of composited filter paper becomes light green, while a negative result when the filter paper remains colourless as shown in Fig 1.In the current study, 19 out of 20 of group (1) samples (95 %) have demonstrated positive results with variable intensity of green-bluish colour, while the remaining %15 showed negative results. While group (2), 24 out of 30 samples (80%) have shown positive results and the remaining 10 % showed negative results as shown in Table 2. All of the blood alcohol concentration estimation techniques depend on the amount of alcohol in the body which is, in turn, depends on many factors such as sex, food, type, quantity of beverage and the rate of alcohol elimination. Using the above techniques has many of disadvantages such as time consuming, requires laboratory skills and expensive. Therefore, the proposed method has overcome all previous difficult and it can rapid, reliable, non-invasive and valid method in comparison with the reported methods. Some of the results 5% and 20% for groups 1 and 2 respectively have shown negative results although all the samples have followed the same procedures, this can be explained by the following reasons. First, the proposed method is capable to detect the blood alcohol concentration of 0.02% or higher than this with excellent accuracy. Thus, any level of blood alcohol concentration less than 0.02% cannot be detected by the proposed method. Second, some alcoholic drinks have a very low alcoholic concentration (less than 5% like beer). While the positive results have shown variable intensities of green-bluish colour and this can be explained by the following facts: these high colour intensities due to consumption of drinks contain high alcohol concentration (>40%). Also, there are many of facts can also influence significantly on the detection method. For example, both of small intestine and the stomach are responsible for the alcohol absorption, therefore, empty stomach and beverage with higher alcohol concentration can lead to fast absorption rate and vice versa. Based on the literature, the maximum of absorption rate can be obtained when the beverage contains 20-45% of alcohol concentration , while the absorption rate is decreased due to consumption beverage with low alcohol concentration like beer (less 5%), high fluid or consume the beverage with food which were all noticed in the groups (1 & 2). Finally, group 1 has shown a high percentage of positive results (95%) than the group 2 (80%) which might be explained as the following: group 1 consumed the alcohol in a short period of time which means there is no enough time for alcohol to be metabolized and hence the high concentrations of alcohol in the blood will be released. While group 2 consumed alcohol with a long period of time which provides ample time for metabolism and lead to slow the absorption rate. All of obtained results are tabulated in Tables 2&3.

Table 2. ***The obtained results by the proposed and reference methods of group 1***

Beverage type	Total number (20)	No of positive sample	No of negative sample	Found in serum of positive samples g/100 ml[a] [24]	Found in serum of negative samples g/100 ml[a]
Whisky	5	5	0	0.02-0.3	NA
vodka	5	5	0	0.09-0.25	NA
Wine	5	5	0	0.06-0.21	NA
Beer	5	4	1	0.02-0.08	0.018

Table 3. ***The obtained results by the proposed and reference methods of group 2***

[a] Determination of Blood alcohol concentration by GC.[24]

Beverage type	Total number (30)	No of positive sample	No of negative sample	Found in serum of positive samples g/100 ml[a] [24]	Found in serum of negative samples g/100 ml[a]
Whisky	8	8	0	0.09-0.25	NA
vodka	8	6	2	0.07-0.23	0.016-0.018
Wine	8	6	2	0.06-0.22	0.016-0.019
Beer	6	4	2	0.02-0.09	0.016-0.018

[a] Determination of Blood alcohol concentration by GC[24].

4.Conclusion

In this paper, a colourimetric saliva-alcohol biosensor has been described, which is prepared by deposition of chromium (VII) Oxide Nanoparticles on the filter paper using an acidic medium (sulfuric acid). The disk is a rapid, practical and feasible method for detect of blood alcohol concentration of 0.02 % or higher throughout saliva for all types of samples even though for those with unconscious state. The above described procedure exhibits the capability of the method for detection of alcohol in saliva on site without any unnecessary pretreatment. The disk is simple low cost and rapid and it must be used in forensic or medicine purposes as a powerful tool for blood alcohol detection. Also, the it can be helpful for screening of individuals or identification of those who might be at risk due to alcohol consumption, which might be serve as a powerful tool against inappropriate consumption of alcohol.

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