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Benzocaine derivative determination via furan-2-carboxaldehyde reagent using differential pulse polarographic method

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A B S T R A C T

Benzocaine (BE) has been determined by the indirect differential pulse polarographic method (DPP). The suggested method is based on the synthesis of benzocaine via furan -2-carboxaldehyde reagent in presence of hydrochloric acid, then the diazotized at 0-5 ^{o}C with sodium nitrite to obtain a new benzocaine derivative. Also finding the values half-wave potential (E½) and electrochemical behavior using the dropping mercury electrode (DME). Optimum conditions affecting a quantitative analysis of benzocaine and its derivative such as solvent, pH, buffer solution and supporting electrolyte effect were explored. The benzocaine derivative showed a clear reduction peak at a potential of (-0.93 V) under optimal conditions. The standard calibration curve prepared for the benzocaine derivative at a concentration range of (5-100) µg.ml^{-1} gave a correlation coefficient of 0.9998, also limit of detection (LOD) and limit of quantitation (LOQ) of the benzocaine drug was 20.096 and 6.949 µg.ml^{-1} respectively, where the average relative standard deviation RSD of the benzocaine derivative was 0.41 % with recovery 100.40% (n=5). Based on the observation, the developed method could be employed for the estimation of benzocaine in pharmaceutical formulations.

Capsule Summary: The benzocaine derivative was prepared and estimated using pulse polarographic method. The developed method is highly precise, rapid and accurate to monitor the benzocaine derivative with LOD and LOQ values of 20.096 and 6.949 µg.ml^{-1}, respectively.

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INTRODUCTION

Benzocaine (BE) is a local anesthetic medication used to relieve pain locally and temporarily (Pysarevska et al., 2018). Benzocaine is a benzoic acid, 4-amino, ethyl ester (ethyl 4-aminobenzoate) acid ester and BE is official in USP2 and BP3 pharmacopeia (Ali, 1983). The BE is a white crystal and odorless (Paczkowska et al., 2018). Also, it is sparingly soluble in water and freely soluble in absolute ethanol, chloroform and ether has the following structure in Scheme 1 (Cheuk et al., 2020). Benzocaine is used as a local to lose the sensitivity and to eliminate the sensation of the hard pain (de Lima et al., 2018). As a result, benzocaine is now widely utilized in the pharmaceutical industry, including as a component of pastilles for sore throat therapy, creams, gels,

and liquids to ease toothache, ear drops (otocol drops), and irritated skin, as well as a local anesthetic for endoscopic procedures (Labidi, 2019).

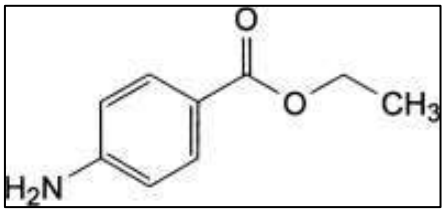
Benzocaine is also used as a long-term local anesthetic for animals such as sheep, cows, horses and pigs. Another significant use of benzocaine is as a sedative for fish and some amphibians such as frogs, adding benzocaine to a fish pond will result in a reduction in ammonia and carbon dioxide, as a result of this impact, the fish's efficacy will be decreased while maintaining a steady water pH (Chen et al., 2019; da Silva et al., 2018).

However, the intake of these aquatic products will cause harm to human health. Therefore, a certain period of withdrawal is needed for those anesthetic fish before they are eaten (Amal, 2018). In addition, the anesthetic residues may spread in water and soils, giving rise to serious risks for the environment (Groff et al., 2014). Therefore, the evaluation and monitoring of trace levels of anesthetics are necessary to ensure human health and to control environmental pollution (Bártíková et al., 2016; Smith, 2011; Sui et al., 2015). Benzocaine is a local anesthetic that inhibits nerve impulses briefly by affecting sodium ion permeability through the neuronal wall (Kurečič et al., 2018; Vadhanan et al., 2015). Because of its shape, it allows for rapid transmucosal and, to a lesser extent, transdermal transport (Pysarevska et al., 2021).

Benzocaine was determined using a variety of techniques, the most frequent of which was spectrophotometry and HPLC on reversed-phase method, which was also described as a stability-indicating method for benzocaine (Aronson, 2008; Plotycya et al., 2016). Additionally, a TLC technique was employed to estimate benzocaine in the presence of the benzocaine common product (Milz and Spangenberg, 2013). Also developed a spectrophotometric technique depends on the synthesis of absorbing complexes (Qadir, 2008). It also used flow injection analysis and coupling derivatization to estimate benzocaine (Paseková and Polášek, 2000). Because the benzocaine molecule has an amino group, it is easily electrochemically oxidizable, allowing electrochemical techniques to be used for the estimation (Taha et al., 2021). In voltametric measurements, the benefit of easy surface renewal of the electrode may be used. In addition, the electrode is clean, safe and unpolluted (Dubenska et al., 2021).

Polarography is the study of oxidizable and reducible materials electrolysis between a dropping mercury electrode (DME) and a reference electrode (RE) (AL-Ameri and AL-Mayahi, 2016). The distinctive characteristics of the polarographic wave are used in the quantitative and qualitative estimate of the material to be analyzed by measuring the diffusion current id, which is directly proportional to the concentration of the analyzed substance as a quantitative estimation (Al-Ameri and Al-Waeli, 2016). The half-wave potential ($E_{1/2}$) is used for qualitative analysis of the material (Lovrić, 2021). Among the many polarographic techniques, the differential pulse polarography (DPP) method is the most commonly utilized (Patil et al., 2019).

The aim of this study was to determine the benzocaine concentration indirectly by precise, rapid, and accurate via synthesis derivative with (furan-2-carboxaldehyde) by using differential pulse polarography (DPP) method.



Scheme 1: The structure of benzocaine (MW =165.2 $g.mol^{-1}$ and chemical formula = $C_9H_{11}NO_2$)

MATERIAL AND METHODS

Apparatus, chemical and reagents

For electrochemical analysis, a polarographic analyzer type 797VA Computrace Metrohm, Herisau, Switzerland was employed. It was used with a working electrode in DME mode, an auxiliary electrode in (Pt) wire mode, and a reference electrode (RE) in Ag/AgCl mode. All of the tests were conducted at a temperature of 25 ^{0}C. A WTW inoLab® IDS – Benchtop pH meter (Germany) was used to measure the pH (AL-Ameri and AL-Mayahi, 2016).

The analyses were performed through applying analytical grade reagents, chemicals substances and solvents. Absolute ethanol was used to prepare the standard solution and commercial drug sample. The pure form of benzocaine standard material was achieved from the state company for drug industries-Samara Iraq (SDI). The Lolite gel (20%) was obtained from local pharmacies.

The benzocaine (100 mg) was used to prepare a standard solution 1000 µg.ml-1 by dissolving in 100 ml volumetric flask with absolute ethanol. The other standard solutions were prepared by sequential dilution with absolute ethanol (Abu-Yamin and Elnawawy, 2021). Supporting electrolytes of 1M lithium chloride (LiCl) was prepared by dissolving 2.1 g in 50 ml of deionized water with continuous stirring also (0.1 and 0.01) M of lithium chloride (LiCl) was prepared by diluting 5 and 0.5 ml respectively of lithium chloride 1M in 50 ml of deionized water with continuous stirring (Ahmed et al., 2018).

The acetate buffer solution (pH = 2,3,4,5,7 and 8) was prepared by dissolving 6.8 g of sodium acetate salt ($C_2H_3NaO_2$) in an appropriate volume of deionized water and add a volume of 2.8 ml of glacial acetic acid and transfer it to a volumetric flask of 1000 ml and the volume to be completed with deionized water to the mark, than adjusted the pH value by adding drops of sodium hydroxide 0.1 N or hydrochloric acid 0.1 N and monitoring the change in the pH value with a pH meter (Bunnell and Mock, 2004).

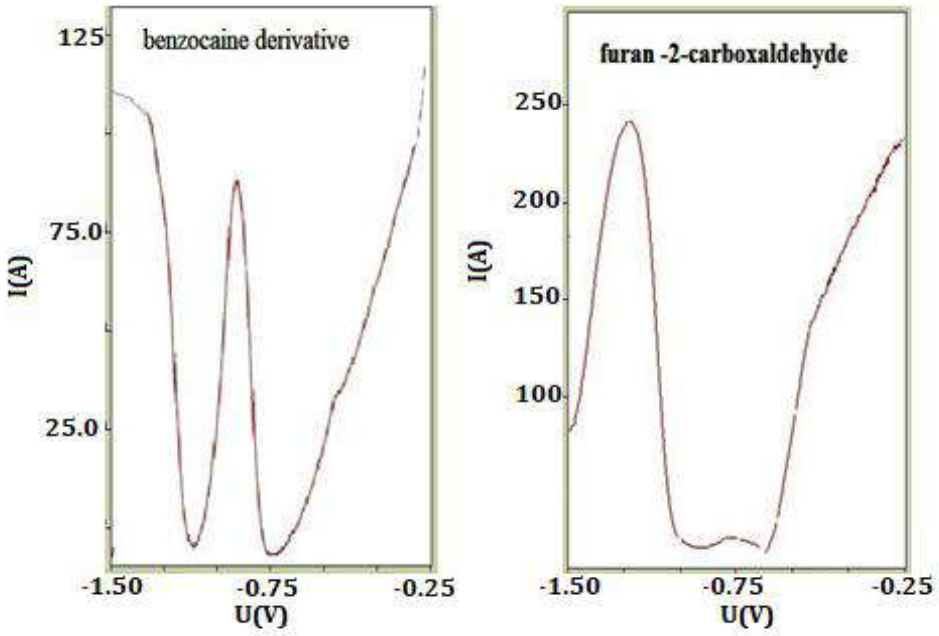
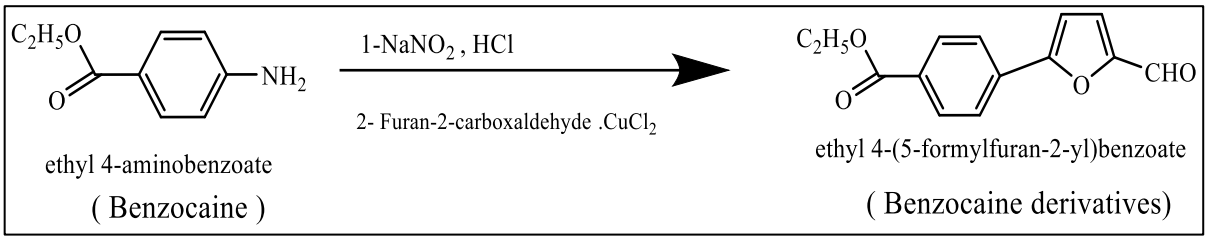
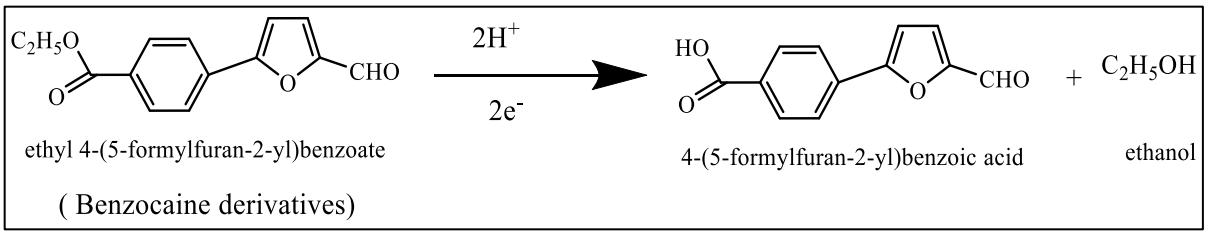


Fig. 1: DPP polargrams of aqueous benzocaine derivative, benzocaine drug and furan-2-carboxaldehyde reagent



Scheme 2: Suggested Mechanism reaction to preparation benzocaine derivatives



Scheme 3: The Suggested reduction mechanism of benzocaine drug

Synthesis of benzocaine

The Benzocaine derivative was prepared by dissolving (0.165 g, 1 mmol) in a mixture of concentrated HCl (33.7 ml, 37%) and H_2O (22.5 ml) and the solution were cooled to 0 ^{0}C, then diazotized at 0–5 ^{0}C with sodium nitrite (9.5 g, 138 mmol) dissolved in H_2O 25 ml. The solution was stirred for another 10 minutes, filtered and then furan-2-carboxaldehyde (15.4 g, 16 mmol) in H_2O (50 ml) was added along with a solution of Cu- $Cl_2.2H_2O$ (5 g, 40 mmol) in (25 ml) at a temperature of 10 -15^{0}C. The reaction mixture was gradually warmed up to 40 ^{0}C and stirred at this temperature for 4 hours. The precipitate was filtered with filter paper No. 45 , washed with water and an aqueous solution of Sodium bicarbonate (5%) and water and then the products was dried at room temperature and recrystallized from ethanol (Sterk and S. Republic, 2004; Tomi et al., 2016).

Standard calibration curve

A stock standard 1000 µg.ml^{-1} benzocaine derivative solution was prepared by dissolving 100 mg weight in an appropriate

volume of absolute ethanol in a 50 ml beaker and transfer the solution to a 100 ml volumetric flask and the volume was completed by adding absolute ethanol to the mark. An aliquot volume of 1000 µg.ml^{-1} benzocaine derivative standard solution was transferred to 20 ml volumetric flasks, and 1 ml of 0.1 *M* acetate buffer solution at pH 3.4 was added, along with 0.2 ml of LiCl 0.01 *M* as a supporting electrolyte, and diluted to the mark with absolute ethanol. The sample was transferred to a polarographic cell and degassed with high purity nitrogen for 300 s to purge the oxygen and analysis at scan rate 5 mVs^{-1} with pulse amplitude 50 (Al-Ameri and Al-Ameri, 2017).

RESULTS AND DISCUSSION

Optimization of DPP

Benzocaine has been studied using the differential pulse polarography (DPP) method. In order to obtain the best performing conditions that provide the best results, by carrying out the analyses at different conditions and selecting the one that resulted in highest value for the diffusion current, the effect of pH solutions, buffers and supporting electrolyte were chosen in the present investigation. Benzocaine drug, preliminary experiments were conducted to evaluate the behavior of the active groups in it and the extent of the best effort to work using (DPP) technique, and the use DEM with 99 percent pure absolute ethanol solvent.

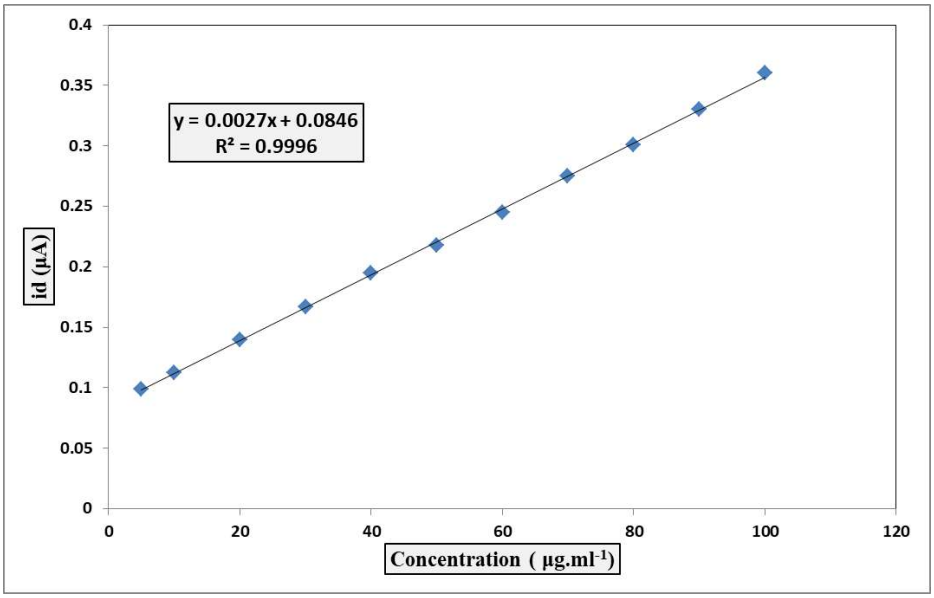


Fig. 2: Standard calibration curve for benzocaine derivative

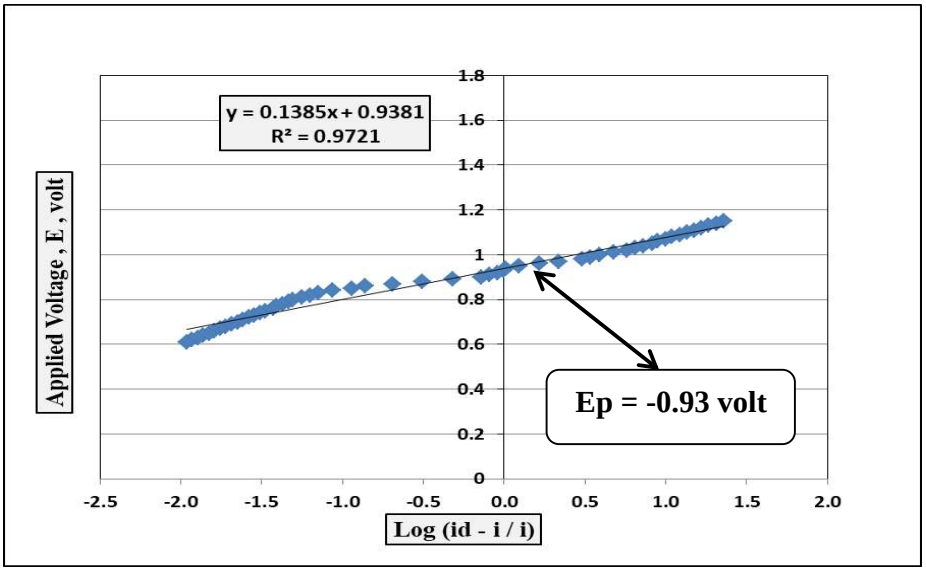


Fig. 3: Correlation between log (id-i)/i verses E for (10 µg. ml^{-1}) benzocaine derivative

Table 1: Analytical data for the calibration curve for the standard benzocaine derivative

Parameters	Benzocaine derivative
Peak potential, Ep, V	-0.93
Concentration range, µg.ml^{-1}	5 - 100
Regression equation, y = bx ± a	y = 0.0027x + 0.0846
Correlation coefficient, r	0.9998
Linearity, R^2	0.9997
Slope b, µA /µg.ml^{-1}	0.0027
Intercept, a, µg.ml^{-1}	0.0846
Standard error of regression line, $S_{y/x}$	0.0019
Standard deviation of intercept, S_a a ± t(n-2)S_a at 95%	0.0011
Standard deviation of slope, S_b b ± t(n-2)S_b at 95%	0.000018
Limit of Detection, LOD, µg.ml^{-1}	2.096
Limit of Quantitation, LOQ, µg.ml^{-1}	6.949

Table 2: Analytical results of standard benzocaine samples

Initial Conc. (µg.ml^{-1})	Found Conc. (µg.ml^{-1})	Absolute Error	Relative Error (%RE)	Recovery %	Standard Deviation (SD)	(RSD%)	C.L = X ± ts/ (√n)
10	9.96	-0.04	-0.37	99.63	0.02	0.21	9.96±0.02
40	40.67	-0.67	1.67	101.67	0.38	0.96	40.67±0.38
80	79.93	0.07-	-0.09	99.91	0.04	0.05	79.93±0.04
n = 5				t_{n-2} = 2.262			

Benzocaine derivative showed a peak at (-0.93 V) applied potential in 0.08 *M* acetate buffer at pH 3.4 as a best buffer solution, also 0.01 M LiCl was found to be the most excellent supporting electrolyte compared with KNO_3 and KCl, where causes precipitation of the benzocaine in solution. The synthesis of the benzocaine derivative was verified at a concentration of 50 µg.ml^{-1} using DPP by the appearance of a polarographic peak at (-0.93 V), while the benzocaine drug showed a polarographic peak at (-0.55 V) and furan -2-carboxaldehyde reagent polarographic wave peak (-1.26 V) (Figure 1).

Benzocaine derivative showed a distinguished peak at (-0.93 V) applied potential versus in 0.08 *M* acetate buffer at pH 3.4 as a best buffer solution, also 0.01 M LiCl was found to be the most excellent supporting electrolyte. Also, the proposed mechanism reaction for the benzocaine derivative prepared with furan -2-carboxaldehyde, Scheme 2.

Method validation

Under optimal measurement conditions, a standard calibration curve prepared via measured diffusion current of benzocaine derivative with the corresponding benzocaine derivative concentration values within the concentration range 5–100 µg.ml^{-1} (Figure 2). The results showed a linear equation statistically treated using the least squares method.

The statistical data for the standard curves showed a straight-line equation well suited for the analysis of benzocaine derivative, also it was used to find the drug concentration in samples. The results showed that the LOD and LOQ found for benzocaine derivative was equal to 2.096 and 6.949 µg.ml^{-1}, respectively (Table 1). The accuracy and precision of the method for the determination of benzocaine derivative were confirmed. Various standard samples were prepared and analysis (n = 5) (Table 2).

Number of transferred electrons and actual $E_{1/2}$

The Ilkovic -Heyrovsky equation was used to calculate the number of electrons transferred during the reduction on the electrode and the actual value for the half-wave potential ($E_{1/2}$) at 25 °C. This equation explains the polarographic wave as reversible/irreversible reaction when the number of electrons is integer and irreversible while (n) is non-integer. Number of electrons (n) can be calculating from the plot of log (i/id -i) versus applied voltage (E) at set group concentrations (Eq. 1) (Al-Ameri et al., 2019).

$$E_{applied} = E_{1/2} - (0.0591/n) \log (i/id - i) \quad (1)$$

The actual half-wave potential ($E_{1/2}$) calculated of benzocaine derivative was -0.93V and two electrons were required for the reduction Figures 3. In benzocaine

derivative have one carboxylic group is one of the active groups with electrochemical activity, this group can be electrically reduced at the surface of the mercury electrode, resulting in the appearance of the electrical diffusion current at the cathode, the results showed easily carboxylic group reduced to give two electrons transfer (Khalil and Hussain, 2010) (Scheme 3).

CONCLUSIONS

Benzocaine was determined by the indirect differential pulse polarographic method and the proposed methods found to be highly precise, rapid and accurate to monitor the benzocaine derivative with LOD and LOQ values of 20.096 and 6.949 µg.ml-1, respectively. The method has illustrated that the DPP technique had several advantages that make it appropriate, for estimating benzocaine in pharmaceutical formulations.

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