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Adsorptive stripping voltammetric determination of pyridostigmine bromide in bulk, pharmaceutical formulations and biological fluid

Rajeev Jain [a], Vinod K. Gupta [b,c,*], Nimisha Jadon [a,b,c], Keisham Radhapyari [a,b,c]

[a] School of Studies in Chemistry, Jiwaji University, Gwalior 474 011, India
[b] Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247 667, India
[c] Chemistry Department, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia

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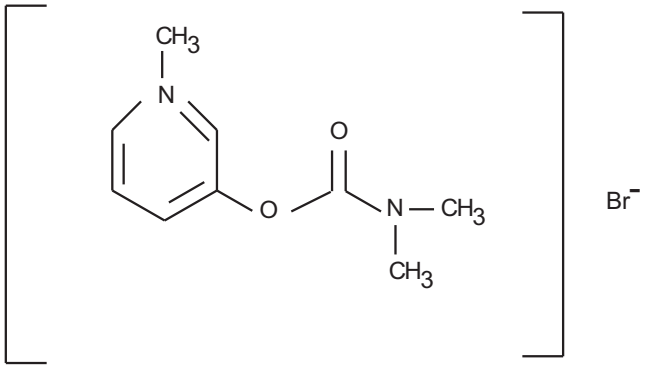
ABSTRACT

Electro-analytical behaviour of pyridostigmine bromide (PB) in BR buffers of pH range 2.4–10 at a hanging mercury drop electrode has been investigated using cyclic voltammetry, differential pulse cathodic adsorptive stripping voltammetry (DPCAdSV) and square-wave cathodic adsorptive stripping voltammetry (SWCAdSV). Voltammograms of the drug exhibited a single two-electron wave and it may be attributed to the reduction of –C=O– centre. Based on the high adsorptive character of PB onto the mercury electrode, a validated direct square-wave cathodic adsorptive stripping voltammetric and differential pulse cathodic adsorptive stripping voltammetric procedure has been developed for the determination of drug in bulk form and pharmaceutical formulations. The proposed SWCAdS and DPCAdS voltammetric methods allow quantitation over the range 100 ng mL^{-1}–72 μg mL^{-1} and 1–80 μg mL^{-1} with detection limit of 20.7 and 32.3 ng mL^{-1}, respectively. The procedure was applied to the assay of the drug in tablets form with mean percentage recoveries of 100.1% with SWCAdSV and 99.99% with DPCAdSV. Precision and accuracy were also checked and were within the limits. The peak current was linear with the drug concentration and percentage recovery was found to be good.

1. Introduction

Pyridostigmine bromide (PB) [3-[[(dimethylamino) carbonyl] oxy]-1-methylpyridinium bromide][A] is a synthetic quaternary ammonium compound belongs to a class of neuro-active compounds called carbamates [1] that contains an amide backbone and a tertiary amine. PB is a powerful and reversible anticholinesterase (AchE) agent, which effectively increases the concentration of acetylcholine at the sites of cholinergic transmission [2]. Pyridostigmine bromide (PB) is one of the drugs currently used for the symptomatic treatment of myasthenia gravis [3] patients, and in neuromuscular blockade [4–6].



[A]: Structure of Pyridostigmine bromide

Perusal of literature reveals that several analytical methods have been used for identification and quantification of the pyridostigmine bromide and their metabolites in bulk, pharmaceutical

* Corresponding author at: Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247 667, India. Tel.: +91 1332 285801.
E-mail addresses: vinodfcy@iitr.ernet.in, vinodfcy@gmail.com (V.K. Gupta).

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formulation and in biological fluid. These methods used high performance liquid chromatography [7,8], gas chromatography [9,10], thermo-spray LC/MS method [11], gas chromatography–mass spectrometry [12,13], capillary electrophoresis [14], enzymatic or immunoassay methods [15,16], high performance liquid chromatography–mass spectrometry [7,17,18], and ion chromatography [19].

However, although the selectivity and the detection limit have been improved in these methods, these are rather time consuming and require large number of complicated steps to follow for analysis. Pyridostigmine bromide molecule has electro-active groups but its electrochemical behaviour has not so far investigated. Literature revealed that electrochemical techniques have demonstrated a large applicability in studies of electrodic reactional mechanisms of pharmaceutical compounds [20–30]. In the present investigation electrochemical investigation of PB has been undertaken to have some insight into its redox process, which is important for our understanding of its property as well as its metabolism in biological system. Furthermore, there appears to be no electro-analytical method for the determination of PB in pharmaceutical formulation and bulk form. The procedure did not require sample pre-treatment or any time-consuming extraction steps other than centrifugal separation of the precipitated protein and other impurities from urine solution prior to the drug assay.

The purpose of the present work is to study the voltammetric behaviour of PB by employing different voltammetric techniques and to establish the methodology for their trace determination using cyclic voltammetry (CV), differential pulse cathodic adsorptive stripping voltammetry (DPCAdSV) and square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) in pharmaceutical formulation.

2. Experimental

2.1. Reagents and materials

Pyridostigmine bromide (99% purity) was obtained from Samarth Pharma Pvt. Ltd., Mumbai, India and was used as received. Pharmaceutical formulation *Distinon* tablets (Product of Samarth Life Sciences Pvt. Ltd., Mumbai, India, B. No. T 2702) labeled to contain 60 mg pyridostigmine bromide per tablet was obtained from commercial sources. For the preparation of standard pyridostigmine bromide stock solution (1 mg/mL), 100 mg pyridostigmine bromide was accurately weighed, dissolved in acetonitrile (ACN) and then adjusted to 100 mL with the same solvent to give the appropriate concentration. Standard working solutions were prepared by appropriate dilutions of the stock solution. The stock solution was stable for at least 1 month when kept in refrigerator. The solution for recording voltammograms was prepared by mixing appropriate volume of stock solution and buffer of varying pH. Britton Robinson buffers in the pH range 2.5–12.0 were used. Solution was stirred after mixing and left overnight to attain equilibrium. The ionic strength was kept constant by adjusting with 1 M KCl. All reagents and solvents were of analytical reagent grade (Merck and Sigma). High purity water was obtained from Millipore (Milford, MA, USA) Milli-Q Plus system.

2.2. Instrumentation

Electrochemical measurements were performed using a μAUTOLAB TYPE III (Eco-Chemie B.V., Utrecht, Netherlands) potentiostat–galvanostat with 757 VA computrace software. A conventional three-electrode system was used consisting of an Ag/AgCl/KCl reference electrode, a hanging mercury drop electrode (HMDE) as a working electrode and a graphite rod as auxiliary electrode. The whole measurements were automated and controlled through the programming capacity of the apparatus.

Controlled potential coulometric experiments were performed using an Autolab Potentiostat/Galvanostat PGSTAT Metrohm 663 VA stand as electrochemical cell, fitted with a PC provided the appropriate GPES 4.2 (General Purpose Electrochemical Software) Software. Coulometric experiments were performed in the potentiostatic mode using Pt foil with large surface area as working electrode and a Pt wire, counter electrode.

Solutions examined by electrochemical techniques were purged for 10 min with purified nitrogen gas after which a continuous stream of nitrogen was passed over the solutions during the measurements. All measurements were carried out at room temperature. The pH metric measurements were made on Decible DB – 1011 digital pH meter fitted with a glass electrode and saturated calomel electrode as reference, which was previously standardized with buffers of known pH in acidic and alkaline medium. Ready made precoated TLC silica gel plates from E Merck, Germany were used for TLC separation. The IR spectrum of solid complex was recorded using KBr pellets on a Shimadzu, Prestige IR 20, IR spectrophotometer. LC/MS spectra was recorded at UPLC/MS–MS from waters (ESI +ive mode).

2.3. Procedure

2.3.1. Operational conditions and electrochemical measurements

For cathodic adsorptive stripping voltammetric measurements, a known volume of pyridostigmine bromide was pipetted into 10 mL volume calibrated flask and then completed to volume with BR buffer (pH 3.8), acetonitrile (ACN) and KCl. After that purified nitrogen was passed for 5 min to remove the dissolved oxygen under stirred conditions. The accumulation potential was applied at the working electrode for a selected time by keeping the constant pulse amplitude while the solution was stirred. At the end of the accumulation time period the stirrer was stopped and 3 s was allowed for the solution to become quiescent. Voltammograms were than recorded by scanning the potential towards the negative direction over the range −1.0 to −1.7 V vs. Ag/AgCl/KCl reference electrode by applying the square-wave waveform and peak current was measured at −1.48 V. The electrode cleaning procedures were carried out for each and every experiment and this required only 5 min. Electrochemical pre-treatment was always performed in the same solution in which the measurement was subsequently carried out. Studies were carried out at 25 ± 0.1 °C.

2.3.2. Construction of calibration curve

Aliquot volumes of pyridostigmine bromide covering the working range 100 ng mL^{-1}–72 μg mL^{-1} were transferred into 25 mL volumetric flasks. It was completed to the mark with ACN. The solution was then transferred into a voltammetric cell and pure N_2 gas was passed for 5 min. Square wave voltammogram and differential pulse voltammogram were recorded in the range −1.0 to −1.7 V. The calibration graph obtained with a known concentration of pyridostigmine bromide was used to convert peak current into sample concentrations. The recovery was calculated using the standard addition method.

2.3.3. Tablets assay procedure

Pyridostigmine bromide determination was performed on commercially available tablet dosage form *Distinon*. Each film coated *Distinon* tablet contains 60 mg of pyridostigmine bromide. Excipients such as colloidal silicon dioxide, lactose anhydrous and stearic acid or alternatively; lactose, starch, silica precipitated, talc and magnesium stearate were added to dosage form, etc. Ten tablets were weighed accurately and thoroughly grounded to a fine powder. A portion of the powder equivalent to the average weight of one tab-