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Electrochemical reduction behavior and differential pulse polarographic determination of Bambuterol HCl in pharmaceuticals and spiked human urine samples

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ABSTRACT

The electrochemical reduction behavior of bambuterol HCl has been studied by using direct current polarography, differential pulse polarography, cyclic voltammetry, millicoulometry and controlled potential electrolysis in universal buffers ranging from pH 2.0 to 12.0 in methanol-water mixtures. Kinetic parameters such as diffusion coefficient (D), forward rate constant (k^{o}_{fh}) values are evaluated and a reduction mechanism is proposed. Quantitative measurements were successful in the concentration range $1.0x10^{-5}$ to $1.25x10^{-9}$M with lower detection limit $1.25x10^{-8}$M. A simple and rapid differential pulse polarographic method has been developed for the determination of bambuterol HCl in commercial capsules containing the drugs using the standard addition and calibration methods.

Key words: Bambuterol HCl, polarography, pharmaceutical and urine sample.

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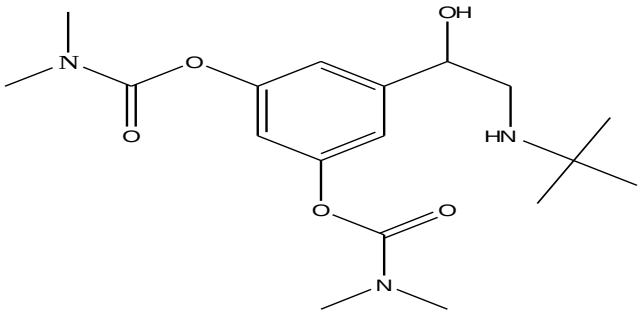
INTRODUCTION

Bambuterol HCl, [(3-[2- (tert - butylamino)- 1- hydroxyethyl]- 5 - (dimethylcarbamoyloxy) phenyl] N,N-dimethylcarbamate] is known to a prodrug of terbutaline but has better therapeutic to toxicity ratio and acts as an endogenous reservoir or terbutaline. Bambuterol is a pro-drug of terbutaline that is slowly converted into the body to the active form, thus providing a prolonged action. Shaise developed a HPTLC method for estimation of bambuterol in tablets [1]. The reverse phase HPLC method for determination of montelukast sodium and bambuterol hydrochloride in tablet dosage form [2] and different HPLC methods were reported for the determination of bambuterol hydrochloride in pharmaceutical dosage form [3-5]. A LC–MS/MS method was developed for the simultaneous screening of 75 basic drugs (bambuterol) using a short LC column coupled with a fast scanning triple-quadrupole mass spectrometer [6]. A high throughput LC–MS/MS method was developed for the simultaneous screening of 75 basic drugs (bambuterol) using a short LC column coupled with a fast scanning triple-quadrupole mass spectrometer [7]. Carbon-13 NMR spectra of the stable polymorphs of solid bambuterol hydrochloride and terbutaline sulfate are reported [8]. Bambuterol was isolated from plasma and urine by solid-phase extraction and analysed by gas chromatography/chemical ionization mass spectrometry [9]. Comparison of oral bambuterol and inhaled salmeterol in patients with symptomatic asthma and using inhaled corticosteroids [10]. Bambuterol is an oral terbutaline prodrug with a prolonged duration of bronchodilator action [11]. A reversed-phase ion-pair liquid chromatographic method was applied to the analysis of bambuterol hydrochloride [12]. The pharmacokinetics of bambuterol in subjects homozygous for the atypical gene for plasma cholinesterase [13]and orally administered bambuterol were investigated in healthy adult subjects [14].



SCHEME 1- Structure of bambuterol HCl.

EXPERIMENTAL

Materials and reagents

Bambuterol HCl was procured from J.INC. company, Ahmedabad, INDIA were obtained from commercially. A stock solution was prepared by dissolving bambuterol HCl in methanol. The universal buffers of pH 2.0 to 12.0 were prepared by using 0.2M boric acid, 0.05M citric acid and 0.1M trisodium orthophosphate. All the reagents used in the experiments were of Analar grade. Triple distilled water was used throughout the experiments. Desired

concentrations of solutions were prepared daily from stock solution. The test solution was purged with purified nitrogen gas for 10 to 15 minutes before the polarograms were taken. All the experiments were carried out at 298±1K temperature.

Instrumentation

D.C. polarography and differential pulse polarography were performed with a model 362 polarographic analyzer supplied by ELICO Ltd, Hyderabad, coupled with a three electrode system consisting of a dropping mercury electrode (DME) (surface area = 0.026 cm^2), the polarographic cell bottom was fitted with a saturated calomel electrode (SCE) was worked as reference electrode and a platinum wire was worked as a auxiliary electrode. EPSON LX-300$^+$ printer were used for D.C. polarography and differential pulse polarographic measurements. Metrohm unit E 506 polarecord coupled with E 612 VA-Scanner, E 648 VA- Controller and digital electronics x-y/t recorder are used for cyclic voltammetry. pH values were measured with pH meter supplied from HENNA Instruments, Italy. A magnetic stirrer (PAR 305) and stirring bar provided the convective transport during pre-concentration.

Preparation of tablet assay

Ten tablets of bambuterol HCl were weighed and then crushed into a fine powdered in a mortar. A suitable amount of this powder was accurately weighed and then dissolved in methanol. It was sonicated for 10 minutes. The content was allowed to settle after stirring magnetically for 10 minutes. The sample solution was filtered through a whatman no.42 filter paper. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquid and diluting them with universal buffer solution in order to obtain a final solution of $1.0X10^{-5}$M bambuterol HCl. Each solution was transferred to a voltammetric cell and the voltammograms were subsequently recorded following the optimized conditions. The content of the drug in tablet was achieved by the standard addition method.

Preparation of urine assay

A quantity of bambuterol HCl was added to a spiked urine sample of 1 ml was dissolved in methanol to achieve final stock solution concentration of $1.0X10^{-5}$M. It was stirred magnetically for 10 minutes and filtered through a filter paper (whatman no.42) and added 9 ml of universal buffer solution. The samples were determined by above described method.

RESULTS AND DISCUSSION

Electrochemical reduction behaviour of bambuterol HCl has been examined over the pH range of 2.0 to 12.0. In acidic and neutral solutions (pH<6) bambuterol HCl was found to give a single well defined cathodic wave/peak which was attributed to the simultaneous reduction of the two carbonyl groups to the corresponding hydroxyl derivative in a four electron process.

Typical differential pulse polarogram was shown in Fig.3. No waves are observed in the basic medium (8<pH<12) due to the precipitation of the electroactive species.

The diffusion controlled nature of the electrode process is evidenced from the linear plots of i_d vs $h^{1\backslash 2}$ and i_m vs $t^{2/3}$ that pass through the origin indicating the absence of adsorption complications. The slight variation of the wave potential values of the title compound was found to be pH dependent and to shift towards more negative values along with an increase in the pH of the buffer system indicating the electrode process to be irreversible. Typical voltammograms for title compound were shown in Fig.1to Fig.3.

Millicoulometry employed at pH 2.0 to find out the number of electrons involved in the electrode process. The results showed the number of electrons to be four. From the slope of the $E_{1/2}$ vs pH plot, the number of protons involved in the rate determining step of the electrode process is found to be two. Controlled potential electrolysis experiments were carried out at -0.27V vs saturated calomel electrode at pH 4.0. The isolated product was identified as hydroxyl product and confirmed by IR spectrometry (absence of C=O stretch 1690 – 1650 cm^{-1}, O-H stretch 3428 cm^{-1}, O-H bend 1364 cm^{-1} and C-O stretch 1248 cm^{-1}).

The values obtained for diffusion coefficient (D) and heterogeneous forward rate constant (k^0_{fh}) at various pH values in D.C. polarography, cyclic voltammetry and differential pulse polarography were given in Table 1. The diffusion coefficient values evaluated from all the techniques are in good agreement. This also indicates absence of absorption complications in the electrode process. The diffusion current and diffusion coefficient values are found to decrease with increase in the pH the solution. This trend shows that the reaction scheme involves successive proton and electron transfers in acidic media. Based on the results obtained, the electrochemical reduction mechanism of bambuterol HCl can be proposed as in Scheme 2.

Differential pulse polarographic peak obtained at pH 4.0 was used in the analytical estimation of bambuterol HCl. Standard addition and calibration methods were used for the analysis of the bambuterol HCl. The peak current was found to vary linearly with the bambuterol HCl concentration over the range of $1.0x10^{-5}$ to $1.25x10^{-9}$M. The lower detection limit was calculated using the expression dl = 3xSd/m (where Sd was the standard deviation and m was the slope of the calibration plot) is found to be $1.25x10^{-8}$M.

Recommended analytical procedure

Bambuterol HCl was analysed by the standard addition method. The standard solution ($1.0x10^{-5}$ M) was prepared by dissolving the appropriate amount of the electroactive species in methanol. One ml of the standard solution is transferred to the polarographic cell and made up to 10 ml with the supporting electrolyte to get the required concentration and then deoxygenated by bubbling nitrogen gas for 10 min. After recording the polarogram, small increments of the standard solutions (0.5ml) were added and then polarograms were recorded

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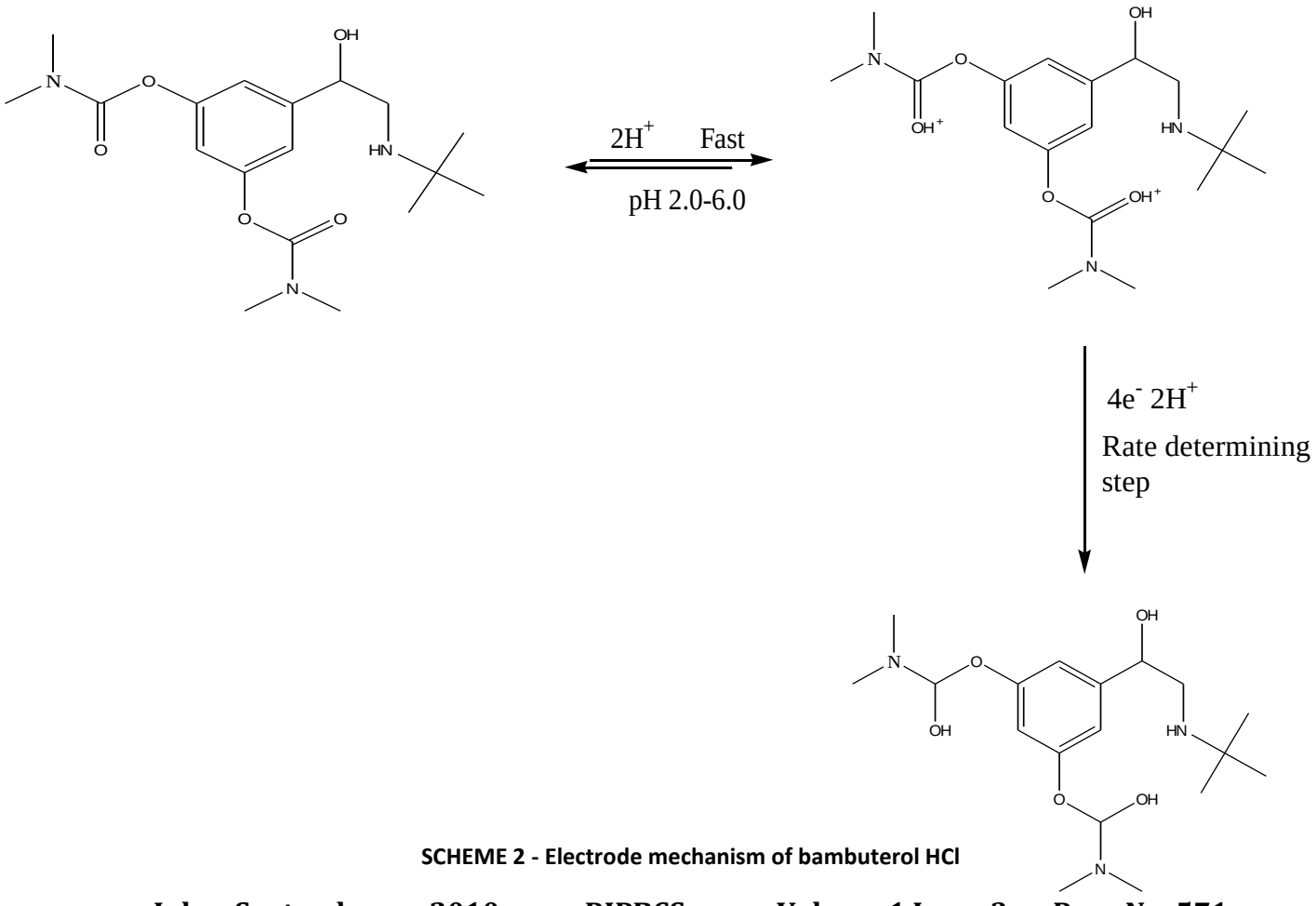
for each addition under similar conditions. The optimum conditions for the determination of bambuterol HCl at pH 4.0 were found to be a drop time of 2 sec, a pulse amplitude of 50mV and an applied potential of -0.27V vs saturated calomel electrode. The relative standard deviation and correlation coefficient values were found to be 1.64%and 0.9423 respectively for 10 replicates.

For analysis of bambuterol HCl in urine sample, different amounts of bambuterol HCl were added to a fixed volume of urine. A small portion of these spiked urine samples were diluted with supporting electrolyte and polarogram recorded. The intension of our experiments with urine samples was to study the various possibilities to overcome a bio-matrix effect and to find the best solution, but to explore the way for direct, simple, reliable and fast determination bambuterol HCl in urine by its dilution by employing differential pulse polarography. The potential interference of some urine ingredients were checked without adding depolarizer. The recovery was found to be 97.6% with relative standard deviation of 1.2% at the lowest concentration level and 99.10% with the relative standard deviation of 0.4% at the concentration of µg/ml and higher. The determination of bambuterol hydrochloride in pharmaceutical formulations and urine samples are presented in Table 2 and 3.



SCHEME 2 - Electrode mechanism of bambuterol HCl

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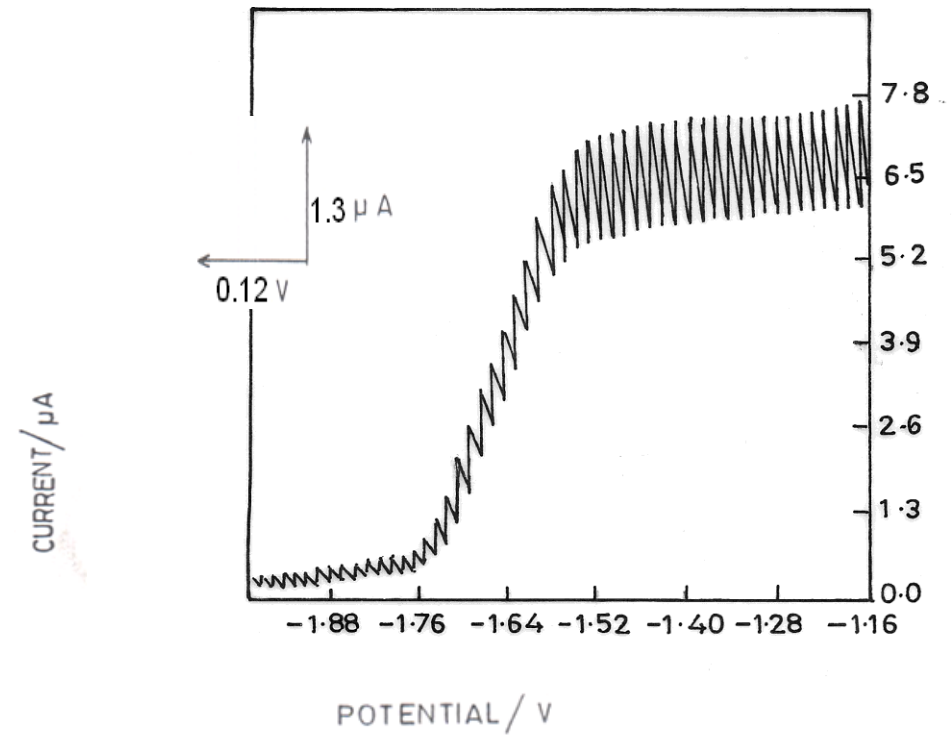

FIGURE 1 - Typical d.c. polarogram of bambuterol HCl at pH 2.0. Concentration: 0.5 mM Drop time : 2 Sec

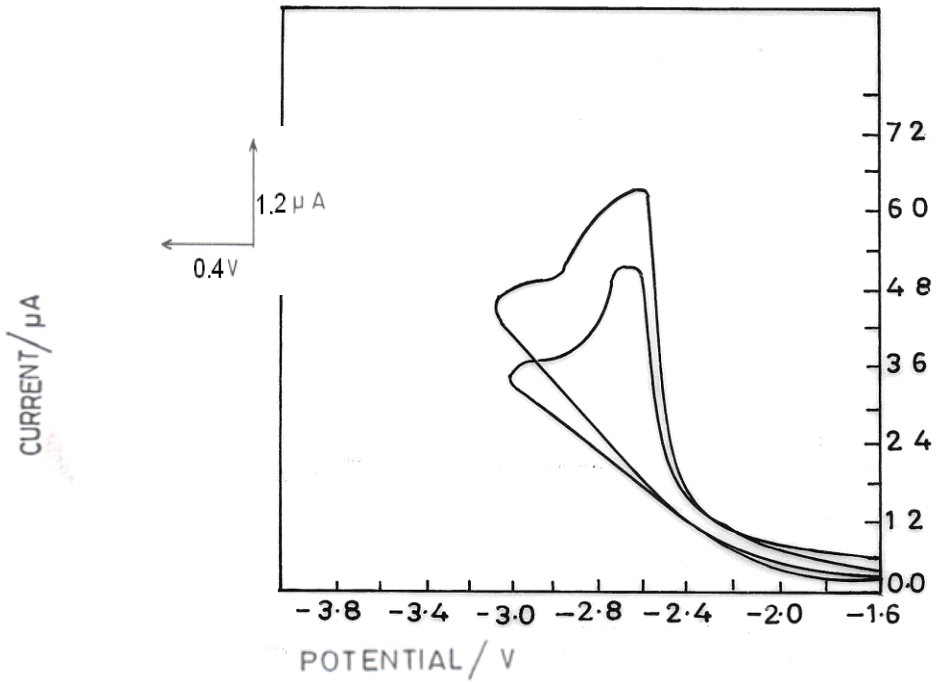

FIGURE 2- Typical cyclic voltammogram of bambuterol HCl at pH 6.0. Concentration : 0.5mM Scan rate : 40mVs^{-1}

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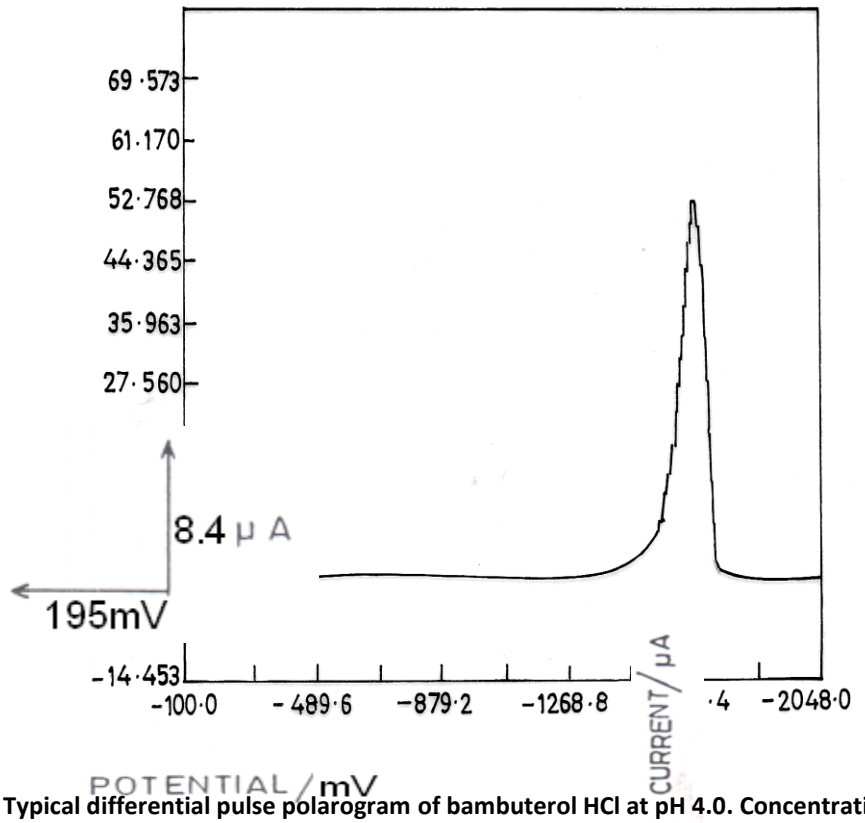

FIGURE 3- Typical differential pulse polarogram of bambuterol HCl at pH 4.0. Concentration : 0.5mM Drop time : 2 sec. Pulse ampliture : 50 mv

TABLE 1- Typical kinetic data of bambuterol HCl Concentration: 0.5 mM, Drop time: 2 sec

pH of the supporting electrolyte	D.C. Polarography			Cyclic voltammetry			Differential pulse polarography		
	$-E_{1/2}$ V	$D \times 10^{-5}$ Cm^2s^{-1}	$K^0_{f,h}$ Cms^{-1}	$-E_p$ V	$D \times 10^{-5}$ Cm^2s^{-1}	$K^0_{f,h}$ Cms^{-1}	$-E_m$ V	$D \times 10^{-5}$ Cm^2s^{-1}	$K^0_{f,h}$ Cms^{-1}
2.0	1.61	8.76	6.52×10^{-5}	1.58	8.08	8.68×10^{-5}	1.21	8.17	6.36×10^{-5}
4.0	1.80	7.45	7.24×10^{-6}	1.64	6.81	7.80×10^{-6}	1.64	6.87	8.16×10^{-6}
6.0	2.81	7.09	6.16×10^{-7}	2.65	6.34	6.96×10^{-8}	2.51	6.50	8.12×10^{-7}

TABLE 2 - Polarographic assay of bambuterol HCl by DPP in pharmaceutical formulations.

S.No.	Sample	Labeled amount mg/L	Average amount found mg/L	Recovery %	Standard deviation	%Relative Standard deviation
1	ABEL	10	09.87	98.70	0.065	0.65
		20	19.76	98.80	0.120	0.60
2	ASTHAFREE	10	09.76	97.60	0.120	1.21
		20	19.60	98.00	0.200	1.01
3	BAMBUDIL	10	09.88	98.80	0.060	0.60
		20	19.79	98.95	0.105	0.52
4	BEMLO	10	09.82	98.20	0.090	0.90
		20	19.80	99.00	0.100	0.50

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TABLE 3- Polarographic assay of bambuterol HCl by DPP in spiked human urine samples.

S.No.	Sample	Labeled amount mg/L	Average amount found mg/L	Recovery %	Standard deviation	%Relative Standard deviation
1	ABEL	10	09.80	98.00	0.100	1.010
		20	19.69	98.45	0.155	0.781
2	ASTHAFREE	10	09.78	97.80	0.110	1.112
		20	19.64	98.20	0.180	0.908
3	BAMBUDIL	10	09.84	98.40	0.080	0.806
		20	19.80	99.00	0.100	0.502
4	BEMLO	10	09.84	98.40	0.080	0.401
		20	19.82	99.10	0.090	0.452

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