ADSORPTIVE CATHODIC STRIPPING DETERMINATION OF MINOXIDIL IN PHARMACEUTICAL, CREAM AND SHAMPOO PRODUCTS

Farhad AHMADI[a1,*], Sahar GHASEMI[a2], Mehdi RAHIMI-NASRABADI[b]

[a] *Department of Medicinal Chemistry, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Islamic Republic of Iran; e-mail: [1] fahmadi@kums.ac.ir, [2] sahar_4171@yahoo.com*

[b] *Faculty of Science, Immam Hossein University, Tehran, Iran; e-mail: rahimi1356@gmail.com*

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The cyclic voltammetric behavior of minoxidil was studied in a buffer with pH 3. Contradictory to that mentioned in a previously published work, the cyclic voltammogram of minoxidil exhibited a single 2-electron irreversible reduction wave in a buffer with pH 3. This wave was attributed to the reduction of the N→O bond. The cathodic differential pulse wave height decreased on the increase of pH till it disappeared in solution with pH 7.2. The quantitative trace determination of minoxidil was studied at a hanging mercury drop electrode by adsorptive cathodic stripping voltammetry. A fully validated sensitive procedure based on controlled adsorptive accumulation of the drug onto a HMDE was developed for its direct determination. Accumulation of minoxidil was found to be optimized in 0.1 M Britton–Robinson buffer with pH 2.0 as supporting electrolyte under the following conditions: accumulation potential –0.2 V, accumulation time 40 s, scan rate 40 mV s^{-1} and pulse height 50 mV. The proposed procedure was applied successfully for determination of minoxidil in its topical solution and illegal shampoo and cream. The mean recoveries of the minoxidil were 99.8, 97.8 and 96.7% and with RSD of 0.86, 1.24 and 1.89% in pharmaceutical topical solution, shampoo and cream, respectively.

Keywords: Adsorptive cathodic stripping voltammetry; Minoxidil; Illegal shampoo; Illegal cream; Topical solution of minoxidil.

Minoxidil, 2,4-diamino-6-piperidinopyrimidine-3-oxide (Fig. 1) is the most common active anti-alopecia and baldness and also recently used in combination with other drugs for treatment of hirsutism and acne[1,2]. In Iran, three approved marketed products are presented in pharmaceutical preparations (tablet 10 mg, topical solutions 2 and 5%) for systemic use. Also, one lotion containing minoxidil–isotrepinoied (2–0.05% w/v) with the name of Minoxidil compound is produced. Conversely, no cosmetic, cream, dermal solution, soap and shampoo containing minoxidil are produced in Iran, therefore, several illegal cosmetic, cream and shampoo products containing

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minoxidil were sold through internet web sites or illegal circuits. These products contain different illegal amounts of minoxidil and other drugs. Cosmetic products have no therapeutic purposes and shall not claim any therapeutic action. According to the report of food and drug organization (FDO) law of Iran, the illegal use of minoxidil, spironolactone, progesterone, estrone, hydrocortisone, triamcinolone and canrenone in cosmetic products is forbidden. Therefore, the analysis of illegal ammount of minoxidil in cosmetic and shampoo products is very important. It was hypothesized that, in order to produce a pharmaceutical effect, an illegal product should contain an amount of minoxidil comparable to the minimum concentration of minoxidil usually administered in pharmaceutical preparation. If an illegal product contains minoxidil at this level of concentration, the usuall techniques such as ion pair-HPLC-UV detection[3], differential pulse polarography[4] derivative spectrophotometry[5], capillary zone electrophoresis[6] and micellar electrokinetic capillary chromatography[7] are usually adequate. Nonetheless, for detection of minoxidil in an illegal product at lower concentrations, the sensetive procedure such as HPLC-ESI-MS should be used[8]. The HPLC-EIS-MS is very expensive and need highly trained persons, therefore, developing sensetive, accurate and inexpensive techniques for determination of minoxidil is of interest. Today different electrochemical methods are widely used in various fields such as: bioelectrochemistry[9–12] analysis of target compounds[13–17], sensors[18,19] and etc. Among of these electrochemical methods, adsorptive cathodic stripping voltammetry (AdCSV) is favor because of easiness, speed of operation and very high sensitivity. To our knowledge, no report exists for determination of minoxidil by adsorptive stripping voltammetry. In this study, an adsorptive cathodic stripping voltammetry on hanging mercury drop electrode (HMDE) was developed to investigate the illegal presence of minoxidil in shampoo, cream and pharmaceutical preparations.

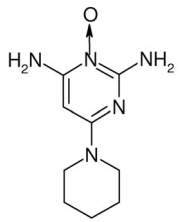


FIG. 1
Structure of minoxidil

EXPERIMENTAL

Materials and Instruments

All solutions were prepared with triple distilled water. Methanol and trifluoroacetic acid (TFA), acetonitrile and HPLC grade methanol were supplied by Merck (Darmstadt, Germany). The stock solution of minoxidil (1.0×10^{-2} mol l^{-1}) was freshly prepared by dissolving appropriate amounts of minoxidil (Sigma–Aldrich) in a 10 ml volumetric flask. All other reagents were purchased from Merck (Darmstadt, Germany). An oil-in-water blank cream containing about 30% fatty substances (water, isopropyl palmitate, octyl octanoate, sucrose cocoate, glycerin, glyceryl stearate, stearyl alcohol, alcohol) free from minoxidil under investigation was prepared in our laboratory according to Iranian Pharmacopoeia and used to prepare calibration standards and quality control samples. Also according to Iranian Pharmacopoeia, a simple shampoo was prepared for validation of procedure. A Metrohm model VA 797 computrace with automated hanging mercury drop electrode was used for the voltammetric measurements. The reference electrode was Ag|AgCl, saturated with AgCl, 3 M KCl and the auxiliary electrode was a platinum wire. Solutions were stirred during purging and deposition steps by a rotating PTFE rod. Obtained scans were evaluated with 797 PC Software (Version 1.2). pH values of solutions were adjusted employing a Metrohm model 827 using a combined glass electrode. High performance liquid chromatography (HPLC) was performed on a KNAUER liquid chromatograph system employing EZ-Chrome Elite software. The variable wavelength UV-Vis detector was operated at 281 nm for minoxidil determination. A 20 µl injection loop and a reversed phase C18 column (250 mm × 4.0 mm i.d., Eurospher 100-5) were used. The mobile phase for analysis of cream and minoxidil solution was a mixture of MeOH–water (80:20% v/v) at a flow rate of 1.0 ml min^{-1}. The mobile phase for analysis of shampoo was a mixture of acetonitrile–0.01 M NaH_2PO_4 solution (45:55% v/v, pH 4.0). Two different products (one cream and one shampoo) were obtained from two local stores, where they were sold as promising remedies for hyperandrogenism-dependent pathologies. The products informations are as follows:

a) 5% minoxidil cream: no hair loss, stop hair loss within 7 days, and hair growth within 15 days, 100% herbal, secret ingredient, brand name YUDA, supplier Kunming Runyan tang, place of origin Yunnan China.

b) Manexil 5 (5% minoxidil shampoo): to treat male pattern baldness and hair loss (2 tubes of 30 g each), manufactered by Fem Care Pharma Ltd. India.

Calibration Standards and Procedure

Diluted dispersions of blank shampoo and blank cream were prepared as follows: 2.5 ml of shampoo were transferred into a 25 ml volumetric flask and dilluted by buffer with pH 2.0, 0.5 g of cream was transferred into 50 ml volumetric flask and taken to volume with methanol. The cream dispersion was subjected to ultrasonic treatment at 40 °C for 10 min. After filtration, the clear solution was collected as blank solution of cream. Calibration standards containing different amounts of minoxidil were prepared by adding suitable amount of standard stock solution to 1.0 ml blank shampoo and blank cream solutions. Then the spiked samples were transferred into a 10.0 calibrating flask and diluted by buffer with pH 2.0. Then 10.0 ml of solution was introduced into the electrolysis cell, and deoxygenated with pure nitrogen for 2.0 min in the first cycle, the nitrogen was then kept over the solution. Preconcentration of minoxidil onto the HMDE was performed at –0.2 V for 40 s while stir-

ring the solution at 1000 rpm. After equilibrium time of 10 s was allowed for the solution to become quiescent, the stripping voltammogram was recorded by scanning the potential toward the negative direction using cathodic differential pulse voltammetry under the optimized conditions.

Sample Preparation

An amount of 2.5 ml of the shampoo sample was transferred into 250 ml calibrating flask and diluted by buffer with pH 2.0. Then 0.1 ml of the solution (1% v/v of shampoo) was pipetted into 100 ml volumetric flask and diluted to achieved a final concentration of 0.001% v/v of shampoo.

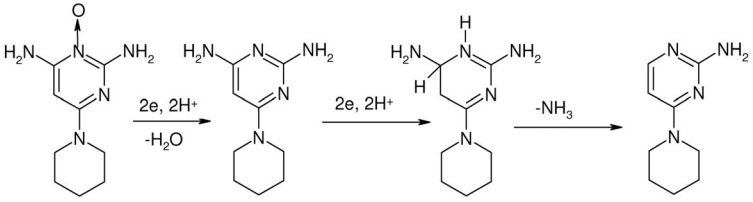
An amount of 0.2 g of cream was transferred into 50 ml volumetric flask and taken to volume with methanol. After treatment of solution as mentioned above, 0.1 ml of solution diluted to 100.0 ml by buffer with pH 2 and 10 ml of solution were analyzed.

An amount of 0.1 ml of Minoxidil solution (2% w/v) was pipetted into 100 ml calibrating flask and made up to the mark with a buffer of with pH 2.0. Then 0.1 ml of this diluted solution was diluted again to 100 ml and followed the proposed procedure.

RESULTS AND DISCUSSIONS

Cyclic Voltammetry Study

For accurate determination of minoxidil in real samples, it was necessary first to proceed with the characterization of the electrochemical processes pertaining to minoxidil by cyclic voltammetry at the surface of HMDE. Amankwa and coworkers in 1983 reported the electrochemical behavior of minoxidil in 0.5 M H_2SO_4 and dimethylformamide–tetraethylammonium bromide solutions[4]. They reported that minoxidil has two differential pulse polarographic waves in –0.95 and –1.2 V and concluded that the first wave is attributed to the reduction of the fully protonated N-oxide while the second probably results from the reduction of the 3,4 carbon–nitrogen double bond. Also, the coulometric analysis of minoxidil in 0.5 M sulphuric acid made them propose an electroreduction process with four electrons per molecule (Scheme 1).



SCHEME 1
DPP reduction mechanism of minoxidile proposed by Amankwa and coworkers[4] in 1983

Minoxidil is slightly soluble in water, but highly soluble under low pH conditions, therefore in this scetion, the buffer with pH 3.0 was used for studying of electrochemical behavior of minoxidil. Figure 2 shows the cyclic voltammograms of 1.0×10^{-6} M minoxidil at different scan rates. In contrast with Amankwa's report[4], in forward sweep potentials (–0.6 to –1.15 V), only one irreversible cathodic peak in potential of –0.96 V in scan rate of 50 mV s^{-1} was obtained and in backward sweep potentials (–1.15 to –0.6) no peak was observed. The reduction peak of minoxidil is probably due to the reduction of oxygen group by two electrons process. As it is observed under selected experimental conditions, by increasing of scan rate, the cathodic peak current (i_{pc}) of minoxidil considerably increased, also the peak potential (E_{pc}) of minoxidil was shifted to negative potentials with increasing scan rate and demonstrated an irreversible electrode process for minoxidil[20]. The linear equations of i_p versus $v^{1/2}$ and v, and E_p versus v and ln v for minoxidil is shown in Table I. As it is observed, the cathodic peak current of minoxidil varies linearly with square root of the scan rate, $v^{1/2}$, rather than with v. The result indicates that the mass transport of minoxidil to HMDE surface is a diffusion controlled process. To measure the symmetry of the energy barrier and all electrons, Eq. (*1*) was used,

$$|E_p - E_{p/2}| = \frac{1.857RT}{\alpha nF} \qquad (1)$$

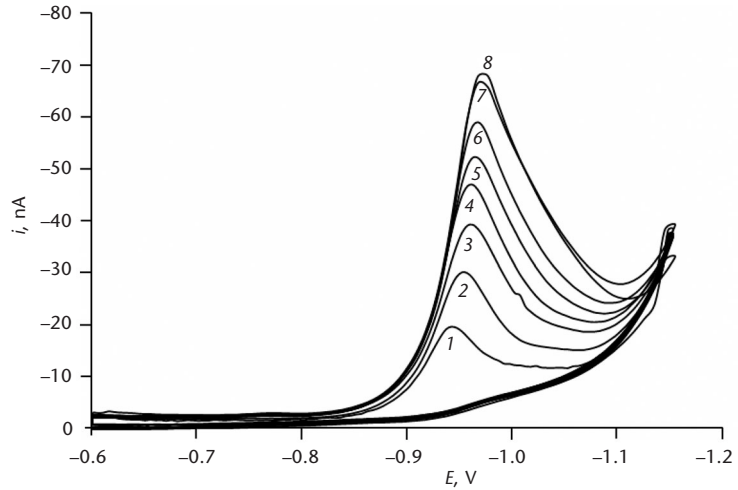
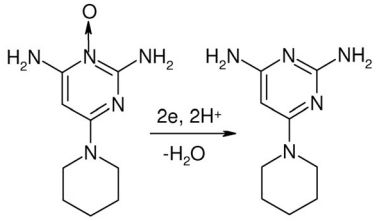


FIG. 2
Cyclic voltammograms of 1.0×10^{-6} M minoxidil with pH 3 at different scan rates (mV s^{-1}): 10 (*1*), 20 (*2*), 30 (*3*), 40 (*4*), 50 (*5*), 60 (*6*), 70 (*7*), 80 (*8*)

where $E_{p/2}$ (mV) is the potential and $i = i_{p/2}$ in cyclic voltammograms of minoxidil. In cyclic voltammograms, the average value of $|E_p - E_{p/2}|$ was 38.8 mV for minoxidil. Generally, α in the totally irreversible electrode process is assumed as 0.5. Hence, 2 electrons are involved in the reduction process of minoxidil. Also the value of α was 0.614 for minoxidil. From the data we proposed the below reduction mechanism for reduction of minoxidil at HMDE surface (Scheme 2).



SCHEME 2
Proposed reduction mechanism of minoxidil with pH 3 at HMDE

Also the Laviron Eq. (*2*) was used for calculation of K_s and E^0,

$$E_p = E^0 + \left(\frac{RT}{\alpha n_\alpha F}\right)\left[\ln\left(\frac{RTK_s}{\alpha nF}\right) - \ln \nu\right] \qquad (2)$$

where E^0 (V) is the formal potential, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T (K) is the temperature, α is the symmetry of the energy barrier, K_s (s^{-1}) is the electrochemical rate constant and F is the Faraday constant (96 500 C mol^{-1}). According to Eq. (*2*), the plot of E_p versus ln v is linear with a slope that allows αn_α to be determined, and an intercept from

TABLE I
Linear equations of voltammetric measurments of minoxidil

Parameter	Linear equation	R^2
i_p (nA) vs ν (mV s^{-1})	$i_p = -0.694\nu - 16.06$	0.983
i_p (nA) vs $\nu^{1/2}$	$i_p = -8.621\nu^{1/2} + 8.259$	0.997
E_p (mV) vs ν (mV s^{-1})	$E_p = -0.360\nu - 948$	0.955
E_p (mV) vs ln ν	$E_p = -15.83 \ln \nu - 905$	0.994

which K_s can be calculated if the value of E^0 is known[12]. The value of E^0 in Eq. (*2*) can be obtained from the intercept of the E_p versus v curve by extrapolation to the vertical axis at $v = 0$. The E^0 and K_s were obtained as –948 mV and 5.52 s^{-1}, respectively, for minoxidil.

Optimization of Parameters

Effect of pH. The shape and characteristics of adsorptive cathodic stripping voltammogram of minoxidil were strongly dependent on pH of electrolyte (Fig. 3a). For controlling of pH, different buffers such as Britton–Robinson (BR), acetate and phosphate buffers were used. The best results with respect to sensitivity accompanied with sharper response were obtained with 0.1 M BR buffer (pH 2). This study was made in the pH range of 1.5–7.2 at a target concentration of 1.0×10^{-6} mol l^{-1} of minoxidil. The dependences of i_p and E_p to pH are shown in Figs 3b and 3c. According to the results, pH 2.0 was chosen as the best one. At pH higher than 2.0, the sensitivity is lower. This is due to a slight dissociation of oxygen from minoxidil. Also the change of E_p versus pH gave a linear equation of $E_p(V) = -0.065pH - 0.797$ ($R = 0.992$) and confirmed the proposed mechanism of minoxidil reduction with two electrons (Scheme 2)[17].

Effect of accumulation potential. The effect of electrolysis potential on the peak current of minoxidil was examined over the range of 0.0 to –1.2 V (Fig. 4). The results revealed that in the potential range of 0.0 to –0.2 V the peak current of minoxidil increased and after that it decreased. An accumulation potential of –0.2 V was used for the optimized analytical procedure.

Effect of accumulation time. The dependence of the cathodic peak current on the accumulation time was also studied (Fig. 5). The peak current was found to increase with increasing accumulation time, indicating an enhancement of minoxidil uptake at the electrode surface. Normally, the increase in the response current continued until the maximum signal level (presumably corresponding to either saturation or an equilibrium surface coverage) was attained. The obtained results indicated that the attainment of a steady-state accumulation level of minoxidil at the electrode surface requires an exposure time of 40 s for 1.0×10^{-6} M minoxidil. An accumulation time of 40 s was used for further studies.

Validation of the analytical procedure. Under the selected conditions the reduction peak current of the minoxidil yields well defined concentration dependence. The voltammograms for solutions with increasing minoxidil concentration and after 40 s are sharp reproducible. The calibration equations in cream shampoo and aqueous solutions were obtained by the least

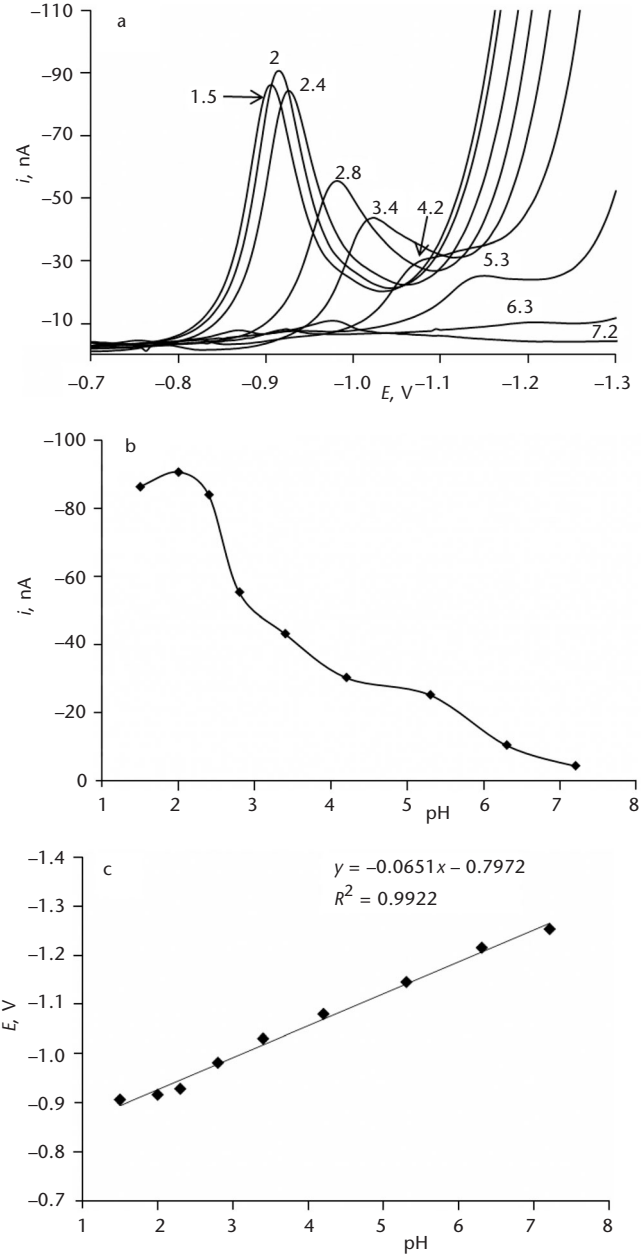


FIG. 3
Effect of electrolyte pH on shape of AdsCSV voltammograms of 1.0×10^{-6} M minoxidil (a), i_p (b) and E_p (c); accumulation potential 0.0 V, accumulation time 10 s

square method and placed in Table II. As the minoxidil concentration was extended above 2.5 × 10^{-6} mol l^{-1} in all calibration samples, a more or less pronounced deviation from the linearity was appeared, which may be attributed to the complete coverage of the mercury electrode surface with the adsorbed minoxidil species. The realiability of the proposed procedure for the determination of minoxidil in shampoo, cream and topical solution was checked, using different spiked shampoo, cream and buffer samples. The free synthesized cream and shampoo in our laboratory were used for

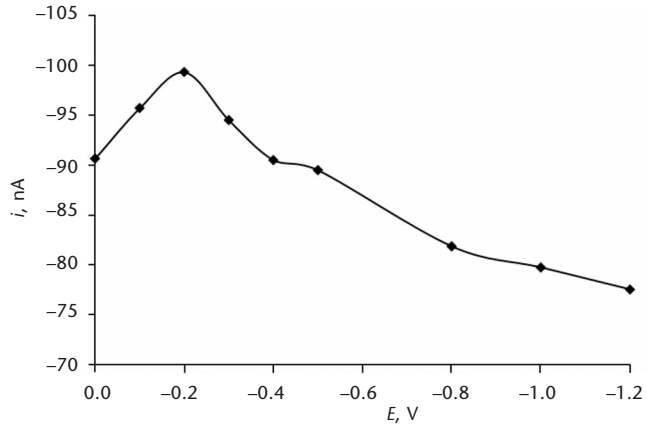


FIG. 4
Effect of accumulation potential on peak current of 1.0 × 10^{-6} M minoxidil with pH 2 and accumulatio time 10 s

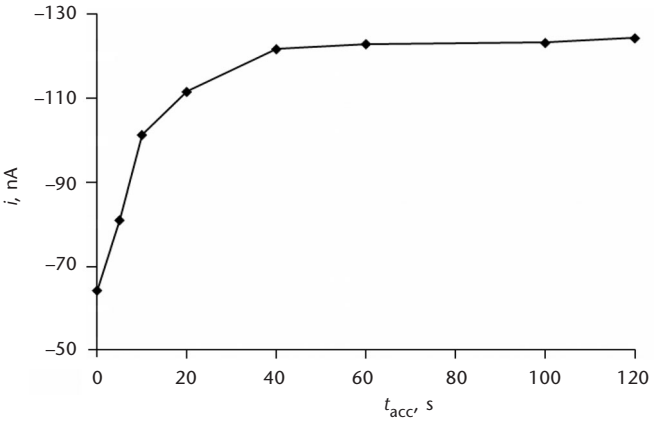


FIG. 5
Effect of accumulation time on peak current of 1.0 × 10^{-6} M minoxidil with pH 2 and accumulation potential –0.2 V

evaluation of the blank signal. The results revealed that no peak current was observed at reduction potential of minoxidil in 0.1% v/v of shampoo and cream blank. The mean recoveries of minoxidil based on the average of five replicate measurements for 1.0×10^{-6} M minoxidil with 40 s accumulation time from shampoo, cream and aqueous buffer were measured and results are shown in Table II. Validation of the optimized procedure for the quantitative assay of minoxidil was examined via evaluation of the limit of detection (LOD) and recovery. The LOD was calculated from the calibration graphs, using the equation LOD = 3SD/*b*. Where SD is the standard deviation of the blank (intercept) and *b* is the slope of the calibration graph. The obtained results are reported in Table II. Repeatability was examined by per-

TABLE II
Characteristics of the calibration graphs of minoxidil in cream, shampoo and water with pH 2.0 under the optimized conditions

Sample	i_p (nA) = bC (nmol l^{-1}) + a	LR (mol l^{-1})	LOD(mol l^{-1})	Recovery	RSD, %	R^2
Shampoo	$i_p = -0.825C - 16.43$	3.0×10^{-8}–2.5×10^{-6}	1.0×10^{-8}	96.7	1.89	0.996
Cream	$i_p = -0.849C - 17.18$	1.0×10^{-8}–2.5×10^{-6}	8.0×10^{-9}	97.8	1.24	0.997
Aqueous buffer	$i_p = -0.875C - 23.16$	5.0×10^{-9}–2.5×10^{-6}	2.0×10^{-9}	99.8	0.86	0.998

TABLE III
Effect of foreign species on the determination of 104.5 ng ml^{-1} of minoxidil under optimum conditions

Foreign species	[Foreign ion]/[minoxidil]	Effect on signal
NO_3^-, Cl^-, Na^+, K^+	800	negative
Ca^{2+}, Mg^{2+}	700	negative
Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}	600	negative
SDS	600	positive
TritonX100	500	negative
CTAB	400	negative
Isopropyl palmitate, octyl octanoate	900	positive
Sucrose cocoate	800	positive

forming of five replicate measurements for 1.0×10^{-6} M minoxidil with preconcentration for 40 s in each solution (Table II). To evaluate the potential effect of foreign ionic and organic species commonly found in real samples on the determination of minoxidil at 5.0×10^{-7} mol l^{-1} (104.5 ng ml^{-1}) level, a systematic study was carried out. A 104.5 μg ml^{-1} level of potentially interfering species was tested first and if interference occurred, the interfering amount was reduced progressively until its effect was less than 5% of tolerance. The tolerance was defined as the amount of foreign species that produced an error not exceeding than 5% in the determination of the analyte (Table III). As can be seen from the data given in Table III, the method offers a practical potential for trace determination of minoxidil with high selectivity, sensitivity, simplicity and speed. The low effect of foreign ions or organic compounds is due to the high level of protons (pH 2.0) in the solution and its competition with cations and organic compounds for interaction with minoxidil.

TABLE IV
Determination of minoxidil in different real samples

Sample	Found by AdsCSV	Found by HPLC
China cream	4.1 ± 0.02[a]% w/w	4.15 ± 0.03% w/w
Indian shampoo	3.5 ± 0.035% w/v	3.45 ± 0.05% w/v
Iranian topical solution	1.95 ± 0.01% v/v	2.01 ± 0.02% v/v

[a] The average of four replications.

TABLE V
Comparsion of proposed procedure with other methods

Method	Linear range, mol l^{-1}	LOD, mol l^{-1}	Ref.
Ion-pair HPLC	9.6×10^{-9}–4.8×10^{-7}	2.4×10^{-9}	3
Differential pulse polarography	1.0×10^{-5}–5.0×10^{-5}	[a]	4
Capillary zone electrophoresis	4.8×10^{-8}–8.9×10^{-7}	[a]	6
HPLC-ESI-MS	2.4×10^{-7}–4.8×10^{-6}	6.7×10^{-8}	8
HPLC-EC	4.8×10^{-9}–1.9×10^{-6}	4.8×10^{-9}	20
AdsCSV	5.0×10^{-9}–2.5×10^{-6}	2.0×10^{-9}	this work

[a] Not reported.

Assay of minoxidil in shampoo, cream and topical solution samples. The optimized procedure was successfully used for determination of minoxidil in cream, shampoo and topical solution of minoxidil. Solutions were prepared as mentioned in section of sample preparation and the results are shown in Table IV. The same results were achieved by HPLC analysis of the real samples (Table IV).

CONCLUSION

Contradictory to the previously published work by Amankwa and coworkers[4] in 1983, minoxidil was found to be reduced at the mercury electrode via a single 2-electron irreversible wave. The drug could be determined directly in pharmaceutical formulations, illegal shampoo and cream with a good success using the adsorptive cathodic stripping voltammetry. The optimized procedure for the direct determination of minoxidil offers a fast response, sensitivity, low cost and easy analysis that have not been presented together in previously reported works (Table V).

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