# SQUARE-WAVE CATHODIC ADSORPTIVE STRIPPING VOLTAMMETRY OF RISPERIDONE

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Electrochemical properties and diffusion-adsorption behavior of risperidone (RPN), an antiphyscotic drug, on hanging mercury drop electrode (HMDE) were carried out in Britton–Robinson (BR) buffer. Some electrochemical parameters such as diffusion coefficient, number of transferred electrons and proton participated to its reduction mechanism and surface coverage coefficient were calculated from the results of cyclic voltammetry, square-wave voltammetry and constant potential electrolysis. RPN was found to be reduced with single two-electron/two-proton quasi-reversible mechanism controlled mainly by adsorption with some diffusion contribution at the potential about –1.58 V (vs Ag|AgCl electrode). Experimental parameters were optimized to develop a new, accurate, rapid, selective and simple square-wave cathodic adsorptive stripping voltammetric (SWCAdSV) method for direct determination of RPN in pharmaceutical dosage forms, spiked human urine and human serum samples without time-consuming steps prior to drug assay. This method was based on the relation between the peak current and the concentration of RPN and it was recognized that peak current of reduction wave linearly changes with the concentration of RPN in the concentration range of 1.5–150 nM, when optimum preconcentration potential –0.65 V and optimum preconcentration time 60 s were applied. In this method, limit of detection (LOD) was found as 5.18 nM (2.12 ppb). The method was successfully applied to determine the RPN content of commercial pharmaceutical preparations, spiked human serum and spiked human urine. The method was found to be highly accurate and precise, having a relative standard deviation of less than 4.80% for all applications.

**Keywords**: Cyclic voltammetry; Electrochemistry; Risperidone; Drug assay; Square-wave adsorptive stripping voltammetry; Electrochemical behavior.

Risperidone (RPN) belongs to a class of antipsychotic drugs known as atypical neuroleptics. It was approved in 1993 for the treatment of schizophrenia. In 2007, risperdal (RPL) was approved as the only drug agent available


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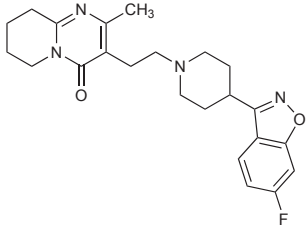
for treatment of schizophrenia in early ages. RPN contains the functional groups of benzisoxazole and piperidine as part of its molecular structure (Scheme 1). It is chemically known as 3-{2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl}-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]-pyrimidin-4-one. Recent evidence from open trials suggests that RPN may be also beneficial in the treatment of schizoaffective and bipolar disorders[1–3].

RPN can be assayed by various methods. In the literature, high-performance liquid chromatography[4–6], liquid chromatography-mass spectroscopy[7–9], liquid chromatography[10], high-performance liquid chromatography and thin layer densitometry[11] methods have been reported for the determination of risperidone in pharmaceuticals or biological fluids. Isolation of degradation products of risperidone[12] and structural studies of impurities of risperidone by hyphenating techniques[13] have also been reported in the literature. Infrared absorption, spectrophotometric and liquid chromatographic methods were described in pharmacopoeias[14] to identify RPN. All these reported methods are either not sufficiently sensitive or tedious and require highly sophisticated instrumentation. Although RPN is an electroactive molecule on mercury surface, in the literature, there are only few studies dealing with electrochemical behavior and electrochemical determination of the RPN [15,16]. Furthermore, reviewing the literature revealed that, up to the present time, there is no square-wave cathodic adsorptive stripping voltammetric (SWCAdSV) method using hanging mercury drop electrode (HMDE) for the assay of RPN in pharmaceutical formulation and bulk form.

The voltammetric techniques, such as cyclic voltammetry, differential pulse voltammetry and square-wave voltammetry have been proved to be very sensitive for the determination of organic molecules including drugs and related molecules in pharmaceutical dosage forms and biological fluids. These methods are faster, easier and cheaper than spectroscopic and chro-




SCHEME 1
Structural formula of RPN

matographic methods. The sensitivity increases when the stripping voltammetry is employed. Adsorptive stripping voltammetry has been shown to be an efficient electroanalytical technique for determination of sub-nanomolar level of a wide range of drugs which have interfacial adsorptive character onto the working electrode surface. Its remarkable sensitivity is attributed to the combination of an effective accumulation step with an advanced measurement procedures that generates an extremely favorable signal to back ground ratio. It usually involves a simple deposition step and most of the excipients used, not interfere in the subsequent determination of the drugs and there are many applications of stripping voltammetric methods[17–24].

The purpose of this study is to establish the experimental conditions for the determination of RPN, to investigate the voltammetric behavior of RPN, and to propose a possible reduction mechanism of RPN on HMDE using cyclic voltammetry and controlled-potential electrolysis techniques. This study also aimed to develop a new, rapid, selective and sensitive electrochemical method for the direct determination of RPN in raw materials, pharmaceutical dosage form, and biological samples which include human serum and human urine without time-consuming extraction, separation and evaporation steps prior to drug assay.

## EXPERIMENTAL

### Apparatus

All voltammetric measurements such as cyclic voltammetry (CV), controlled potential coulometry (CC) and square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) were carried out by using a CH-instrument electrochemical analyzer (CHI 760). A three electrode cell system incorporating the HMDE (BAS, Controlled Growth Mercury Electrode, CGME, USA) as a working electrode, platinum wire auxiliary electrode (BAS MW-1034) and an Ag|AgCl reference electrode (MF-2052 RE-5B) were used in all experiments.

A three electrode combination system for bulk electrolysis was consisted of mercury pool (55.4 $cm^2$) as a working electrode, coiled platinum wire auxiliary electrode (23 cm) (BAS MW-1033) and Ag|AgCl reference electrode (BAS MF-2052 RE-5B).

All pH measurements were made with Thermo Orion Model 720A pH ion meter having an Orion combined glass pH electrode (912600) which had been calibrated with pH 4.13 and 8.20 stock buffer solutions before measurements. All the data were obtained at ambient temperature (25 ± 3 °C).

The deionized water was supplied from Human Power $I^+$, Ultra Pure Water System.

### Materials

Standard sample of RPN (99.0%, from Janssen–Cilag) was used to plot the calibration curve. Stock solution of RPN ($4.0 \times 10^{-3}$ mol $l^{-1}$) was prepared in 25.0 ml of ethanol (from Merck).

Calibration solutions were prepared by appropriate dilution of the stock solution over the range of desired concentrations with BR buffer.

All chemicals used both in preparation of BR buffer solution, such as phosphoric acid (Riedel), boric acid (Riedel), acetic acid (Merck), sodium hydroxide (Merck) and in the preparation of $NH_3/NH_4Cl$ (both Merck) buffer solution were analytical reagent grade and these chemicals were used without further purification. Double-distilled deionized water was used in preparations of all the solutions.

Risperdal (Eczacibaşi Co.) tablets were used as pharmaceutical dosage form which contains 1.0 mg of RPN and some amount of lactose monohydrate as excipients per tablet. To prepare the solutions of tablets, initially the drug content of ten tablets was weighed, finely powered and mixed. The average mass per tablet was determined. A sample equivalent to one tablet was weighed and transferred in to a 100.0 ml calibrated flask and completed to the mark with ethanol. The contents of the flasks were sonicated for 30 min to achieve complete dissolution. After the solution step, the content of the flask was centrifuged 30 min at 1500 rpm. The sample from the clear supernatant liquor was withdrawn and quantitatively diluted with BR buffer. This solution was used for determination of RPN in tablets using direct calibration methods.

Voltammetric Procedure

In cyclic voltammetry, 10 ml of RPN solution in BR buffer were placed into the electrochemical cell for each time. The solution was deoxygenated with purified argon (99.99% purity) for 2 min before running. After deareation, a hanging mercury drop was formed and the voltammograms were recorded applying a negative-going scan from –1.00 to –1.75 V.

In controlled-potential electrolysis, 50.0 ml of 120 μM RPN solution in BR buffer were placed into the cell with the mercury pool electrode (55.4 $cm^2$). The solution was deoxygenated for 25 min before running electrolysis. The applied potential was hold constant at –1.85 V and the electrolysis was performed for 5 h with stirring continuously.

## RESULTS AND DISCUSSION

### *Electrochemical Behavior of RPN*

Electrochemical behavior, diffusion and adsorption properties of RPN were studied using the results of cyclic voltammetry (CV), square-wave voltammetry (SWV) and controlled-potential electrolysis (CPE). In CV studies, a single reduction peak was observed at a potential of about –1.58 V at pH 10.3 (Fig. 1). There is no peak when blank BR was scanned at the same conditions, and peak current increases linearly with increasing concentration of RPN (Fig. 1 inset), concluded that this cathodic reduction peak is related with the reduction of RPN molecules on HMDE. As can be seen from Fig. 2, there is also an anodic peak at reverse scan indicating that electrode reaction could be reversible.

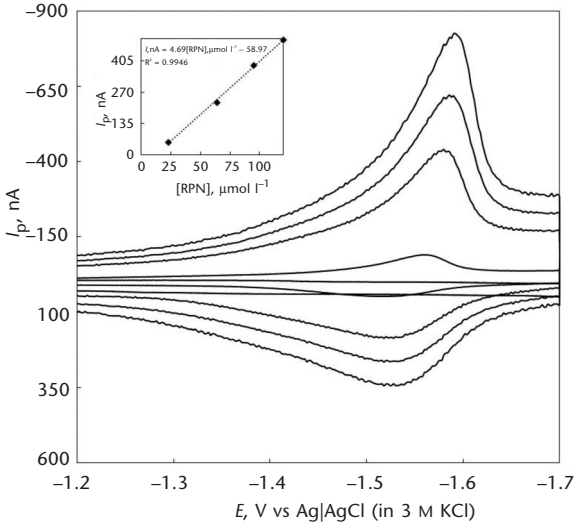



FIG. 1
Cyclic voltammograms of RPN in BR solution at pH 10.3 with scan rate of 0.100 V s$^{-1}$. From inner to outer: blank BR, 23, 64, 95 and 120 μmol l$^{-1}$. Inset: $I_p$ (in nA) vs [RPN] (in mol l$^{-1}$)

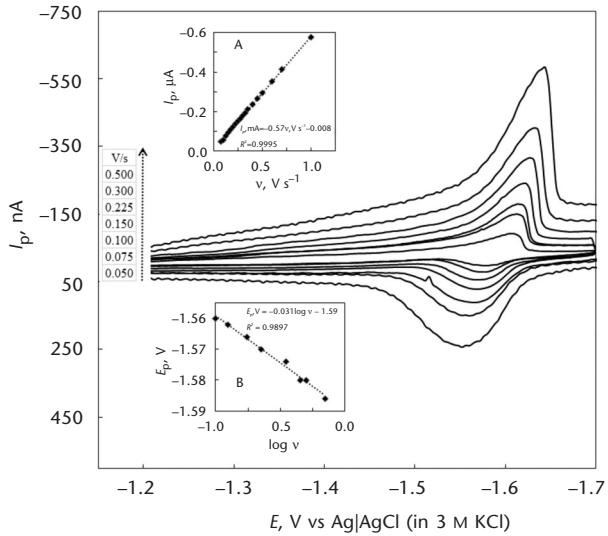



FIG. 2
Cyclic voltammograms of 120 μM RPN in BR solution at pH 10.3 at different scan rates. From inner to outer: 0.050, 0.075, 0.100, 0.150, 0.225, 0.300 and 0.500 V s$^{-1}$. Inset A: $I_p$ (in nA) vs ν (in V s$^{-1}$); Inset B: $E_p$ (in V) vs log ν (ν in V s$^{-1}$)

The influences of the potential scan rate on cathodic peak current ($i_{p,c}$) were investigated for $1.2 \times 10^{-4}$ M RPN in the 0.005–1.000 V $s^{-1}$ range. In this range of scan rate, a linear dependence of cathodic peak current, $i_{p,c}$ (in mA), upon the scan rate, ν (in V $s^{-1}$), was found as given equation $i_{p,c} = -0.574\nu - 0.008$ with $R^2 = 0.9995$ (Fig. 2 inset A). Linearity of this plot shows us that electrode reaction should be adsorption-controlled. Also a plot of logarithm of peak current (in A) versus logarithm of scan rate (in V $s^{-1}$) was constructed. This relation was found to be linear with a slope of 0.961. This value of slope is very close to the theoretical value of 1.0 for adsorbed species[17]. The plot of peak current versus square root of scan rate was also constructed and this graph is linear with an equation $i_p$ (μA) = $-0.596\nu^{1/2} + 0.1386$ ($R^2 = 0.9927$). Linearity of this relation indicates the contribution of diffusion on electrochemical reaction.

Some extra studies were carried out to investigate the adsorption phenomena according to literature[25,26]. As a result, the value of the ratio of cathodic peak current to concentration ($i_{p,c}/C$) decreases with increasing concentration, value of the ratio of cathodic peak current to multiplication of concentration and scan rate ($i_{p,c}/C\nu$) decreases at the beginning and then nearly constant with increasing scan rate, and value of the ratio of cathodic peak current to multiplication of concentration and square root of scan rate ($i_{p,c}/C\nu^{1/2}$) increases with increasing scan rate.

As can be seen from Figs 1 and 2, there is also an anodic peak at reverse scan. Anodic peak is wider than cathodic peak but both have approximately the same area. This behavior may show the strong adsorption of product and weak adsorption of reactant. According to experimental results, following explanations may be proposed: RPN molecules are adsorbed and reduced at electrode surface and then reduced form of RPN diffuses to solution. When the concentration difference between the surface and solution is high enough (i.e. concentration of RPN is relatively small), diffusion rate is high and current of anodic peak decreases. But when the concentration of RPN is high enough to minimize the diffusion effect on releasing the reduced form of RPN from the surface, current of anodic peak is found to be very close to that of cathodic one. Dependence of the existence of anodic peak to the concentration of RPN may be explained in this way.

In the present study, effect of potential scan rate on cathodic peak potential ($E_{p,c}$) was also investigated. The peak potential shifts to more cathodic values with increasing scan rate (Fig. 2). Relation between peak potential (in V) and logarithm of scan rate (V $s^{-1}$) was found to be expressed by the equation $E_p = -0.030 \log \nu - 1.59$ with $R^2 = 0.9897$ (Fig. 2 inset B). Potential shifting with scan rate supports the irreversibility of electrochemical reac-

tion under investigation. According to literature[27], slope of the curve of peak potential versus logaritm of scan rate has the value of 0.0296 V per unit ($n\alpha_c$), and the difference between the peak potential and half peak potential is 0.0477 V per unit ($n\alpha_c$) (here $\alpha_c$ is cathodic charge transfer coefficient, $n$ is the number of electrons). As can be seen from the Fig. 2 inset B, the curve of peak potential versus logaritm of scan rate has a slope value of –0.031. Using these experimental results, value of $n\alpha$ was calculated to be 0.955. This value was calculated to be 0.915 from the difference of peak potential and half peak potential. These findings were also supported by the results of frequency studies in SWV. In such studies, cathodic peak potential shifts to more cathodic values as frequency increases and peak current linearly increases with increasing frequency.

In electrochemical studies, pH is one of the variables that commonly and strongly influence the electrochemical behaviors of molecules under investigation. Therefore, electrochemical behavior of RPN was studied as a function of pH. At pH values lower than 7.0, there was no electrochemical signal. As can be seen from the SWCAdSV measured from the solutions having different pH (Fig. 3), the potential of the cathodic peak shifts linearly to more negative values with increasing pH as can be expressed by the equation: $E_{p,c}$ (V) = –0.052 pH – 0.975 with $R^2$ = 0.9936 (Fig. 3 in inset A).

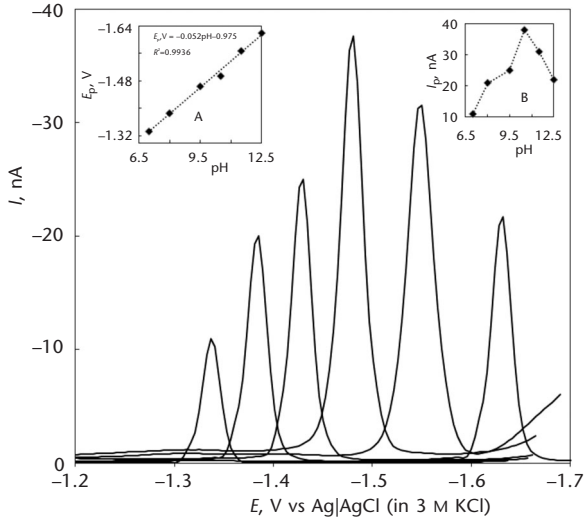



FIG. 3

Influence of pH on peak potential and peak current in SWCAdSV for 15 nM RPN. pH values from left to right: 6.8, 8.2, 9.5, 10.3, 11.4 and 12.5; $E_{acc}$ = –0.65 V; $t_{acc}$ = 60 s. Insets A: $E_p$ (in V) vs pH; Inset B: $I_p$ (in nA) vs pH

The experimental value of the slope of this curve was found to be 52 mV per unit pH in pH range between 7.0 and 12.4. The slope is very close to theoretical value of 60 mV per unit pH required for assuming 2 e/2 $H^+$ or 4 e/4 $H^+$ process[22,28] of the electroreduction of RPN. Based on literature, Eq. (*1*) was used in SWV to find the ratio of number of protons to number of electrons ($\delta/n$) in electrode mechanism[29].

$$E_p = E^0 + RT/nF \ln ([Ox]/[Red]) - \delta RT/nF \ln [H^+] \quad (1)$$

In this equation, $E_p$ (in V) is peak potential (vs Ag|AgCl), $E^0$ (in V) is standard peak potential, $R$ is ideal gas constant taken as 8.31 J $mol^{-1}$ $K^{-1}$, $T$ is absolute temperature taken as 298.15 ± 3 K, $F$ is Faraday constant taken as 96485 C $mol^{-1}$, [Ox] is the molar concentration of oxidized species, [Red] is the molar concentration of reduced species, δ is number of proton participated in reaction mechanism, $n$ is number of transferred electrons in electrochemical step and $[H^+]$ is the molar concentration of hydrogen ions. The ratio of number of protons to number of electrons was found to be 0.87 from the slope value of the plot of $E_p$ vs pH value. As a result, the same number of electrons and protons participates in electroreduction of RPN molecules.

As a result of constant potential bulk electrolysis carried out at –1.85 V with 120 μM RPN, there is no significant change in the peak current and peak potential before and after electrolysis. Catalytic adsorptive reduction mechanism may be proposed when these experimental results were concluded. As mentioned above, it is impossible to find out the number of electrons transferred in electrochemical step from the data of bulk electrolysis. To find out the number of electrons, following relations proposed for adsorption process[17] were used in CV studies

$$i_p = n^2F^2\Gamma A\nu/4RT \quad (2)$$

and the relation

$$Q = nFA\Gamma \, . \quad (3)$$

In these equations, Γ (in mol $cm^{-2}$) is the surface coverage of adsorbed substance, and the others are commonly known quantities (in this study $A$ = 0.0145 $cm^2$)[17]. By substitution the Γ term of Eq. (*3*) to Eq. (*2*), it is easy to get a new relation for $n$

$$n = 4i_pRT/FQ\nu \, . \quad (4)$$

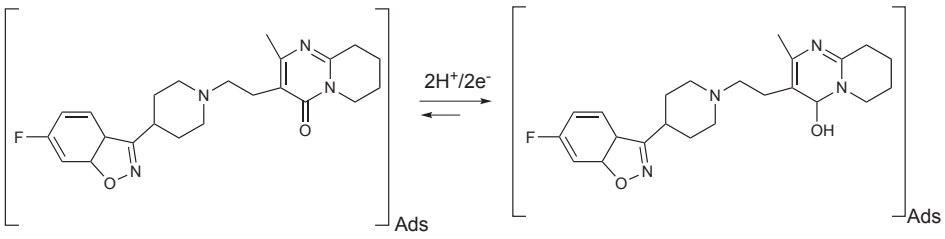
In the potential scan rate range from 0.010 to 0.500 V $s^{-1}$, number of electrons ($n$) transferred in electrode reaction was calculated using directly Eq. (*4*) for each scan rate value, and mean value for the number of electrons was found to be nearly 2. The surface coverage (Γ) of adsorbed substance was found using the slope of the curve of the peak current (in A) versus potential scan rate (in V $s^{-1}$) according to Eq. (*2*). According to potential scan rate studies, slope of the curve of peak current (in A) versus potential scan rate (in V $s^{-1}$) has the value of $1.06 \times 10^{-6}$. Using this value of slope and value of 2 for number of electrons transferred in electrochemical step, and the value of other common constants, value of Γ was calculated as $1.94 \times 10^{-11}$ mol $cm^{-2}$ between the potential scan rates from 0.010 to 0.500 V $s^{-1}$. At scan rates higher than 0.500 V $s^{-1}$, shapes of cyclic voltammograms are distorted. Thus it was not aimed to calculate the electrochemical parameters of RPN at scan rates higher than 0.500 V $s^{-1}$.

The peak current for an adsorption-desorption couple (at 298.15 K) is given by the equation[30]

$$i_p = (1.09 \times 10^6) n^2 \, ACD^{1/2} \nu t^{1/2}. \qquad (5)$$

Diffusion coefficient of RPN was calculated using the slope of the plot of peak current versus potential scan rate ($i_p$ vs ν). Diffusion coefficient was found as $1.84 \times 10^{-8}$ $cm^2$ $s^{-1}$.

According to these investigations, a quasi-reversible, 2 e/2 $H^+$ charge transfer reaction that includes the adsorption of product and reactant with different strength to electrode surface may be proposed.




Since the structure of RPN molecule contains carbonyl group, activated by the neighboring nitrogen and based on the observed transfer of 2 electrons with 2 protons, it can be postulated that the electrode reaction is due to the reduction of carbonyl group activated by the neighboring nitrogen. This postulate is supported by literature[31].

*Square-Wave Analytical Study of RPN*

*Type and pH of supporting electrolyte*. In the present study, SWCAdSV of 15 nM RPN in BR (pH 2–12) and $NH_3/NH_4Cl$ (pH 7.5–10.5) buffers was recorded following its preconcentration onto the HMDE by adsorptive accumulation at –0.65 V for 60 s. Sharp peak and much enhanced peak current were achieved when the BR buffer as a supporting electrolyte was used, and optimum concentration of each component included in BR (acetic, phosphoric and boric acids) was found as 0.04 mol $l^{-1}$. The peak current increased with the increase of pH of the supporting electrolyte till it reached the maximum value over the pH range 6.8–10.3 and peak current decreased with increasing pH at pH values higher than 10.3 (Fig. 3). Therefore, the BR buffer of pH 10.3 was chosen as a supporting electrolyte in the rest of the present study.

*Instrumental parameters*. The square-wave (SW) response markedly depends on the parameters of the excitement signal. In order to obtain a well-defined square-wave voltammetric peak shape, the optimization studies were carried out for instrumental parameters such as frequency (*f*), scan increment ($\Delta E_i$) and pulse amplitude ($\Delta E_a$) for 15 nM RPN in a BR buffer of pH 10.3 at a HMDE. Peak current increases with increasing $\Delta E_i$ from 1 to 4 mV and begins to decrease at higher values. At values of $\Delta E_i$ higher than 4 mV, shape and symmetry of voltammograms were distorted. As a result, optimum $\Delta E_i$ was chosen as 4 mV. In optimization studies for $\Delta E_a$, it was recognized that there is no correlation between peak current and pulse amplitude, but peak boarding was observed at $\Delta E_a$ values higer than 15 mV. In frequency studies, it was observed that peak potential shifts to more cathodic values with increasing frequency and peak current increases linearly with increasing frequency. At higher frequency peak shape and peak symmetry were distorted because of high scan rate. The optimum frequency value was selected to be 25 Hz.

*Accumulation parameters*. The effect of varying accumulation potential range between 0.0 and –1.20 V on the peak current intensity of the SWCAdSVs recorded for 15 and 105 nM RPN in a BR buffer of pH 10.3 under the optimal instrumental parameters following its preconcentration onto the HMDE for 60 s was also evaluated (Fig. 4a). A much enhanced peak current and symmetry were achieved at –0.65 V, hence, it was used throughout the present study. On the other hand, SWCAdSV measurements for 15 and 105 nM RPN solutions were recorded under the optimal instrumental parameters at various preconcentration times from 5 to 180 s using –0.65 V as deposition potential. The magnitude of the peak current

depended linearly both on the analytic concentration of RPN and accumulation time. Apparently, lower concentration of the RPN requires longer preconcentration time to maintain adsorption-desorption equilibrium. This meant that the choice of preconcentration time was dictated by the sensitivity required. In the present analytical procedure, the most suitable preconcentration time was selected as 60 s (Fig. 4b).

*Method Validation*

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. The elements required for method validation are linearity range, limits of detection and quantitation, accuracy, reproducibility, stability, selectivity and robustness[32].

*Linearity*: To establish the linearity range (working concentration range) of RPN in stripping studies, eight RPN solutions in concentration range from 0.60 to 250 nmol $l^{-1}$ were used at the optimum experimental and instrumental conditions. Result of concentration studies showed that an average peak current of reduction wave changed linearly with RPN concentration in the range from 1.5 to 150 nmol $l^{-1}$ by obeying the calibration equation $I_p$ (nA) = 0.51[RPN] (nmol $l^{-1}$) + 10.65 with $R^2$ = 0.9992. Value of $R^2$ is the evidence of good linearity between peak current and concentration of RPN (Table I).

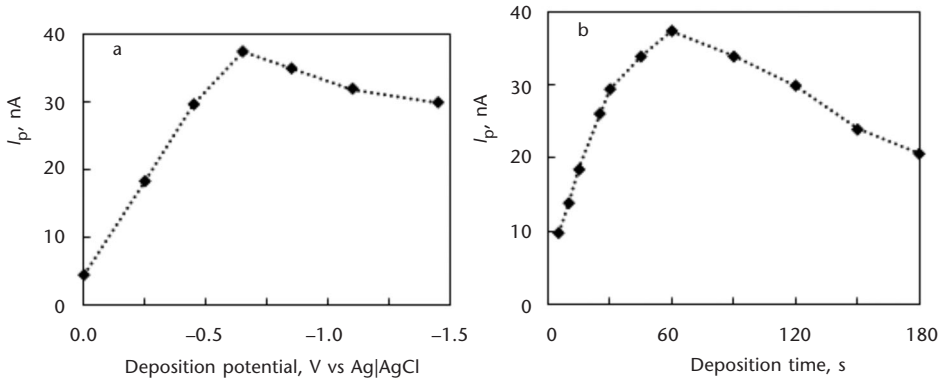



FIG. 4
Effect of deposition potential on stripping peak current for 15 nM RPN at pH 10.3 with deposition time of 60 s (a). Effect of deposition time on stripping peak current of 15 nM RPN at pH 10.3 with deposition potential of –0.65 V (b)

*Limits of detection and quantitation*: Limit of detection (LOD) and limit of quantitation (LOQ) values for RPN were calculated using the relations LOD = 3*s*/*m* and LOQ = 10*s*/*m* [23,24]. The abbreviation of *s* is the standard deviation of intercept of calibration curve and *m* is the slope of the related calibration curve, LOD and LOQ values were found 5.18 nmol $l^{-1}$ (2.12 μg $l^{-1}$) and 17.25 nmol $l^{-1}$ (7.07 μg $l^{-1}$), respectively. Both LOD and LOQ values confirmed the sensitivity of the proposed methods.

*Accuracy*: The accuracy of measurements by means of the described SWCAdSV procedure was checked by calculating the recovery of a known concentration of RPN following deposition onto the HMDE by adsorptive measurement at optimum instrumental and experimental conditions. Recovery values range between 99.6 and 101.22% for tablet analysis (Table II), found between 99.40 and 101.60% for urine analysis, and between 97.60 and 102.00% for serum analysis (Table III).

*Reproducibility*: This analytical performance was evaluated from five repeated measurements of electrochemical signal of four different RPN solutions following deposition onto the HMDE by adsorptive measurement as described in accuracy section. The precision of the described method is excellent. The relative standard deviation of recovery values is between 0.95

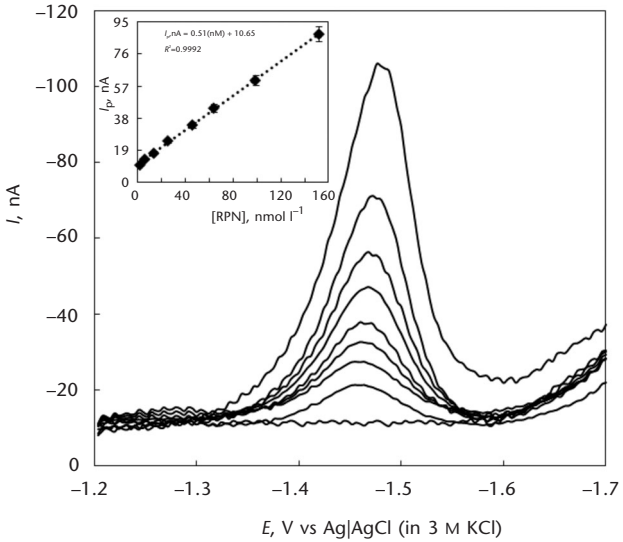



FIG. 5
Stripping voltammograms of RPN solutions with different concentrations. From inner to outer: blank BR, 1.5, 5.9, 14, 25, 46, 64, 99 and 150 nmol $l^{-1}$. Inset: calibration curve for related concentrations

and 4.74% for all measurement including tablets, urine and serum samples (Tables II and III).

*Stability*: The stability of RPN in a BR buffer of pH 10.3 was evaluated under the optimal procedural conditions by monitoring the changes in the cathodic peak height of standard RPN solution following its deposition onto the HMDE for 60 s at different days. The electroanalytical signal showed no significant difference in the peak current intensity and peak potential which confirms the stability of RPN over the time period of measurements (Table I).

*Robustness*: The robustness[31] of the proposed procedure was examined by studying the effect of small variation of some important procedural conditions such as pH, accumulation potential, accumulation time and temperature. Small changes (±1%) in such conditions do not affect the results of procedure.

Validation parameters are given in Tables I and II.

### *Assay of RPN in Real Samples*

In order to evaluate the applicability of the proposed method to pharmaceutical preparations and biological samples, RPN was determined in the pharmaceutical preparation (risperdal tablets), human urine and human se-

TABLE I
Validation parameter for proposed method obtained at optimum instrumental and experimental conditions such as $E_{acc}$ = –0.65 V, $t_{acc}$ = 60 s, pH 10.3

| Parameter | Value |
|---|---|
| Linearity range, mol $l^{-1}$ | $1.50 \times 10^{-9}$–$1.50 \times 10^{-7}$ |
| Slope, A l $mol^{-1}$ | 0.51 |
| Intercept, A | $1.14 \times 10^{-8}$ |
| Regression coefficient, $R^2$ | 0.9992 |
| Standard deviation (SD) of regression, A | $8.12 \times 10^{-10}$ |
| SD of slope, A l $mol^{-1}$ | $5.83 \times 10^{-3}$ |
| SD of intercept, A | $8.82 \times 10^{-10}$ |
| Limit of detection (LOD), mol $l^{-1}$ | $5.22 \times 10^{-9}$ |
| Limit of quatitation (LOQ), mol $l^{-1}$ | $1.74 \times 10^{-8}$ |
| Repeatability of current (RSD[a]), % | 0.84 |
| Repeatability of potential (RSD[a]), % | 0.17 |

[a] Relative standard deviation of 5 serial measurements.

TABLE II

Results of RPN assay in tablets using calibration method at optimum instrumental and experimental conditions such as $E_{acc}$ = –0.65 V, $t_{acc}$ = 60 s, pH 10.3

| Sample | RPN content mg | Found values, mg | | | | | Average value[a] | Average recovery[a] | RSD, %[b] |
|---|---|---|---|---|---|---|---|---|---|
| A (1st day I) | 0.50 | 0.49 | 0.50 | 0.50 | 0.52 | 0.48 | 0.49 ± 0.02 | 99.60 ± 3.29 | 2.66 |
| B (1st day II) | 1.00 | 1.02 | 0.99 | 1.00 | 0.99 | 1.00 | 1.00 ± 0.01 | 100.00 ± 1.36 | 1.10 |
| C (2nd day I) | 2.22 | 2.25 | 2.23 | 2.19 | 2.20 | 2.22 | 2.22 ± 0.03 | 99.88 ± 1.19 | 0.95 |
| D (2nd day II) | 2.43 | 2.54 | 2.47 | 2.44 | 2.40 | 2.45 | 2.46 ± 0.06 | 101.22 ± 2.33 | 1.85 |

[a] $(\bar{x} \pm ts/\sqrt{N})$ at 95% confidence level. [b] Relative standard deviation for percentages of recovery values.

TABLE III
Results of RPN assay in spiked serum and spiked urine using calibration method at optimum instrumental and experimental conditions such as $E_{acc}$ = –0.65 V, $t_{acc}$ = 60 s, pH 10.3

| Sample | Spiked amount µg | Found values, µg | | | | | Average value[a] | Average recovery[a] | RSD %[b] |
|---|---|---|---|---|---|---|---|---|---|
| A (urine, s)[c] | 2.50 | 2.45 | 2.50 | 2.55 | 2.60 | 2.60 | 2.55 ± 0.05 | 101.60 ± 2.90 | 2.29 |
| A (urine, t)[d] | 2.50 | 2.30 | 2.40 | 2.40 | 2.45 | 2.50 | 2.40 ± 0.10 | 96.40 ± 3.29 | 2.75 |
| C (serum, s) | 2.50 | 2.50 | 2.50 | 2.55 | 2.60 | 2.60 | 2.55 ± 0.05 | 102.00 ± 2.22 | 1.75 |
| C (serum, t) | 2.50 | 2.25 | 2.40 | 2.45 | 2.50 | 2.60 | 2.45 ± 0.15 | 97.60 ± 5.76 | 4.74 |

[a] $(\bar{x} \pm ts/\sqrt{N})$ at 95% confidence level. [b] Relative standard deviation for recovery values. [c,d] For each sample in first row standard (s) RPN sample was spiked and second row sample prepared using RPL tablets (t) was spiked.

rum using direct calibration method. Analytical and statistical parameters obtained in voltammetric studies for standard RPN were used in the assay studies.

*Assay of RPN in tablets*. Proposed method was firstly applied to assay of RPN in risperdal tablets. The results of these applications were given in Table II. As seen from this table, average recoveries are in good agreement with the RSD values less than 3%, which is a good evidence of validity of method. Thus, the precision is satisfactory for the analysis of pharmaceutical preparations as well as bulk samples.

*Assay of RPN biological samples*. In order to investigate the applicability of proposed methods to biological samples, the method was applied to spiked human urine and spiked human serum samples. In both applications, analytical and statistical parameters found or calculated in studies of standard RPN were used. Analytical studies in biological samples were performed as direct calibration method.

*Application to spiked urine*. Urine samples obtained from healthy individuals were centrifuged at 4000 rpm. Into a set of 10 ml volumetric flasks, separate aliquots of urine (1.0 ml) were spiked with varying amounts of RPN. The volumes were adjusted to 10.0 ml with a BR buffer at pH 10.3. A 1.0 ml aliquot from each solution was diluted to 10 ml with the same buffer and transferred into the measuring vessel. Voltammograms were recorded as in construction of calibration curve. The results are given in Table III.

*Application to spiked serum*. Serum samples, obtained from healthy individuals, were stored frozen until assay. After gentle thawing, an aliquot volume of urine (2.0 ml) was spiked to 8.0 ml of BR buffer. Then, 1.0 ml of stock solution of RPN ($4.0 \times 10^{-3}$ mol $l^{-1}$) dissolved in ethanol was added to mixture of serum and BR solution. The mixture was centrifuged for 15 min at 4000 rpm to remove the precipitated serum proteins. 1.0 ml clear sample was transferred in the 25.0 ml volumetric flask and volume was completed with BR solution. Appropriate volumes (5.0, 10.0, 30.0, 50.0 and 75.0 µl) from this liquor were spiked to electrochemical cell containing 10.0 ml of BR solution. In the sample solution thus obtained RPN was determined. The results are given in Table III.

As seen in Table III, average recoveries are in good agreement with low RSD values less than 4.8% both for human serum and human urine, which is a good evidence of validity of the method.

*Interference Studies*

During an application of proposed method to biological samples and tablets, before adding a standard solution of molecule under investigation, voltammetric base line of biological medium was measured applying the same procedures as applied to calibration studies with standard samples. In such applications, there exist no extra voltammetric signals were found, indicating that there is no significant interferences of various inorganic cations, anions and some organic substances found in pharmaceutical preparations (tablets) and biological mediums (human urine and human serum). Because of these investigations, no further interference studies were carried out.

## CONCLUSION

The study of electroactive compound (RPN) in BR buffer medium provides a new assay method based on the calibration of the current signal due to the reduction process as a function of RPN concentration at optimum instrumental parameters and experimental conditions. Adsorptive preconcentration of surface with RPN at optimum conditions enhances the reduction current signal. The proposed method provides a very sensitive and selective method of RPN assay without further purification of compounds in pharmaceutical dosage forms and two different biological liquids. The developed method has a detection limit of 5.18 nmol $l^{-1}$ (2.12 ppb). The method can be used especially to assay of the trace amount of RPN in biological samples. The method is more sensitive than already reported different spectrophotometric, chromatographic and electrochemical methods given in references[4–16]. The proposed method has distinct advantages over other existing methods regarding sensitivity, time-requirements and lower detection limit. In addition, no sophisticated instrumentation is required. Consequently, the proposed method has the potential of a good analytical alternative for determining RPN in different mediums.

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