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Electrochemical study of spiramycin and its determination in pharmaceutical preparation

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Spiramycin (SPY) is a medium-spectrum antibiotic with high effectiveness against Gram-positive bacteria. The voltammetric behaviour of spiramycin was studied using differential pulse polarography (DPP) and square wave polarography (SWP). The drug in Britton-Robinson buffer (pH 11.5) is reduced at −1.45 V, giving rise to a well-defined cathodic peak using hanging mercury drop electrode (HMDE) versus Ag/AgCl electrode. This peak is attributed to the reduction of the aldehyde group. The results proved that the reduction of SPY is an irreversible diffusion-controlled process. The diffusion current-concentration relationship was shown to be rectilinear over the range of 20–80 and 0.8–80 μg ml^{-1} using DPP and SWP modes, respectively, with detection limit of 8.5 μg ml^{-1} (1.01 × 10^{-5} M) and 0.46 μg ml^{-1} (5.46 × 10^{-7} M) for DPP and SWP modes, respectively. A mechanism is postulated for the reduction of SPY. The proposed techniques were successfully applied to the determination of the studied compound either in pure form or in its formulation. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: spiramycin; differential pulse polarography; square wave polarography; pharmaceutical preparation

Introduction

Spiramycin (SPY) is a 16-membered macrolide antibiotic used to treat infections of the oropharynx, respiratory system and genitourinary tract as well as cryptosporidiosis and toxoplasmosis.[1] Literature survey reveals that several methods have been reported for the determination of SPY in pharmaceutical preparations or in biological fluids including spectrophotometry,[2] LC,[3–7] electrophoresis,[8] TLC,[3,9,10] and voltammetry.[11] Analytical reviews of SPY did not reveal any reported polarographic studies concerning the reduction at the mercury electrode. Also its electrochemical behaviour has not been studied. In the present work, simple and sensitive differential pulse polarographic (DPP) and square wave polarographic (SWP) techniques were developed for the determination of SPY in bulk and tablet forms.

Experimental

Apparatus

The polarograms were obtained with a Metrohm 693 VA Processor. A Metrohm 694 VA Stand was used in the hanging mercury drop electrode (HMDE) mode. The three-electrode system was completed by means of an Ag/AgCl (3M KCl) reference electrode and a Pt auxiliary electrode. For pH measurements, a Jenway 3310 digital pH meter was used.

Materials and reagents

Pharmaceutical grade SPY (batch no: 20061209) was kindly supplied as a gift sample by Pharonia Pharmaceuticals (New Borg El-Arab City, Alexandria, A.R.E.) and used without further purification; it was certified to contain a potency of 4435 I.U. mg^{-1} of SPY. All materials used were of analytical reagent grade. High purity distilled water was used throughout the study. Rovamycin® tablets (Batch No. 7621006), manufactured by Aventis, France, were labelled to contain 3 M.I.U. SPY per tablet. The studies were carried out in Britton–Robinson (B-R) buffer (0.04 M of each of acetic, *o*- phosphoric and boric acids) adjusted to the required pH with 0.2 M sodium hydroxide solution.

Solutions

Stock standard solution of SPY, 1 mg ml^{-1}, was prepared in water. A working solution of 0.1 mg ml^{-1} was prepared by dilution of aliquot of the stock solution using B-R buffer (pH 11.5).

Construction of calibration curves

Aliquots from the working solution, covering the working range (Table 1), were transferred into set of 10-ml volumetric flasks and completed to volume with B-R buffer pH 11.5. The content of each flask was transferred into the measuring vessel and purged with pure nitrogen for 5 min. The DP and SW polarograms were then recorded using the HMDE as working electrode with optimum instrumental parameters (Table 2). The potential range scanned was from 1.0 to 1.8 V. Measurements were carried out at 20 ± 0.5 °C and the ionic strength was that provided by B-R buffer used.

Procedure for pharmaceutical preparation

Ten tablets of Rovamycin® were crushed. Adequate amounts of homogeneous powder corresponding to stock solution 100 mg%

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Table 1. Statistical data of the regression equations for the determination of Spiramycin (SPY) by the proposed method

Parameters	DPP	SWP	
Concentration range ($\mu g\ ml^{-1}$)	20–80	0.8–9	10–80
Slope (b)	8.164×10^{-3}	0.171	0.015
Intercept (a)	−0.014	−0.103	0.477
Correlation coefficient (r)	0.9953	0.9989	0.9985
Standard deviation of slope (S_b)	4.58×10^{-4}	5.73×10^{-3}	5.85×10^{-4}
Variance around the slope ($S_b{}^2$)	2.00×10^{-7}	3.28×10^{-5}	3.00×10^{-7}
Standard deviation of intercept (S_a)	2.32×10^{-2}	2.64×10^{-2}	2.69×10^{-2}
LOD ($\mu g\ ml^{-1}$)	8.50	0.46	
LOQ ($\mu g\ ml^{-1}$)	20.5	1.54	

were individually weighed and transferred into a 25-ml volumetric flask, and then dissolved in water by shaking for 20 min. The solution was directly diluted to obtain the appropriate concentrations (similar to standard working solutions). Then the procedure was completed as mentioned earlier.

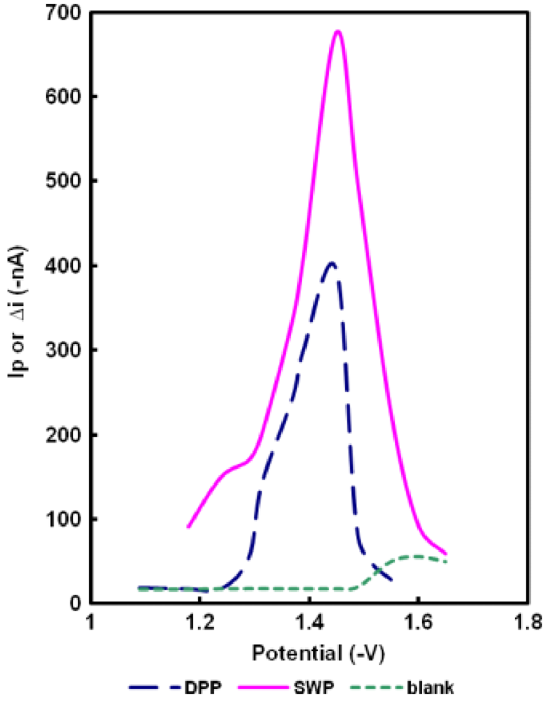


Figure 1. (A) Differential pulse polarography (DPP) polarogram of 50 $\mu g\ ml^{-1}$ SPY and (B) square wave polarogram (SWP) of 30 $\mu g\ ml^{-1}$ SPY at pH of 11.5 and instrumental parameters mentioned in Table 2 **(A) (B)**.

Results and Discussion

Figure 1 shows typical DP and SW polarograms of SPY in Britton-Robinson buffer of pH 11.5. SPY exhibits a well-defined cathodic peak at −1.45 V using HMDE versus Ag/AgCl electrode. This peak is attributed to the reduction of the aldehyde group.

Characteristics of electrode reaction

The reversibility of the electrode reaction was checked using the method reported by Birke *et al.*[12] The DP polarogram was recorded with a negative going potential pulse (−100 mV), then with a positive going potential pulse (+100 mV). It was found that SPY reduction process corresponded to irreversible criteria:

$\mathbf{E_p{}^c - E_p{}^a < /\Delta E/}$, where ΔE is the pulse magnitude, $E_p{}^c$ is cathodicaly scanned potential and $E_p{}^a$ is anodicaly scanned potential

$\mathbf{I_p{}^a/I_p{}^c = 1}$**,** where $I_p{}^a$ is current measured at anodicaly scanned potential and $I_p{}^c$ is current measured at cathodicaly scanned potential for SPY.

These values were $E_p{}^c - E_p{}^a = 20$ mV ($\Delta E = 100$ mV) and $I_p{}^a/I_p{}^c = 0.95$.

A linear Randles-Senvik plot (plot of peak current $\{I_P\}$ against the square root of scan rate $\{\nu^{1/2}\}$) with correlation coefficients of 0.988, was obtained indicating that diffusion is the mean of mass transport.[13] This finding was further confirmed by applying logarithmic analysis (Log I_p versus log ν) yield linear plot with slope of 0.42 and correlation coefficients of 0.979. This slope value is close to that theoretically expected (0.5) for ideal reaction of solution species.[14] Therefore, the reduction of SPY at HMDE is an irreversible diffusion controlled process.

Figure 2A shows the effect of pH values (in the range of pH 9–12) on potential (Ep) of the reduction peaks of SPY. The peak potential shifted linearly to more negative values with increasing pH with slope (S) of 173 $mVpH^{-1}$. According to Zuman,[15] this Ep-pH dependent behaviour indicated that a transfer of protons was coupled to the electrode process, and the number of protons, Z, could be evaluated by Equation 1.

$$\mathbf{S = 60\,Z/\alpha n} \tag{1}$$

The value of αn was calculated by the method proposed by Laviron.[16] According to Laviron, the half-peak width, $W_{1/2}$, is given by Equation 2:

$$\mathbf{W_{1/2} = 62.5/\alpha n\ (mV)} \tag{2}$$

α is the transfer coefficient and n is the number of electrons transferred in the electrode process. The value of $W_{1/2}$ in Figure 1 was 100 mV. The number of protons participated in the electrode process was found to be 2. Values of n and Z indicated that the electrode process was coupled with a transfer of two protons and

Table 2. Optimum operational parameters selected for the determination of Spiramycin (SPY) by DPP and SWP

Parameters	pH	Scan rate (mVS^{-1})	Pulse amplitude (mV)	Modulation frequency (Hz)	Working area (mm^2)	Rotating speed (rpm)
DPP	11.5	120	−100	–	0.6	2000
SWP	11.5	40	+50	100	0.6	2000

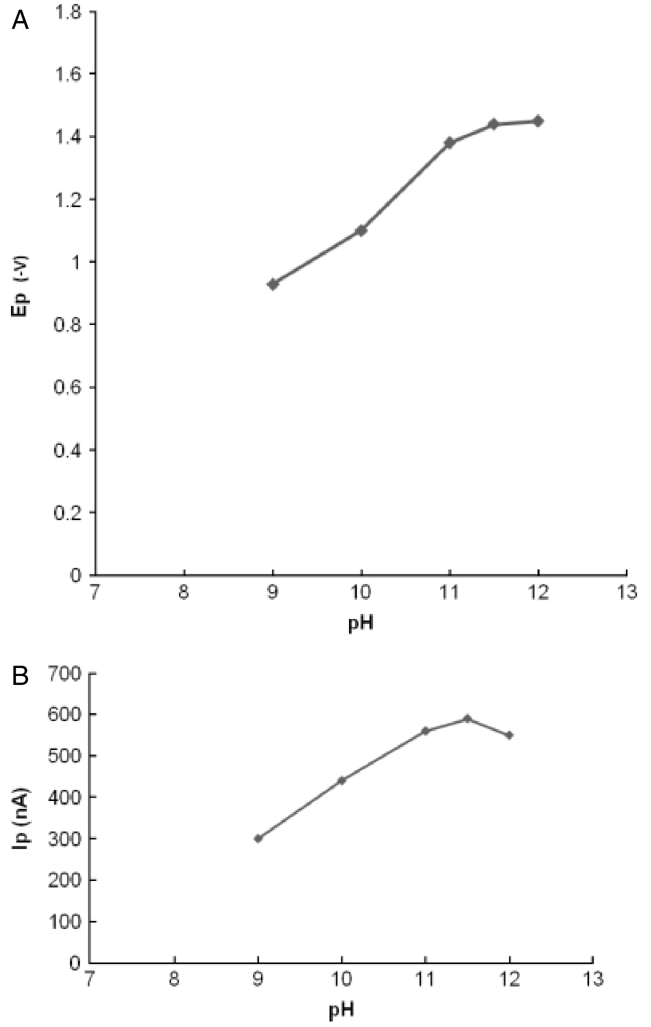


Figure 2. Influence of pH on the peak potential (A) and peak current (B) of SPY (40 µg ml^{-1}). Instrumental parameters as in Table 2.

two electrons. The transfer coefficient (α) was found to be 0.342 (less than 1) confirming the irreversibility of the reduction reaction.

Mechanism of electrode reaction

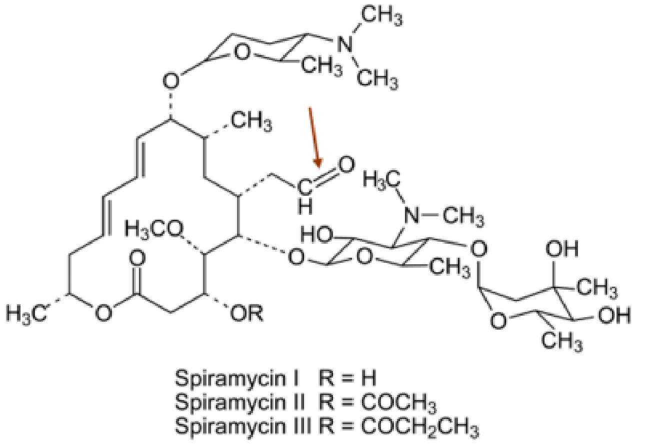
SPY shows polarographic peak at -1.45 V, using HMDE versus Ag/AgCl electrode in Britton–Robinson buffer pH 11.5, pointing to a two-electron transferred process. This cathodic peak is attributed to the reduction of the carbonyl group. It has been reported that compounds including carbonyl group are polarographically reducible.[17,18]

The reduction of carbonyl group involves two electrons and two protons. The following mechanism (Schematic 1) is proposed for the reduction behaviour.

Optimization of analytical conditions

Factors affecting the peak current were studied and optimized. Britton-Robinson buffer was chosen as supporting electrolyte for DP and SW polarographic determination of the drug. The effect of pH on the peak current was studied over the pH range 8–12. This study showed that the reduction signal appears only for pH values higher than 8.5. This is because reduction only occurs at very negative potentials, as predicted for this type of compound.[18] Consequently, the reduction peak can only be expected to be observed when very high pH value supporting electrolytes are used because in those experimental conditions, the negative potential range for HMDE is increased up to -2.0 V *vs* Ag: AgCl, and the reduction peak can be recorded. Maximum SPY peak current was observed at pH 11.5 (Figure 2B). The effect of pH on the peak potential (Ep) was also studied. Ep values were shifted to more negative potential upon increasing the pH. The relationship was almost linear with slope of 173 mVpH^{-1} and correlation coefficient of 0.9998.

$$R' - CHO + 2e^{-} + 2H^{+} \longrightarrow R' - CH_2OH$$



Scheme 1. Proposed mechanism for the reduction behaviour of spiramycin.

Instrumental conditions affecting the peak current were also optimized. Maximum response was obtained at scan rate (ν) of 120 mV S^{-1} and 40 mV S^{-1} for DPP and SWP, respectively (Figure 3). Pulse amplitudes of -100 mV and $+50$ mV were chosen for analytical measurement of SPY in DPP and SWP, respectively. The influence of working electrode area on the peak current was also tested. As expected, an increase of the area yielded an increase in the peak height, so a large area (0.6 mm^2) was considered suitable. In case of the SW technique, modulation frequency is a very important parameter. A wide interval was studied from 25 to 150 Hz, and the best signals were achieved at 100 Hz. At higher frequencies the measurement was not reproducible.

Validation of the proposed method

Linearity

Using the optimum conditions reported in Table 2, the determination of SPY was carried out, based on the linear dependence of the peak current (µA) on concentration (µg ml^{-1}). For DPP, the linear range was between 2.37×10^{-5} and 9.49×10^{-5} M according to the equation cited in Table 1. For SWP, two linear calibration graphs were obtained between 9.49×10^{-7} and 9.49×10^{-5} M and between 1.19×10^{-5} and 9.49×10^{-5} M with different slopes according to the Equations cited in Table 1. Other important statistical parameters are also given in Table 1.

Limit of detection and limit of quantification

In accordance with IUPAC,[19] the limit of detection (LOD) $= 3S/b$ and the limit of quantification (LOQ) $= 10S/b$, where S is the

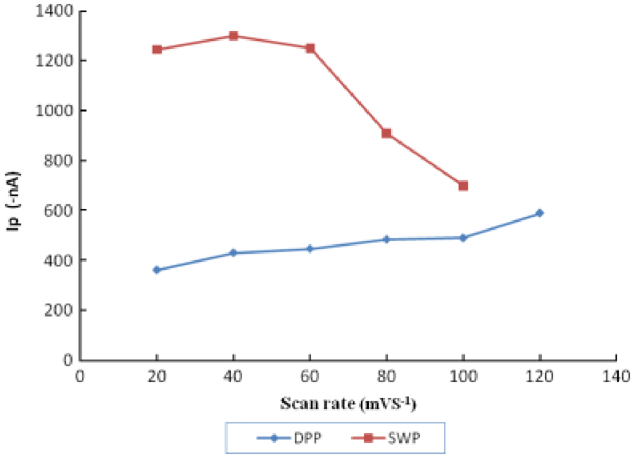


Figure 3. Effect of scan rate on the peak current of SPY (55 µg ml^{-1}) in BR buffer at pH 11.5. Instrumental parameters as in Table 2.

standard deviation of replicate blank responses (under the same conditions as for the sample analysis). Using this formula, LOD and LOQ are calculated and cited in Table 1. Experimentally, LODs were found to be 8 µg ml^{-1} (9.49×10^{-6} M) and 0.5 µg ml^{-1} (5.93×10^{-7} M) by DPP and SWP, respectively while LOQs were 20 µg ml^{-1} (2.37×10^{-5} M) and 0.8 µg ml^{-1} (9.49×10^{-7} M) for DPP and SWP, respectively

Selectivity

The selectivity of the proposed procedures for the assay of SPY was identified by studying the effect of excipients that often accompany SPY in its tablets. An attractive feature of analytical procedures is its relative freedom from interference by the commonly used tablet excipients (anhydrous colloidal silica, gelifiable maize starch, magnesium stearate, talc, hydroxypropyl cellulose, sodium crocarmellose, and microcrystalline cellulose). The results indicated no significant interference from excipients, thus the procedure was able to assay SPY in their presence and hence could be considered selective.

Accuracy and precision

The accuracy study was performed by the addition of known amounts of SPY to known concentration of the commercial capsules (standard addition method). The resulting mixtures were assayed and the results obtained for SPY standard addition method (Table 3) suggest good accuracy of the proposed methods and no interference from the tablet's excipients, hence the proposed method was applicable for the quantitative determination of SPY in its dosage form.

The intra-day and inter-day variation for the determination of SPY were carried out at three different concentration levels namely: 10, 40 and 80 µg ml^{-1} for DPP and 0.8, 3.0 and 9.0 µg ml^{-1} for SWP. Method repeatability was indicated from RSD% values obtained by repeating the assay five times on the same day for intra-day precision (Table 3). Intermediate precision was assessed by the assay of the sample sets on three different days (inter-day precision). The calculated RSD% values are mentioned in Table 3. The results indicated satisfactory precision of the proposed techniques.

Robustness

Robustness, as a measure of procedure, is the capability to remain unaffected by small variations in the operational parameters. This was evaluated by analyzing SPY at three different concentration levels, as used under the evaluation of precision. This was performed under intentional slight variation of the selected parameters. These parameters were pH values of the buffer [11.5–12.0], pulse amplitudes [(−90)–(−100) mV)] for DPP and [45–50 mV] for SWP, potentiostat models, and different analysts. It was found that variation in the above parameters had no significant influence on the determination of SPY using both polarographic techniques. The low values of RSD% of current amplitudes of SPY obtained after introducing small deliberate changes in the method parameters indicated the robustness of the developed polarographic techniques (Table 3).

Table 3. Accuracy, precision and robustness of the proposed method

	Recovery ± SD[a]		
Parameters	DPP	SWP	Conditions
Accuracy	98.5 ± 1.98	98.3 ± 1.77	*pH value = 11.5*
Repeatability (Intra-day precision)	99.6 ± 1.80	99.0 ± 1.65	*Pulse amplitude of −100 mV for DPP and +50 mV for SWP*
Intermediate precision (Inter-day precision)	98.1 ± 1.55	99.7 ± 1.70	*pH-meter jenway 3310*
Robustness			
❖ pH of the medium			*Pulse amplitude of −100 mV for DPP and +50 mV for SWP*
(11.5 and 12.0)	98.9 ± 0.70	99.3 ± 0.95	*pH-meter jenway 3310*
❖ Pulse amplitudes			*pH value = 11.5*
(−90 mV and −100 mV)	101.0 ± 0.50	–	*pH-meter jenway 3310*
(45 mV and 50 mV)	–	100.6 ± 1.50	
❖ Potentiostate models[b] lab (1) and lab (2)	99.0 ± 1.65	101.0 ± 1.75	*pH value = 11.5*
			Pulse amplitude of −100 mV for DPP and +50 mV for SWP
❖ Two analysts	98.5 ± 1.20	99.7 ± 1.90	*pH value = 11.5*
			Pulse amplitude of −100 mV for DPP and +50 mV for SWP
			pH-meter jenway 3310

[a] Average of three concentrations 10, 40 and 80 µg ml^{-1} for DPP and 0.8, 3.0 and 9.0 µg ml^{-1} for SWP, respectively. (n = 5).
[b] pH-meter jenway 3310 and pH-meter cyberscan 510.

Table 4. Determination of Spiramycin (SPY) in commercial tablets by the proposed method

Preparations	Recovery ± SD[a] DPP	SWP	Reference method[b]
Rovamycin® tablets *(3 MIU SPY per tablet)*	98.59 ± 1.20	97.59 ± 0.62	98.49 ± 0.70
t[c]	0.288	2.14	
F[c]	2.86	1.30	
Standard addition	99.5 ± 0.80	99.0 ± 0.95	

[a] Mean ± standard deviation of five determinations.
[b] HPLC[20].
[c] Theoretical values of t and F are 2.31 and 6.39, respectively, at 95% confidence limit.

Application to commercial tablets

Assays of SPY in its tablet form were carried out as described under tablet preparation in the Experimental section. Five replicate determinations were made. Excipients in the preparation did not interfere with the analysis. Satisfactory results were obtained for both techniques and were in a good agreement with the label claims (Table 4). Moreover, to check the validity of the proposed method, the standard addition method was applied by adding different concentrations of SPY to the previously analyzed tablets. Drug recovery was calculated. The results of the analysis of the commercial tablets and the recovery study (standard addition method) of SPY (Table 4) suggested that there was no interference from any excipients normally present in tablets. These results were compared with those of the official High Performance Liquid Chromatography (HPLC) method.[20] Statistical comparison of the results was performed with regard to accuracy and precision using Student's *t*-test and the F-ratio at 95% confidence level (Table 4). There was no significant difference between the proposed method and official HPLC method with regard to accuracy and precision.

Conclusion

The reduction of Spiramycin (SPY) occurs at very high potentials, which means that it can be studied only at pH values higher than 9. Two electro-analytical techniques based on DPP and SWP have been developed. These methods are quick and relatively cheap to operate compared with alternative chromatographic methods. They are suitable for routine analysis in quality control laboratories, to be applied for the analysis of SPY in pure form and in tablets. Taking into account the results obtained for the calibration graphs, SWP technique is more sensitive than DPP technique.

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