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# CATHODIC ADSORPTIVE STRIPPING VOLTAMMETRY FOR THE DETERMINATION OF ATORVASTATINE DRUG IN PHARMACEUTICAL FORMULATION AND SPIKE HUMAN PLASMA

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## ABSTRACT

Adsorptive stripping voltammetry is used for the determination of atorvastatine. The dependence of the peak current and potentials on pH, concentration, accumulation potential, accumulation time, scan rate were investigated on the hanging mercury dropping electrode. The reduction of atorvastatine was irreversible on the hanging mercury dropping electrode (HMDE). The response is linear from $10^{-8}$ mol.L$^{-1}$ to $10^{-7}$ mol.L$^{-1}$ and $10^{-7}$ mol.L$^{-1}$ to $10^{-6}$ mol.L$^{-1}$. The detection limits (LOQ) of a standard solution are estimated to be 9.6 x $10^{-10}$ mol.L$^{-1}$ and quantitation limits (LOQ) are 3.2.$10^{-9}$ mol.L$^{-1}$ (ater accumulation for 90 s). The methods were fully validated and successfully applied to the high through put determination of the drug in pharmaceutical formulation, human plasma with good recoveries.

**Key words**: Atorvastatine, Voltammetry, Adsorptive, Accumulation, Pharmaceuticals, Human plasma.

## INTRODUCTION

Atorvastatine (AT), (3*R*, 5*R*)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid (Fig. 1), is a second generation HMG-CoA reductase inhibitor recently approved for clinical use as a cholesterol lowering agent[1]. Cholesterol and triglycerides circulate in the blood stream as part of lipoprotein complexes. Triglycerides and cholesterol in the liver are incorporated into very-low-density lipoprotein and released into the plasma for delivery to peripheral tissues. Clinical and pathological studies show that elevated plasma levels of total cholesterol, low density lipoprotein-cholesterol and are risk factors for developing cardiovascular disease, while

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increased levels of high-density lipoproteins are associated with a decreased cardiovascular risk[1,2].

This drug is absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism in the liver. Atorvastatine 98% is bound to plasma proteins. This drug is excreted mainly in feces via the bile, with a smaller proportion excreted in urine[3-5].

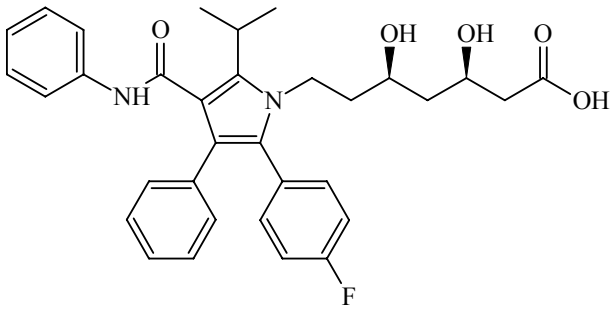



**Fig. 1: The structure of atorvastatine**

Several analytical techniques have been used for the determination of atorvastatine such as spectrophotometry[6-8], spectrofluorimetry[9], high performance liquid chromatography[10,11], and liquid chromatographic - MS/MS[12,13]. However, these methods may be difficult to run and expensive instrumentation and are time-consuming. Due to the high sensitivity and selectivity of electrochemical methods, the determination of atorvastatine has been interested in developing[14-16].

In this study, low concentrations of atorvastatine are determined by adsorptive stripping voltammetry. The analyte is preconcentrated by adsorption on the surface of the electrode and the surface-active species is then determined by a voltammetric scan method. Adsorptive stripping voltammetry has been shown to be a sensitive analytical method for a wide range of pharmaceutical compounds that can be adsorbed on the electrode surface. In this paper, the cyclic voltammetry was used to study electrochemitry of atorvastatine on hanging mercury dropping electrode. The square-wave voltammetry method has been applied to the determination of atorvastatine in pharmaceutical formulation and spike human plasma.

## EXPERIMENTAL

### Apparatus

All measurements were performed with an μAutolab type III (Netherlands) and a hanging mercury drop electrode (VA 663, Metrohm, Switzerland), controled by software

757 VA computrace. The reference electrode (Metrohm, Switzerland) was a double-junction Ag/AgCl electrode with 3M KCl in the salt bridge, and the counter electrode (Metrohm, Switzerland) was a glassy carbon rod. All measurements were carried out in solutions, which were thoroughly deaerated with high-purity nitrogen for at least 5 min.

## Reagents

Stock solutions of atorvastatine ($1 \times 10^{-3}$ mol.L$^{-1}$) were prepared by dissolution of precisely weighed amounts of the standard in methanol. Standard solutions were prepared daily by further dilution of the standard stock solution with methanol:water (1:4) to produce $10^{-4}$ mol.L$^{-1}$ and $10^{-6}$ mol.L$^{-1}$ atorvastatine solutions. The standard stock and the working solutions were kept in dark bottles immediately after preparation and stored at $4^0$C. All other reagents, of p.a. quality, were purchased from Merck and Sigma-Aldrich. All solutions were prepared using double-distilled water. For optimization of electrolyte pH, several universal buffers (phosphate, acetate and Britton-Robinson) between pH 2.0-12.0 were tested.

## Analysis of tablets

Atorvastatine tablets were obtained in a local pharmacy. Ten atorvastatine tablets was weighed and finely ground in an agate mortar (amount declared of atorvastatine per tablet 10.0 mg) and finally the correct amount of powder (about 100 mg ± 0.1 mg) was dissolved in 15 mL methanol, shake about ten min then dilute to volume 25 mL with double-distilled water, after that the mix solution was filtered through a 0.4 μm with Millipore filter (Gelman, Germany). A volume of 1.0 mL of the filtrate in a 100 mL volumetric flask was dilute with double water. Take 0.25 mL that solution into 25 mL volumetric flask containing 10 mL BR buffer, an aliquot was then transferred to a voltammetric cell. The square-wave voltammetry method was record, and atorvastatine concentration in drugs was determined by standard addition method.

## Spiked human plasma samples

Human plasma samples, obtained from healthy volunteers, were collected and stored frozen until the assay. A 1.0 mL aliquot of the human plasma sample, spiked standard solution of atorvastatine in that with differential concentration, add 1 mL BR buffer pH = 9.0. The mix solution was passed through C18 extraction column (Water Sep-Pak Vac C18, Lot. No. 036133008A, 3-mL size, US). Before analysis, the column was washed twice with methanol and twice with double-distilled water. Then the sample was applied and allowed to run through, and the columnwas washed 1 mL with water and 1 mL metanol : water (5:95 v/v). The drug was eluted from the column into 25.0 mL volumetric flask with 1 mL of methanol : buffer (BR, pH = 9) (95:5 v/v).

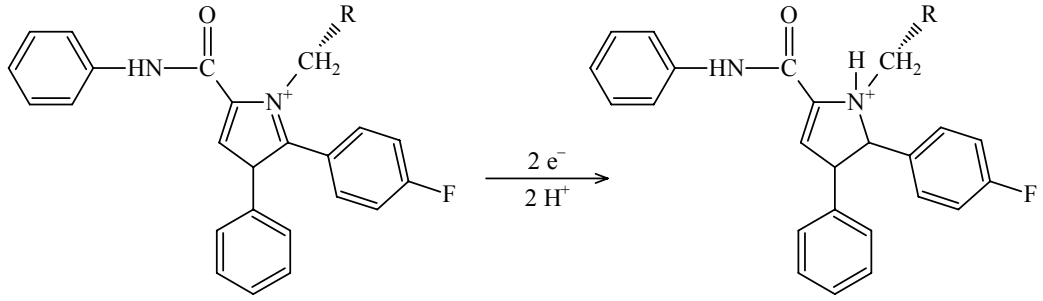
### Analytical stripping procedure

Ten millilitres of BR buffer of pH 9.0 containing atorvastatine drug at an appropriate concentration was introduced into the electrolysis cell, through which a pure nitrogen stream was passed for 5 min before recording the voltammogram. A selected accumulation potential $E_{acc}$= - 0.9 V (vs. Ag/AgCl/KCls) was applied to the HMDE during the preconcentration of the drug ($t_{acc}$= 30 s) while the solution was kept under stirring. After the accumulation time had elapsed, the stirrer was stopped and 5 s was allowed for the solution to become quiescent. Then the voltammogram was recorded by scanning the potential towards the negative direction applying the differential pulse waveform. A calibration graph was constructed under the optimized conditions of the procedure.

## RESULTS AND DISCUSSION

### Cyclic voltammetry

The influence of pH on the cyclic voltammogram of the drug was studied in BR buffers of pH from 4.0 to 11.0 at an hanging mercury dropping electrode (HMDE). The voltammogram exhibited a single irreversible cathodicpeak over the entire pH range due to the reduction of the C=N group of the reactant molecule[14].




(ATOR)

**Scheme 1**

To investigate the adsorptive character of atorvastatine at the mercury surface, cyclic voltammograms for $10^{-7}$ mol.$L^{-1}$ solution were conducted in BR buffers of pH = 9.0. Fig. 2, displays voltammograms for $10^{-7}$ mol. $L^{-1}$ atorvastatine in BR buffer of pH 9.0 at the HMDE, (1) blank solution, (2) without preconcentration ($t_{acc.}$= 0 s), (a) after preconcentration for 60 s, (b, c) the repetitive cycle of a, b, respectively. The peak current after preconcentration is higher 3.4 times compared to that without preconcentration. The repetitive cathodic peak current (curve b) decreased rapidly compared to that of the first

cathodic cycle (curve a). The repetitive cathodic peak current (curve c) decreased slowly compared to that the second cathodic cycle (curve b) but higher than stripping peak current without preconcentration. Which may be attributed to desorption of the drug species from the mercury electrode surface.

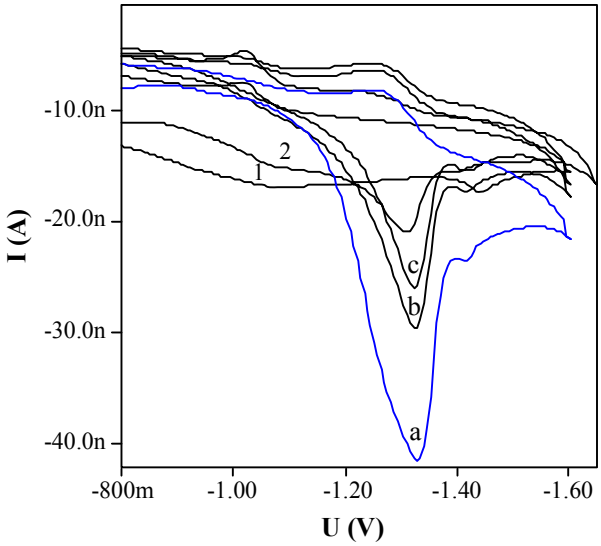



**Fig. 2: Cyclic voltammograms for $10^{-7}$mol.$L^{-1}$ atorvastatine (1) blank solution pH = 9.0; (2) without preconcentration $t_{acc.}$ = 0 s, (a) preconcentration for 60 s (b, c) repetitive cycle of (a, b respectively) at the same mercury drop; scan rate = 100 mV $s^{-1}$ and $E_{acc.}$= –0.9 V**

The peak current ($i_p$) increased linearly with scan rates of 10 - 1000 Mv.$s^{-1}$ according to the equation log $i_p$ = 0.946 logν - 0.679; $R^2$ = 0.998. For such a relation, the slope values of 1.0 and 0.5 were expected for an ideal surface and solution reaction species, respectively.

However in most cases the voltammetric behaviour of the surface confined species was not ideal[15,17]. Accordingly, the slope value (0.946) of the present log$i_p$ vs. logν plot indicated that atorvastatine possesses a strong adsorptive character onto the hanging mercury drop electrode surface. Moreover, peak potential ($E_p$) of stripping peak shifted to more nagative values when the scan rate was increased.

## Choice of the applied voltammetric waveform

After preconcentration of atorvastatine onto the HMDE in BR buffer of pH 9.0, the application of either differential-pulse or square-wave voltammetric scan in the negative direction gave rise to a well-defined stripping reduction peak. As shown in Fig. 3, for

$2 \times 10^{-6}$ mol $L^{-1}$ atorvastatine, a large response was obtained after preconcentration of the drug for 30 s. On the contrary, the solution without preconcentration ($t_{acc.}$= 0, curve a), showed a smaller reduction peak current.

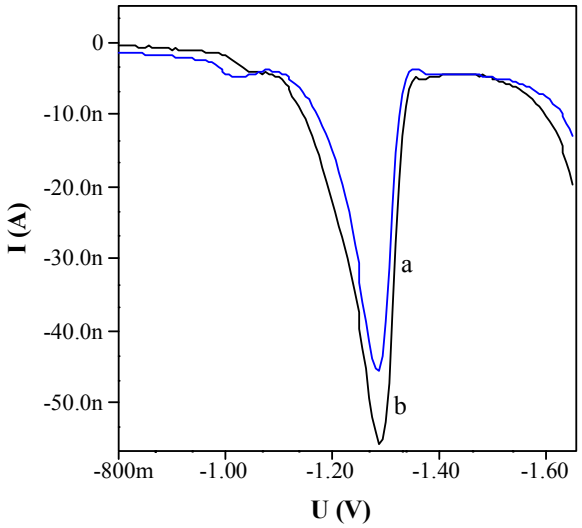



**Fig. 3: Differential pulse (DP) adsorptive stripping voltammograms for $2 \times 10^{-6}$ mol $L^{-1}$ atorvastatine in BR buffer of pH 9.0: (a) $t_{acc.}$ = 0 s and (b) $t_{acc.}$= 30 s. Experimental conditions: $E_{acc.}$= –0.8 V, pulse height = 50 mV, and scan rate = 10 mV $s^{-1}$**

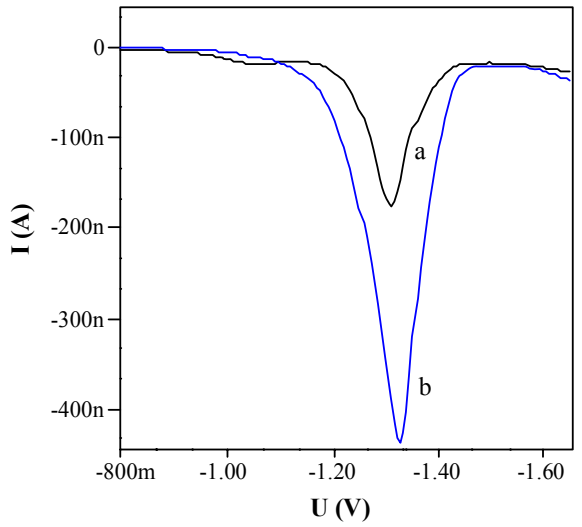



**Fig. 4: Square-wave (SW) adsorptive stripping voltammograms (SW) for $2 \times 10^{-6}$ mol $L^{-1}$ atorvastatine in BR buffer of pH 9.0: (a) $t_{acc.}$ = 0 s and (b) $t_{acc.}$= 30 s. Experimental conditions: $E_{acc.}$= –0.8 V, pulse amplitude=50 mV, f=100 Hz, and ΔE= 10 mV**

However, as shown in Fig. 4, application of square-wave waveform (scan increment ΔE = 10 mV, pulse amplitude a = 50 mV, frequency f = 100 Hz, $t_{acc.}$= 30 s, $E_{acc}$ = –0.8 V) gave a cathodic peak of current intensity $E_p$ = -1.31 V and $i_p$= 422 nA, which was about 8 times higher compared to that obtained by application of the differential-pulse technical ($E_p$= –1.29 V and $i_p$ = 52.2 nA). Thus, the square-wave waveform was preferred to the differential-pulse one for the rest of study because of its highest sensitivity, moreover, its high scan rate provided an economy of time.

The pH had a effect on both the peak current and peak potential. Fig. 5 show that, a higher peak current was observed in Britton-Robinson buffer of pH 8.5-9.0. Hence, a BR buffer of pH 9.0 was considered as a supporting electrolyte for the drug assay.

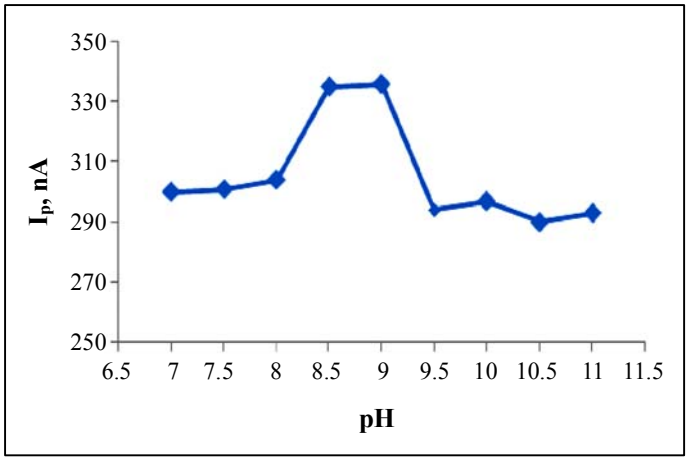



**Fig. 5: Effect of pH (7.0– 11.0) on the cathodic adsorptive stripping square-wave peak current of atorvastatine 5 × $10^{-7}$mol. $L^{-1}$, $E_{acc.}$ = –0.5 V, A=50 mV, f = 50 Hz**

The sensitivity of the adsorptive stripping voltammetry procedure depends on both the preconcentration of time period and potential of the analyte at the working electrode surface. Fig. 6 shows a rapid increase of the peak current after a short preconcentration time period, and then decreased after longer periods depending on the concentration level of the drug. In this case, the decrease of the peakcurrent may be attributed to the complete coverage of the electrode surface, that was reached rapidly at concentrations higher than 5 × $10^{-7}$mol. $L^{-1}$ atorvastatine. At solution of 5 × $10^{-7}$mol. $L^{-1}$ atorvastatine, the maximum peak current reached at 90s preconcentration time and decreased after longer period time. Similarly with $10^{-8}$ M atorvastatine, when increasing preconcentration time from 10 s to 120 s, stripping peak current increase rapidly, after that very slowly increased up to 300s and then decreased peak current if continuing precontration time. So, preconcentration of the

drug for 30 s and 90 s were chosen for the assay of higher and lower concentrations of the drug than $5 \times 10^{-7}$mol $L^{-1}$, respectively.

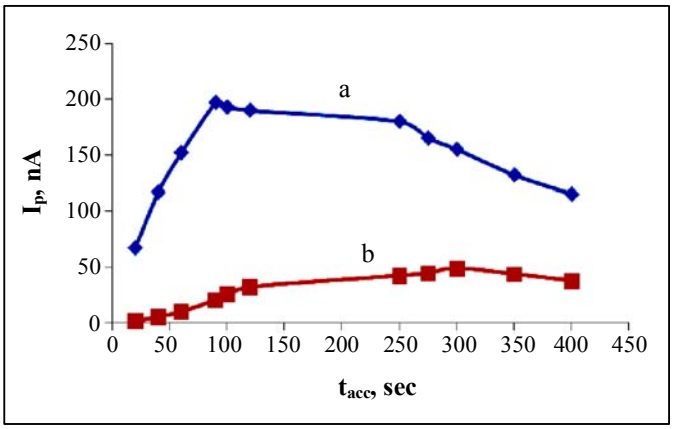



**Fig. 6: Effect of accumulation time ($t_{acc.}$) on the cathodic adsorptive stripping square-wave peak current of two different concentrations of atorvastatine: (a): $5 \times 10^{-7}$ mol $L^{-1}$ and (b) $10^{-8}$ mol .$L^{-1}$, $E_{acc.}$= –0.4 V, a = 50 mV, f = 50 Hz**

## Accumulation potential ($E_{acc}$)

The dependence of the stripping peak current on the accumulation potential was also studied over the range 0 V to –1.0 V for $5 \times 10^{-7}$ mol.$L^{-1}$ atorvastatine after preconcentration for 60 s. The peak current was practically independent of the accumulation potential up to –0.9 V vs. Ag/AgCl (Fig. 7). Therefore, an accumulation potential of –0.9 V was chosen for the rest of measurements.

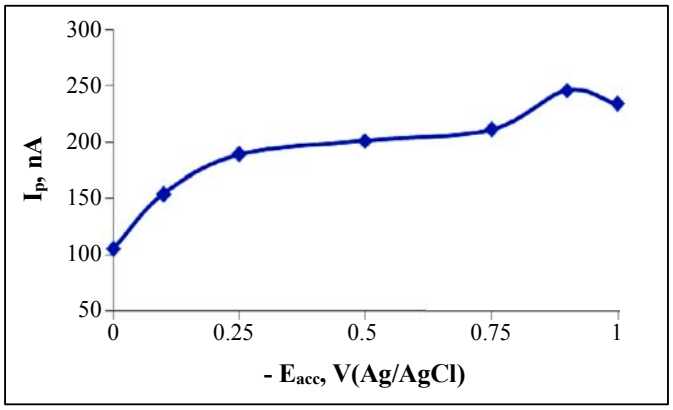



**Fig. 7: Effect of accumulation potential ($E_{acc.}$) on the cathodic adsorptive stripping square-wave peak of atorvastatine $5 \times 10^{-7}$ mol. $L^{-1}$, a = 50 mV, f = 50 Hz**

Other conditions were studies such as frequency f, pulse amplitude (A), potential scan rate (ν), equal time of atorvastatine on the hanging mercury dropping electrode. The optimal square-wave parameters for the assay of atovastatin were established as f = 50 mV, a = 50 mV, ν = 300 mV/s. Two linear concentration ranges were obtained from $1 \times 10^{-8}$ mol.L$^{-1}$ to $1 \times 10^{-7}$ mol.L$^{-1}$ ($t_{acc.}$= 90 s) and from $1 \times 10^{-7}$ mol.L$^{-1}$ to $1 \times 10^{-6}$ mol.L$^{-1}$ ($t_{acc.}$ = 30 s). The variation of $i_p$ versus atorvastatine concentration C was represented by the straight line equation $i_p$ = 10.71 C.$10^{-8}$ mol.L$^{-1}$ -0.033 and 30.47 C.$10^{-7}$ mol.L$^{-1}$ + 0.41, respectively.

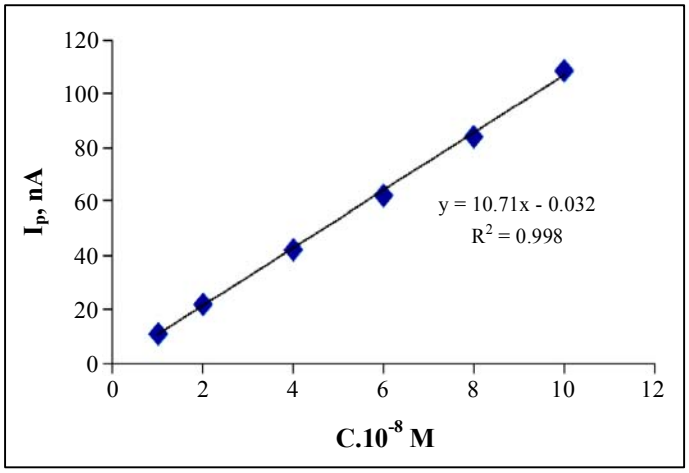



**Fig. 8: Calibration of atorvastatine from $1 \times 10^{-8}$ mol.L$^{-1}$ to $1 \times 10^{-7}$ mol.L$^{-1}$**

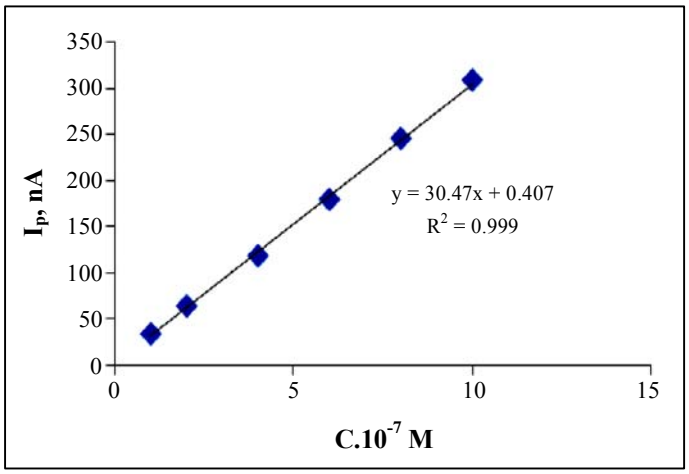



**Fig. 9: Calibration of atorvastatine from $1 \times 10^{-7}$ mol.L$^{-1}$ to $1 \times 10^{-6}$ mol.L$^{-1}$**

## Validation of the procedure

The limit of detection (LOD), limit of quantitation (LOQ) calculated from the calibration curves as $k.S.D_a/b$[19,20] where k = 3 for limit of detection and k = 10 for limit of quantitation, $S.D_a$ is the standard deviation of the intercept and b is the slope of the calibration curve. The caculated LOD is 9,6. $10^{-10}$ mol.L$^{-1}$ and LOQ is $3.2.10^{-9}$ mol.L$^{-1}$ater accumulation for 90s.

Repeatablity was measured for $5.10^{-7}$ mol.L$^{-1}$ solution after 30s accumulation, relative standard deviation (RSD) is 0.42%.

## Applications

The proposed procedure was successfully applied to the determination of atorvastatine in drugs such as lipivastin 10 mg of Mekofar, Avastor 10 mg of Boston pharma. The atorvastatine concentration in drug was determined by a standard method (Fig. 10 and Fig. 11). The mean recovery based on six replicate measurement was found to be 98.2% and 97.6%, respecctively[18,20-22]. This results was compared with HPLC method 99.6% and 98.4%, respectively.

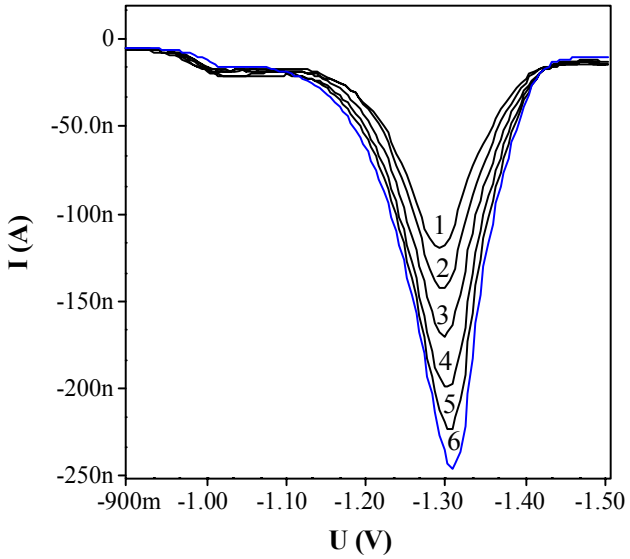



**Fig. 10: Cathodic adsorptive stripping square-wave voltammograms of atorvastin in drug samples: (1)$C_x$, (2) $C_x$ +5 x $10^{-8}$, (3) $C_x$ + 1.0 x $10^{-7}$, (4) $C_x$ + 1.5 x $10^{-7}$, (5)$C_x$ + 2.0 x $10^{-7}$, (6) $C_x$ + 2.5 x $10^{-7}$ molL$^{-1}$, $E_{acc.}$ = - 0.9 V, $t_{acc.}$ = 30 s, a = 50 mV, f = 50 Hz and BR buffer of pH = 9.0**

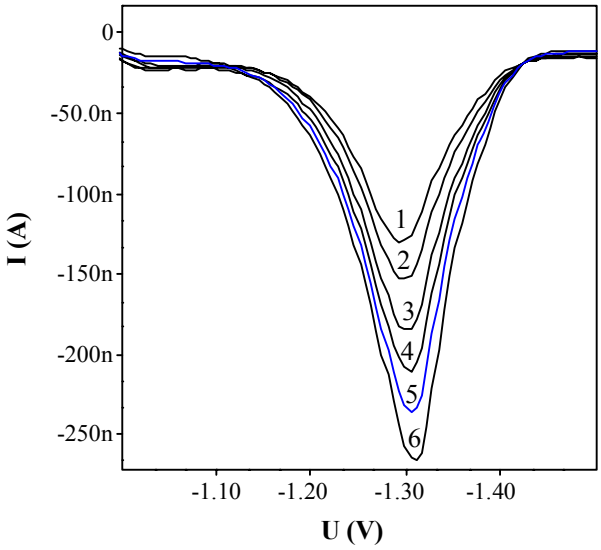



**Fig. 11: Cathodic adsorptive stripping square-wave voltammograms of atorvastin in drug samples: (1) $C_x$, (2) $C_x$ +5 x $10^{-8}$ , (3) $C_x$ + 1.0 x $10^{-7}$, (4) $C_x$ + 1.5 x $10^{-7}$, (5) $C_x$ + 2.0 x $10^{-7}$, (6) $C_x$ + 2.5 x $10^{-7}$ $molL^{-1}$ , $E_{acc.}$ = - 0.9 V, $t_{acc.}$ = 30 s, a = 50 mV, f = 50 Hz and BR buffer of pH = 9.0**

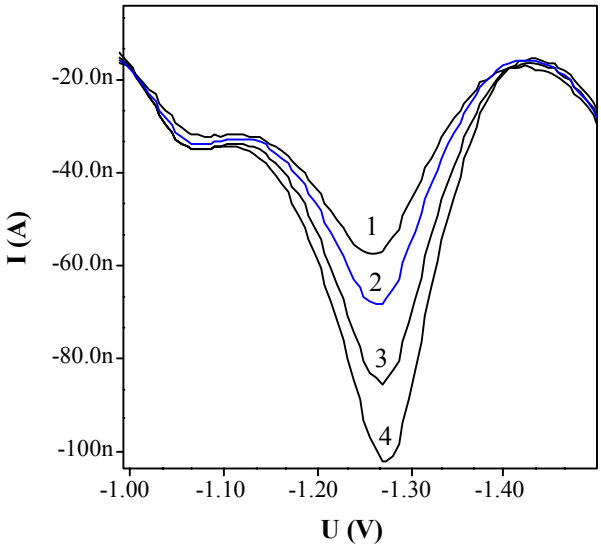



**Fig. 12: Cathodic adsorptive stripping square-wave voltammograms of atorvastin spiked in plasma samples: (1) Atorvastatine 1.0 x $10^{-7}$, (2) 1.5 x $10^{-7}$, (3) 2.0 x $10^{-7}$, (4) 2.5 x$10^{-7}$ $molL^{-1}$ , $E_{acc.}$ = - 0.9 V, $t_{acc.}$ = 30 s, a = 50 mV, f = 50 Hz and BR buffer of pH = 9.0**

The quantitative determination of concentrations of atorvastatine in human plasma was carried out by using the proposed adsorptive stripping square-wave voltammetry method. Fig. 12 display, curve (1) was human plasma containing atorvastatine concentration 1.0 x $10^{-7}$ mol.$L^{-1}$, curve (2) 1.5 x $10^{-7}$ mol.$L^{-1}$, curve (3) 2.0 x $10^{-7}$ mol.$L^{-1}$, curve (4) 2.5 x $10^{-7}$ mol.$L^{-1}$. The results showed that, tripping peak current increased linear with atorvastatine concentration in human plasma. The variation of $i_p$ versus atorvastatine concentration C in human plasma was represented by the straight line equation $i_p$ = 29.88 x C.$10^{-8}$ mol.$L^{-1}$ + 13.26 with $R^2$ = 0.996. The reliability of the method for determination of atorvastatine in human plasma was checked using different spiked human plasma samples. The recovery of atorvastatine based on the average of five replicate measurements was found to be 94.70 ± 0.93%

## CONCLUSION

A fully sensitive, selective, fast, validated and low-cost cathodic adsorptive stripping square-wave voltammetry procedure was developed for trace determination of atorvastatine drug in pharmaceutical formulation and spiked human plasma. This method could be applied for the pharmacokinetic as well as quality-control laboratories.

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