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Voltammetric determination of dopamine in the presence of ascorbic and uric acids using partial least squares regression: determination of dopamine in human urine and plasma

Research Article

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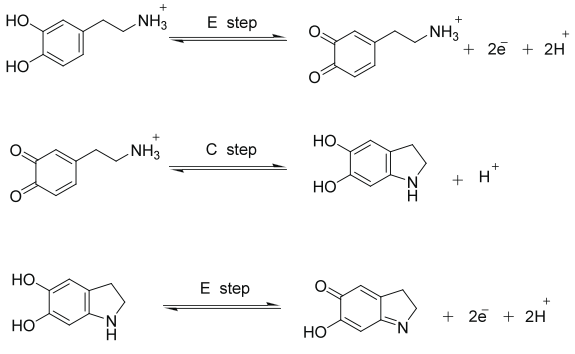
Abstract: A new differential pulse voltammetric method for dopamine determination at a bare glassy carbon electrode has been developed. Dopamine, ascorbic acid (AA) and uric acid (UA) usually coexist in physiological samples. Because AA and UA can be oxidized at potentials close to that of DA it is difficult to determine dopamine electrochemically, although resolution can be achieved using modified electrodes. Additionally, oxidized dopamine mediates AA oxidation and the electrode surface can be easily fouled by the AA oxidation product. In this work a chemometrics strategy, partial least squares (PLS) regression, has been applied to determine dopamine in the presence of AA and UA without electrode modification. The method is based on the electrooxidation of dopamine at a glassy carbon electrode in pH 7 phosphate buffer. The dopamine calibration curve was linear over the range of 1 – 313 μM and the limit of detection was 0.25 μM. The relative standard error (RSE %) was 5.28%. The method has been successfully applied to the measurement of dopamine in human plasma and urine.

Keywords: *Partial least squares • Differential pulse voltammetry • Dopamine*

1. Introduction

3, 4 - Dihydroxyphenyl ethylamine, commonly known as dopamine (DA), is an important neurotransmitter in the mammalian central nervous system [1]. It is an unstable phenolic compound which undergoes oxidation by O_2 in neutral or alkaline solutions to dopaminochrome and other polymeric compounds [2-4]. DA presents as a cation in acidic solution (pK_a *ca*. 8.87) [5]. The electrochemical oxidation of DA in aqueous solution occurs as a two-electron ECE reaction [6]:



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DA plays a significant role in the cardiovascular, renal, hormonal and central nervous systems. DA dendrites extend into various regions of the brain, controlling different functions through the stimulation of α and β adrenergic and dopaminergic receptors (D1 and D2) [7-9]. It is thought to control processes as diverse as movement and drug addiction.

Quantitative determination of DA in human physiological fluids is of considerable significance in both biochemical investigations and clinical diagnoses. Methods include chemiluminescence [10], fluorimetry [11], ultraviolet-visible spectrometry [12], and capillary electrophoresis - luminescence [13]. Because of its electrochemical activity, DA can also be determined by electrochemical methods. These have attracted great interest because they can be fast, low cost, and give low detection limits and high accuracy [14].

DA, ascorbic acid (AA) and uric acid (UA) usually coexist in physiological samples such as blood and urine [15], and the AA concentration (0.2–0.4 mM) is generally 100 to 1000 times that of DA. This makes it difficult to detect DA electrochemically because AA and UA can be oxidized at a potential close to that of DA at bare electrodes. Additionally, oxidized DA mediates AA oxidation, and the electrode surface can be easily fouled by the AA oxidation product [16]. Therefore, it is essential to develop simple and rapid methods for their determination in routine analysis. A major problem in DA determination is the resolution between DA and coexisting species such as (AA) and (UA). The concentration of AA is generally much higher than that of DA (100 to 1000 times) [12]. UA and AA also coexist in biological fluids, such as blood and urine [19]. The significant problem encountered with the detection of DA arising from the low concentration levels of DA and the primary interference from AA (0.2–0.4 mM) and UA, which largely co-exist with DA and have overlapping voltammetric response at bare electrodes. However, in assay of DA, the electrochemical methods suffer from inferior selectivity because of the presence of AA and UA that have higher concentrations than DA in physiological fluids and whose oxidation potentials always are close to that of DA. Therefore it is important to separate the oxidation peak potentials of DA and AA or UA [17]. Electrochemical methods have been widely used for determination of DA in the presence of AA and UA. All require modification of the electrode surface [18-20].

The present work is an effort to develop a simple and accurate electrochemical procedure for the determination of DA using an umodified glassy carbon electrode as a conventional working electrode. A chemometrics method, partial least squares (PLS) regression, was used for modeling and prediction. Since the charging due to background current is a limiting factor in the analytical determination of any electroactive species, all experiments were carried out using differential pulse voltammetry (DPV).

1.1 Theory of PLS regression

PLS regression (PLSR) can allow simultaneous electrochemical determination of several species as well as improve data analysis for complex chemical systems [21-22]. It generalizes and combines features from principal component analysis (PCA) and multiple regression. It is particularly useful when we need to predict a set of dependent variables from a (very) large set of independent variables (*i.e*. predictors) [23]. PLSR is a particular type of multivariate analysis which uses the two-block predictive PLS model to model the relationship between two matrices, X and Y. Because PLSR models the structure of X and of Y it gives richer results than the traditional multiple regression approach [24-26]. PLSR is of particular interest because it can analyze data with strongly collinear (correlated), noisy, and numerous X-variables, and also simultaneously model several Y-response variables [23].

In electrochemical methods such as DPV, the measured current at a given potential is a function of analyte concentration. By recording DPVs of several samples a two-dimensional data matrix (matrix: number of samples × number of recorded potentials) can be obtained.

The first step in principal component regression (PCR), is a PCA that decomposes the data matrix into two small matrices, Score and Loading (eigenvectors). The second step of PCR is a regression of the score matrix against the analyte(s) concentration(s). In comparison, PLS actually uses the concentration information during the decomposition process and the decomposition and regression are done simultaneously. The main idea of PLS is to get as much concentration information as possible into the first few loading values.

The main tool in both PCR and PLS is regression of the concentration matrix (Y) against the data matrix (X). PCR approximates X by a few (R) *principal components* and regresses Y on these R components. PCR can thus be written as:

$$X = T_x P' + E_x$$
$$Y = Ty\ B + E_y$$

where T_x and P' are the Score and Loading matrices of the response matrix, X, and T_y and B are the Score and Loading matrices of the concentration matrix, Y, respectively.

PLS regression finds components that compromise between fittings of X and predicting Y. The general idea of PLS regression is to approximate X by a few (R) *specifically constructed components* and to regress Y on the R components. Hence, PLS regression tries to model X and Y using the common components T:

$$X = T P' + E_x$$
$$Y = T Q' + E_y$$

Where T is a matrix of Score, P is a matrix of X-loading, Q is a matrix of Y-loading, E_x and E_y are residual matrices [27].

PLS calibration of a multi-component system can be performed in two different ways, PLS-1 and PLS-2. The use of PLS-2 has a few advantages. First, there is one common set of PLS factors for all analytes, simplifying the procedure and interpretation and enabling simultaneous graphical inspection. Second, when the analyte concentrations are strongly correlated the PLS-2 model is more robust than separate PLS-1 models. Finally, when the number of analytes is large, the development of a single PLS-2 model is done more quickly than development of many separate PLS-1 models. Practical experience, however, indicates that PLS-1 calibration usually performs equally well or better in terms of predictive accuracy. Thus, when the best possible prediction is required a separate PLS-1 regression for each analyte is advised [27].

In data analysis, there is a strong tendency to delete variables that do not fit the current model. This is risky and should usually be avoided. In particular, PLS modeling is little affected by noise variables in the model, as long as a small set of variables supports the model [28]. PLSR is a very versatile data analysis approach which can be even more useful with extensions / modifications required by special types of data.

2. Experimental Procedures

2.1. Materials and reagents

AA, DA and UA were obtained from E. Merck (Darmstadt, Germany). Stock solutions of these components (10^{-3} M) were freshly prepared. Phosphate buffer (1 M, pH=7) was prepared by dissolving suitable amounts of K_2HPO_4 and KH_2PO_4. Human plasma and urine samples were obtained from a nearby hospital. All reagents were of analytical grade and solutions were prepared using doubly distilled water.

2.2. Apparatus

An Autolab PGSTAT30 potentiostat-galvanostat equipped with a Metrohm Model 663 VA Stand was used to record the voltammograms. Three-electrode systems with a glassy carbon wire counter electrode, an Ag | AgCl | 3 M KCl reference electrode and a 2 mm glassy carbon (GC) working electrode were purchased from Metrohm. The working electrode was polished with alumina powder (0.05 μm) for 1 min and washed with water before use. The pH was measured with a Metrohm digital pH meter using a combination glass electrode.

2.3. Procedure

Suitable amounts of 10^{-3} M stock analyte (AA, UA and DA) were transferred into an electrochemical cell. 1 mL buffer solution (phosphate, pH = 7) was added and the mixture was diluted to 10 mL using doubly distilled water. The solution was mixed thoroughly and the potential was scanned from –70 to 298 mV *vs.* the Ag | AgCl reference electrode. DPV with pulse amplitude of 50 mV and increment steps of 5 mV was used. The current at 75 potential values was measured for each sample and used for PLS calibration. The peak current of each analyte was used to construct individual calibrations.

2.4. Analysis of biological samples

Plasma samples were deproteinized with 2 M phosphoric acid [33] and centrifuged for 5 minutes before voltammetric measurements.

3. Results and Discussion

3.1. The effect of pH on oxidation of AA, UA and DA

On increasing the pH the oxidations of AA, UA and DA shifted toward negative potentials. As can be seen in Fig. 1, the peak current for AA increases up to pH 4.0 and then decreases. Maximum peak currents for DA and UA appear at pH 7.0 and 9.0, respectively. Since our objective was DA, pH 7 was selected. The effect of buffer concentration on AA, UA and DA peak currents was also studied; 0.1 M phosphate was the optimum.

3.2. Effect of ionic strength

At pH 7 and in the absence of phosphate buffer, the effect of ionic strength was studied by changing the concentration of KCl and NaCl over the range 0.005–0.5 M. The analyte peak currents increased with increasing KCl or NaCl concentration up to 0.1 M and remain nearly constant at higher concentrations. There was no significant difference between KCl and NaCl. The peak

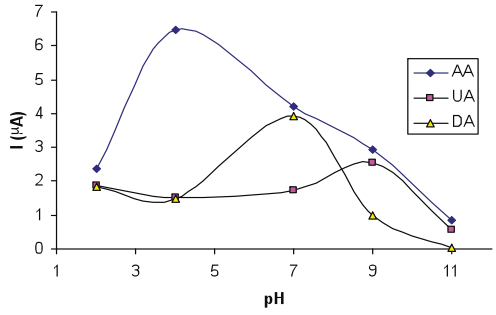

Figure 1. Effect of pH on peak currents of AA, UA and DA.

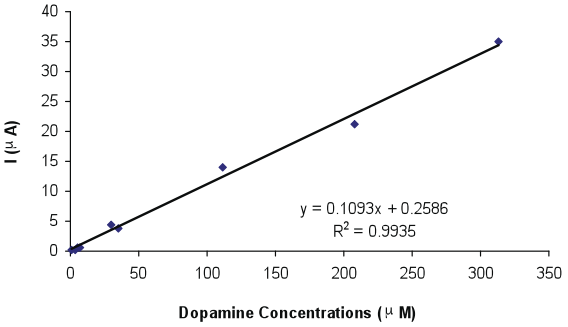

Figure 3. Dopamine individual calibration.

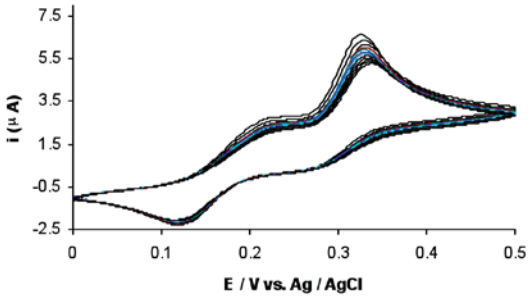

Figure 2. Cyclic voltammograms of a mixture of DA (30 μM), AA (200 μM) and UA (45 μM) in 0.1 M pH 7 phosphate buffer, (10 cycles). Scan rate = 100 mV s^{-1}, step potential = 2.5 mV.

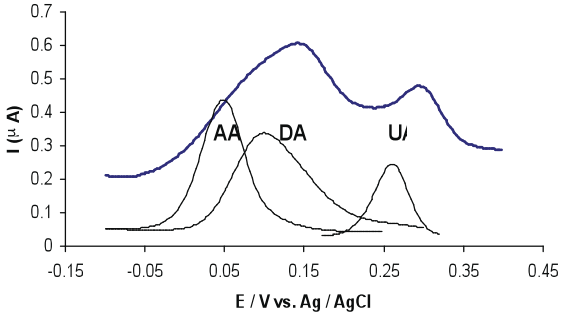

Figure 4. Differential pulse voltammograms of DA (3.5 μM), AA (60 μM) and UA (2 μM) and mixture (7.2 μM DA, 66.5 μM AA and 4 μM UA) at a bare glassy carbon electrode in 0.1 M pH 7 phosphate buffer, step potential = 2.5 mV.

currents in these solutions were equal to those in 0.1 M phosphate buffer. Therefore the ionic strength was fixed using the 0.1 M phosphate buffer.

3.3. Voltamogram reproducibility

The GC electrode is fouled by adsorption of oxidized AA [29-31]. Rueda *et al*. [32] studied the oxidation of AA on a gold electrode over a wide pH range and proposed that AA is oxidized to dehydroascorbic acid (DHAA) *via* the radical anion intermediate monodehydroascorbic acid. The DHAA carbonyl adds water forming hydrated dehydroascorbic acid, DHAA•H_2O, which is electro-inactive [32]. Therefore, it is reasonable that adsorption of oxidized AA on a GC electrode may disturb the oxidation of UA and/or DA.

The cyclic voltammograms obtained under these optimized conditions (Fig. 2) show that acceptable results for 30 μM DA in the presence of 200 μM AA and 45 μM UA could be obtained for 10 repeated cycles using a bare electrode without polishing. As can be seen, no significant changes in peak currents were obtained (RSD < 5%).

Because differential pulse voltammetry was used the electrode was polished before each scan to prevent fouling.

3.4. Individual calibration

Individual calibration graphs were constructed as peak current *vs.* concentration. The curve for DA was linear over the range of 3 – 313 μM. The characteristics of the individual DA calibration are shown in Table 1 and Fig. 3. The linear ranges for individual determinations of AA and UA were 3 – 150 and 5 - 250 μM, respectively.

Table 1. Characteristics of the dopamine individual calibration

Analyte	Regression Equation[a]	R^2	LOD (μM)[b]	LOQ (μM)[c]	Linear Range (μM)
Dopamine	Y = 0.1093C + 0.2586	0.9935	1.1	3	3 – 313

[a] *Y is the peak current (μA) vs. C, dopamine concentration (μM). The standard deviations were 0.0033 and 0.433 for the slope and intercept, respectively.*
[b] *Limit of detection*
[c] *Limit of quantification*

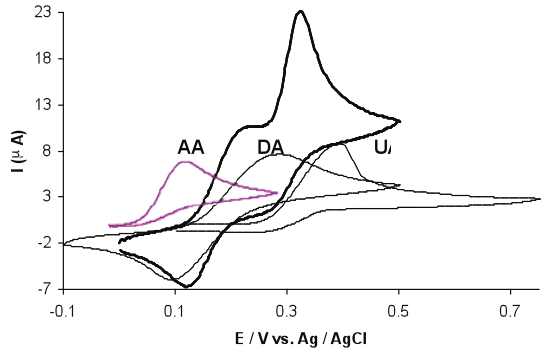

Figure 5. Cyclic voltammograms of DA (84 μM), AA (850 μM) and UA (65.5 μM) and mixture (120 μM DA, 1055 μM AA and 180 μM UA) at a bare glassy carbon electrode in 0.1 M pH 7 phosphate buffer, step potential = 2.5 mV, Scan rate = 100 mV s^{-1}.

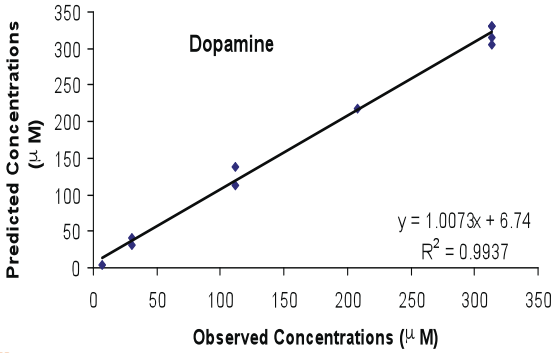

Figure 7. Predicted vs. known concentration of DA using PLS-1 model with 5 components.

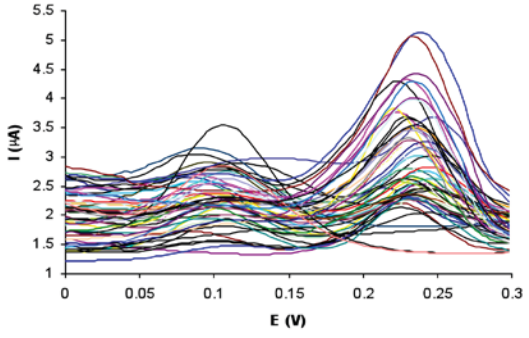

Figure 6. Differential pulse voltmmogrms of mixtures of AA, UA and DA used as calibration set for PLS modeling, step potential = 2.5 mV.

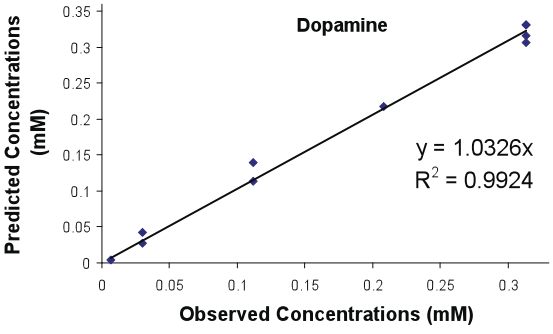

Figure 8. Predicted *vs.* known concentration of DA using PLS-1 model with 5 components (intercept set equal to zero).

3.5. PLS Calibration

Typical differential pulse and cyclic voltammograms of AA, DA and UA are in Figs. 4 and 5. As can be seen, the voltammograms overlap. In such cases, voltammetry using modified electrodes has been used for their simultaneous determination in real samples [33-34]. In this work, instead of a time consuming modification step, PLSR was applied to the determination of DA in the presence of AA and UA.

The first step is construction of the calibration set for the ternary mixture AA-UA-DA. The components in the calibration mixtures must span all dimensions. Correlation between the calibration samples must be avoided because collinear components in the training set cause under-fitting in the PLS models.

The potential region between -66.9 and 298 mV, with 75 experimental points per *i* - E curve was selected. A training set of 30 standard samples was used (Table 2). Fig. 6 shows their differential pulse voltammograms. The model was validated with a test set of 10 synthetic mixtures containing different proportions of AA, UA and DA. All standard and test solution concentrations were randomly chosen within the linear range for each analyte. Table 3 presents the synthetic ternary mixtures, and their predicted concentrations and recoveries (%) for DA using the PLS-1 model constructed. Fig. 7 shows the predicted *vs.* known DA concentrations. The slope and R^2 are close to 1 and the intercept is negligible, demonstrating the validity of the model's predictions. Fig. 8 shows the predicted vs. known DA concentrations given by this model when its intercept was set equal to zero. The slope and R^2 remain close to 1; the conclusion is unaltered.

The applicability of a calibration model can be evaluated in various ways. The prediction error of a single component in the mixtures was calculated as the relative standard error (RSE) of the predicted concentration [35-36]:

Table 2. Calibration solution composition.

Calibration Solution	AA (μM)	UA (μM)	DA (μM)
1	100	20	5
2	133	226	111
3	50	16	313
4	50	226	313
5	0	6.6	0
6	133	10	5
7	133	113	313
8	3.3	20	3.3
9	100	226	6.6
10	66	23	3.3
11	66	6.6	208
12	100	6.6	3.3
13	3.3	20	111
14	66	6.6	0
15	66	20	1
16	66	113	0
17	66	113	111
18	0	23	313
19	3.3	113	208
20	133	23	3.3
21	3.3	16.6	3.3
22	0	0	3.3
23	3.3	16.6	0
24	133	26	3.3
25	100	0	35
26	116	113	111
27	3.3	6.6	30
28	116	23	111
29	83	6.6	5
30	0	6.6	1

$$R.S.E.(\%) = \left(\frac{\Sigma_{j=1}^{N} (\hat{C}_j - C_j)^2}{\Sigma_{J=1}^{N} (C_j)^2} \right)^{1/2} \times 100 \qquad (1)$$

where N is the number of samples, C_j the concentration of the component in the j^{th} mixture and $\hat{C}_j$ the estimated concentration.

To select the number of factors in the PLS algorithm a cross-validation method leaving out one sample at a time was employed. For the set of 30 calibration curves, PLS-1 calibration on a 29-member calibration set was performed and using this calibration the concentration of the sample omitted was calculated. This process was repeated 30 times; each sample was omitted once. The predicted concentration of each sample was then compared with its known value and the prediction residual error sum of squares (PRESS, Eq. 2) was calculated. Fig. 9 shows the plot of the PRESS versus the number of factors for each individual component. The F test showed the optimal number of factors was 5.

$$PRESS = \sum_{i=1}^{N} (C_i^{added} - C_i^{found})^2 \qquad (2)$$

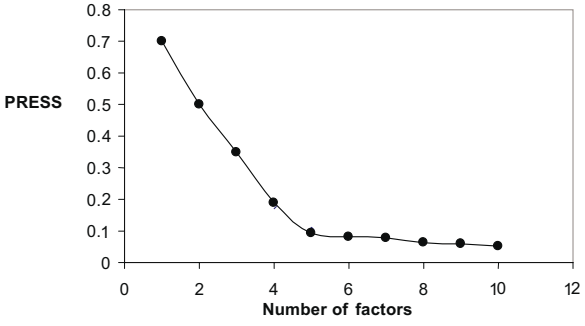

Figure 9. Plot of PRESS against the number of factors for dopamine (calculated according to Eq. 2).

3.6. Application of the method

The PLS method was successfully applied to the measurement of DA in the presence of AA and UA in human plasma and urine. Results (average of three replicate determinations) are presented in Table 4. The recoveries are close to 100% and indicate that the method was successful.

Table 3. Composition of synthetic ternary mixtures of DA, UA and AA, the prediction of DA, its recovery obtained by the PLS-1 model and the statistical parameters for the system.

	Synthetic samples (μ M)			Prediction Samples (μ M)	Recovery (%)
	DA	AA	UA	DA	DA
1	111.6	133	20	111.3	99.73
2	111.6	66	23	139	124.55
3	30	83	10	33	110
4	313.3	133	13	331.5	105.8
5	313.3	133	23	315.7	100.76
6	30	133	26	28	93.33
7	6.6	66	26	4.7	71.21
8	313.3	83	23	306.1	97.7
9	313.3	116	26	330.6	105.52
10	208.3	133	100	217.5	104.41
Mean Recovery	-	-	-	-	101.301
RSE[a] (%)	-	-	-	-	5.28

[a] *calculated using Eq. 1*

Table 4. Results of analysis of DA in human plasma and urine samples.

Sample	Spiked (μM)			Found ± std, (n=3)	Recovery (%)
	DA	AA	UA	DA	DA
Plasma sample (a)	3.59	100	20	3.72 ± 0.176	103.62
Plasma sample (b)	30	133	26	28 ± 1.2	93.33
Urine sample (a)	9.79	66	10	9.53 ± 0.257	97.34
Urine sample (b)	30	133	26	32.2 ± 1.8	107.33

Table 5. Comparison of the linear range and detection limit with previous work.

Method	Linear Range	Electrode	Detection Limit	References
differential pulse voltammetry	0.8 - 8 μM	Oracet blue modified glassy carbon electrode	0.02 μM	[1]
differential pulse voltammetry	2 - 1500 μM	Carbon - ionic liquid electrode	1 μM	[8]
differential pulse voltammetry	40 nM to 3 μM	poly(acrylic acid)-multiwalled carbon-nanotube composite-covered glassy-carbon electrode	20 nM	[17]
RRDE voltammetry	80 - 2080 μM	ruthenium oxide modified electrode	Not reported	[19]
differential pulse voltammetry	5 - 25 μM	poly (p-nitrobenzenazo resorcinol) modified glassy carbon electrode	0.3 μM	[38]
differential pulse voltammetry	0.5 - 160 μM	palladium nanoparticle-loaded carbon nanofibers modified electrode	0.2 μM	[39]
linear sweep voltammetry	19.5 - 2285 μM	bare glassy carbon electrode	Not reported	[37]
differential pulse voltammetry	0.1 - 200 μM	poly(eriochrome black T) modified glassy carbon electrode	20 nM	[40]
differential pulse voltammetry	1 - 313 μM	bare glassy carbon	0.25 μM	This work

4. Conclusion

This method for measurement of DA in the presence of AA and UA using PLS regression is new, reliable, simple, cheap and precise. The time and reagent consuming electrode modification has been avoided. The method successfully determined DA in human serum and urine over a wide range of concentrations, showing its applicability to real samples. The reproducibly of the method is good; RSE % for determination of DA in the presence of AA and UA was 5.28%.

The method was compared with several recent attempts to determine DA in the presence of AA and/or DA (Table 5). In most of this work the overlapping DA, AA and UA peak currents have been separated using a modified electrode, while this method solves the problem by using a conventional glassy carbon electrode and applying PLSR. The method shows a higher sensitivity than some methods [8,23,27] or moderately less than others [1,19,37,40]. The limit of detection (LOD) of the method was 0.4 μM dopamine, comparable with the reported methods.

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