A simple and cost-effective method, as an appropriate alternative for visible spectrophotometry: development of a dopamine biosensor

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In this study, a new, simple, fast and inexpensive method as an alternative to visible spectrophotometry is developed. In this method the cells containing the sample solution were scanned with a scanner, then the color of each cell was analyzed with software written in visual basic (VB 6) media to red, green and blue values. The cells were built by creating holes in the Plexiglas® sheet. The dimensions of identical cells were examined by Cr (III) solution with known concentrations. The validity of this new method was studied by determination of dopamine (DA) without using any other reagent. The parameters which affect the system were optimized. The comparison between the current and traditional UV-Vis spectrophotometry methods was studied and the results revealed similar trends in both methods. The developed method was successfully applied to the determination of dopamine in serum and urine without using any pretreatment. Finally comparing the results obtained in the developed method showed that microwave irradiation of the solution can decrease the experimental time, increase sensitivity and improve the limit of detection.

1. Introduction

In the past decade many methods in analytical chemistry based on spectrophotometry have been presented. Spectrophotometric techniques are based on the measurement of transmitted light by an analyte. In the visible spectrophotometric methods the sample must not be turbid and the species should often have a sharp λ_{max} to obtain the precise determination in the visible region. The linearity of the Beer–Lambert law is limited by chemical and instrumental factors. One of the instrumental deviations occurs when the incident radiation is non-monochromatic, which can be minimized by using λ_{max}.

For the first time paptode was developed in 2004 in our research group for speciation of iron (II) and iron (III)[1] and then it was used for determination of ascorbic acid.[2] Nóbrega *et al.*[3] used a flatbed scanner instead of CCD (charge-coupled device) for determination of ascorbic acid. They use a plastic sheet which contains holes with a cylindrical volume of approximately 8 mL. As the extension of paptode, in this proposed method instead of using of a TLC (Thin Layer Chromatography) strip as the reaction substrate, the reaction was done in the solution. The transparency of the solution in this method is not important, because the light reflection is measured, and the light radiation does not pass across the solution. Also, the sharp λ_{max} of the species are not important, because in this method the intensity of the color of the solution was analyzed by software to three main color (red, green and blue) values.

It is known that dopamine is a neurotransmitter that is released from the synaptic cleft between two neurons and is found in many discrete regions throughout the central nervous system. When dopamine presents in low concentrations is likely to give rise to neurodegenerative diseases such as Parkinson's and Alzheimer's,[4] among others.

In the past some methods for the determination of dopamine in authentic and dosage forms were developed. Nevado[5] *et al.* proposed a flow injection method for determination of dopamine based on the hydrolysis of this compound in alkaline medium. The absorbance of the product of dopamine hydrolysis was measured at λ 390 nm. Also a stopped flow method for the determination of dopamine based on aerial oxidation of this catecholamine in strong alkali medium was proposed by Berzas *et al.*[6] In this method the absorbance of the oxidation product was measured at λ 360 nm. Filho *et al.*[7] developed a spectrophotometric method based on using the crude extract of sweet potato root (*Ipomoea batatas* (L.) Lam.) as an enzymatic source of polyphenol oxidase. The oxidation of dopamine to dopaminequinone is catalyzed by this enzyme. This compound is converted by a rapid spontaneous auto oxidation to dopaminechrome, has a strong absorption at 470 nm. Barnum[8] reported a method based on nitration of dopamine with sodium nitrite in slightly acidic solution. In this method, tungstate or molybdate ions increase the color intensity, probably by stabilizing the nitrated phenol by complex formation. After nitration, the solution was made basic; and absorption was measured at about 500 nm. Also Based on the formation of a nitroso derivative followed by the formation of an azo red compound with sulfamic acid in the presence of alkali a method for determination of dopamine was developed by Murthy and Sundaram[9] and Nagaraja *et al.*[10] proposed two spectrophotometric methods for the determination of catecholamine derivatives (pyrocatechol, dopamine, levodopa and methyldopa). The first method involves the oxidation of *o*-dihydroxybenzene derivatives by *N*-bromosuccinimide followed by oxidative coupling with isoniazid leading to the formation of red-coloured products with

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a maximum absorbance around 480–490 nm. The second method is based on the formation of green to blue complexes (λ_{max} 635–660 nm) between *o*-dihydroxybenzene derivatives and sodium nitroprusside in the presence of hydroxylamine hydrochloride. Li *et al.*[11] established a method for determination of dopamine using a condensation reaction of sodium 1,2 naphthoquinone-4-sulfonate and tetradecyl benzyl dimethyl ammonium chloride with dopamine. The product of this reaction was a pink compound. A method for estimation of catechol and its derivatives like dopamine based on the interaction of diazotised sulfanilamide with catechol derivatives in the presence of molybdate ions in acidic medium was developed by Nagaraja *et al.*[12] Absorbance of the resulting red coloured product was measured at 490 nm for pyrocatechol and at 500 nm for other catechol derivatives. Wang *et al.*[13] described a fluorimetric method for determination of dopamine. The measurement of relative fluorescence intensity was carried out at 315 nm with excitation at 279 nm. In all of these methods not only a sharp λ_{max} and an expensive instrument are needed, but many reagents are also involved.

In the current method the cells containing the sample solution were scanned with the scanner, then the color of each cell was analyzed with software to red, green and blue values. To examine the validity of the proposed method, dopamine was determined by this method only in alkaline media. The results showed the acceptable precision and accuracy in determination of dopamine. Although, this method basically saves time and money and shows good sensitivity, the solution is also irradiated with microwaves to improve the detection limit and sensitivity.

2. Experimental

2.1 Apparatus

The cells were built by using a sheet of Plexiglas®. A CanoScan 4200F (Canon) scanner with a cold cathode fluorescent lamp (CCFL) and CCD (charge coupled device) as a light source and detection system respectively were used for scanning the Plexiglas® sheet. The CCFL is a three wavelength source (for red, green and blue regions). It should be mentioned that the scanners usually work based on reflection for non-transparent materials, however, in this study since a non-turbid solution was used it is expected that a combination of both reflection and absorption will occur. The resolution of the scanner was regulated at 150 ppi. We wrote software in VB 6 media to convert the recorded pictures of color of cells to RGB (red, green and blue) data. An eppendorf micropipette was used for injecting samples into the cells. The pH values of buffer solutions were measured by a metrohm (780) pH meter. The domestic microwave (Moulinex) was used for radiation of the sample by microwaves. The absorbance spectrum of the dopamine decomposition product was measured by the spectrophotometer Uv/Vis (Philips PU 8720).

2.2 Materials

All of the chemicals used were of analytical reagent grade. Deionized water was used to prepare the buffer, reagent and stock solutions. A 1.0×10^{-2} M stock solution of dopamine was prepared by dissolving the required amount of dopamine hydrochloride (Sigma) in Deionized water. This solution was kept in a dark, cold place and was freshly prepared every day. A 0.1 M phosphoric acid was prepared by diluting concentrated H_3PO_4. 0.1 M NaOH was prepared by dissolving an appropriate amount of NaOH in the deionized water, and phosphate buffer solutions (PBS) with required pH were made by mixing these solutions in appropriate fractions. The 0.1 M Cr (III) solution was prepared by dissolving the appropriate amount of $Cr(NO_3)_3.9H_2O$ (Riedel Dehaen) in deionized water.

2.3 Principles of the red, green and blue (RGB) color system

The RGB color model is an additive color model in which red, green, and blue light are added together in various ways to reproduce a broad array of colors. In computing, the color values are often stored as integer numbers in the range 0 to 255, the range that a single 8-bit byte can offer (by encoding 256 distinct values). In the RGB system, any color is represented in the form of (R, G, B), in which the (0, 0, 0) and (255, 255, 255) refer to black and white respectively. Therefore by increasing the intensity of colors, the color values are decreased. In this system 16777216 colors can be made. Any color can be described by the following formula:

$$V = R + 256\,G + 256^2\,B$$

Where, R, G and B are red, green and blue values of the main color. For black and white, V is equal to 0 and 16777216, respectively. By using the following flowchart, R, G and B values of V for any color can be extracted:

$$R = V \text{ Mod } 256$$

$$G = \left((V - R) \text{ Mod} \left(256^2\right)\right)/256$$

$$B = (V - R - G*256)/(256^2)$$

"Mod" is a numeric function which returns the remainder when dividing two numbers.

2.4 Preparation of cells array

In this method we used the cylindrical cells (Fig. 1). These cells were built by creating holes (id 1.5 cm) in the sheet of Plexiglas® (thickness 0.6 cm) using a laser. In order to close the bottom of the holes and make the cells, this holed sheet was stuck to another sheet of Plexiglas® (thickness of 0.1 cm).

In our design the cells were aligned to 3 columns and 7 rows, giving a total of 21 cells in the Plexiglas® sheet.

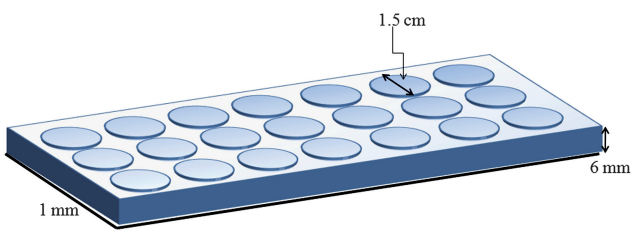
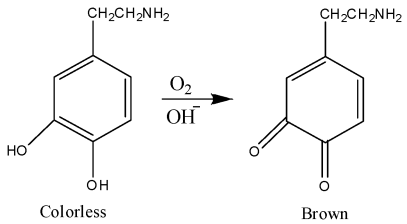


Fig. 1 Cell array on the Plexiglas® sheet.

2.5 Procedure

Dopamine is oxidized by oxygen in alkaline media to form an oxidation product (Benzoquinone) according to following reaction.[13]



Based on this reaction, we could determine the concentration of dopamine by measuring the intensity of the color of the reaction product (benzoquinone).

The appropriate volume of sample was injected to the cell and the pH of this solution was adjusted to be alkaline. The dopamine in the samples can be easily degraded with dissolved O_2 in the alkaline medium. Therefore there is no need to supply any O_2 into the solution. After an appropriate period of time, the cells were shaken to obtain the homogenous color and the Plexiglas® sheet was scanned with the scanner. The image was transferred to the computer and any color changes in each cell were analyzed using a program written in VB 6 media (Fig. 2). In this program, color of each cell is analyzed based on the RGB system into R, G and B values. It should be noted in the color analysing programs, we select a specific area for analysis and the number of pixels that could be indicated by this area was about 10000–300000, and this program can average these pixels. Therefore the signal to noise ratio can be dramatically increased.

The effective intensity for any color values was calculated as follows:

$$A_r = -\log(R_s / R_b)$$
$$A_g = -\log(G_s / G_b)$$
$$A_b = -\log(B_s / B_b)$$

Where A_r, A_g and A_b are the effective intensity for red, green and blue, respectively. R_s, G_s, B_s and R_b, G_b and B_b are the red, green and blue color values of a sample and a blank, respectively. It should be noted, that the word "intensity" here differs from that usually used in spectrophotometry. Actually, the intensity here is the amount of any color values in the 8 bit RGB color system in the computer not the intensity of reflection light. To obtain a calibration curve, the effective intensity of any color values were plotted *vs.* concentration.

Fig. 2 color analysing program.

3. Results and discussion

3.1 Study of similarity among the cell dimensions

The similarity of the cell dimensions was studied by using a solution of 0.1 M Cr (III). 600 μL of Cr (III) solution was injected into each cell. After scanning the sheet, the color of each cell was analyzed to R, G and B values. The results in Table 1 show the acceptable RSD (relative standard deviation) for cells and for all color values.

3.2 Effect of the injection volume and resolution of scanner

The effect of sample volume injected into the cell was studied. For this purpose, different volumes of 0.1 M Cr (III) solution were injected into each cell. As Fig. 3 (a, b) shows by increasing the volume of injected sample from 300 to 1000 μL (the calculated volume of each cell is 1060 μL), the color values decreased (in other word the color intensity increased) (Fig. 3a) and RSD% ($n = 21$) up to 600 μL increased and beyond 600 μL it levelled off (Fig. 3b). For the facility of shaking the Plexiglas® sheet to obtain the homogeneous color and less sample consuming, 600 μL was chosen as the optimum injected volume in all experiments.

The main parameter that controls the speed of analysis is resolution of the scanner. Therefore it is expected that at high resolution the speed of scanning will decrease or *visa versa*. Practically, we observed that by changing the resolution of the

Table 1 The color values for all cells using a 0.1 M Cr(III) solution (injection volume 600 μL)

	Color value		
Cell	R	G	B
1	133	113	133
2	131	113	132
3	133	114	133
4	133	115	134
5	130	111	131
6	131	111	132
7	132	113	133
8	131	112	133
9	132	111	132
10	133	112	131
11	130	113	132
12	133	112	133
13	132	111	132
14	131	112	131
15	130	114	133
16	133	113	132
17	132	112	131
18	131	113	133
19	130	112	131
20	132	113	132
21	133	112	133
Average	132	112	132
RSD%	0.89	0.96	0.64

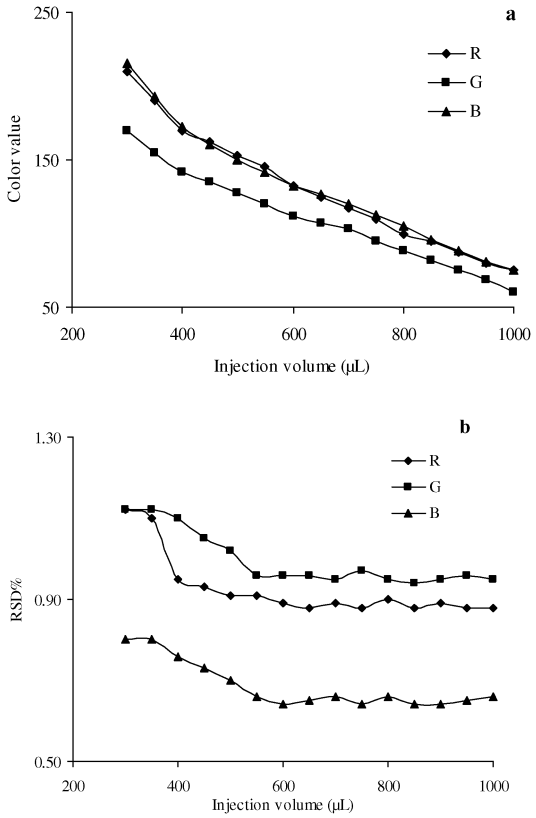


Fig. 3 Effect of injection volume on the color values (a) and RSD% (n = 21) (b) of 0.1 M Cr (III) solution (resolution was 150 ppi).

scanner no significant change will be observed in color values and precision. Results showed that the resolution of 150 ppi was enough to obtain a good speed of scanning and quality of image (Table 2).

3.3 Effect of pH on the dopamine degradation

In order to study the effect of pH on the validity of the developed method at first the oxidation of dopamine was investigated. For this purpose, the solutions containing 5 mM dopamine at the different pHs (0.1 M PBS) were prepared. After 1 h, the color of the samples indicate the completion of the dopamine degradation reaction. Then 600 μL of each sample was transferred into the cells. After scanning the cells, the image of each cell was analyzed with the software. As a result Fig. 4 shows, by increasing the pH, the effective intensity is increased, which means that the color is deeper. Therefore the pH 13 was selected as optimum pH. Fig. 5 represents the effect of pH on the dopamine degradation obtained by spectrophotometer. As is obvious from these figures (Fig. 4 and 5) a similar trend was observed in both methods, which indicates that for this system the scanner can behave similarly to the spectrophotometer. For the next experiments, adjusting the pH was performed only by NaOH or KOH to 13.

Table 2 The color values and RSD% (n = 21) of 0.1 M Cr(III) solution scanned with different resolutions (injection volume 600 μL)

	Color values (average of 21 inject)			RSD% (n = 21)		
Resolution (ppi)	R	G	B	R	G	B
100	132	112	132	0.88	0.99	0.64
150	133	113	131	0.88	0.97	0.63
200	132	112	132	0.89	0.96	0.64
250	131	111	132	0.89	0.96	0.65
300	132	112	132	0.88	0.97	0.64
350	132	112	133	0.89	0.96	0.65
400	131	113	132	0.90	0.95	0.64
450	132	112	133	0.88	0.96	0.65
500	131	113	133	0.89	0.96	0.64
550	132	112	133	0.89	0.96	0.64
600	131	113	133	0.88	0.96	0.65
650	131	112	133	0.89	0.96	0.65
700	131	113	133	0.89	0.96	0.65
1000	131	112	133	0.89	0.96	0.65
1200	131	113	133	0.89	0.96	0.65

3.4 Effect of time

The degradation of dopamine in alkaline medium also depended on time. In order to study the effect of time on the degradation of dopamine, a 300 μL solution containing 2 mM dopamine was injected into the cell. Then a 300 μL solution of NaOH 0.2 M was injected into cell in order to adjust the pH to 13. The cell was scanned in different times. As Fig. 6 shows, after 40 min the color values levelled off.

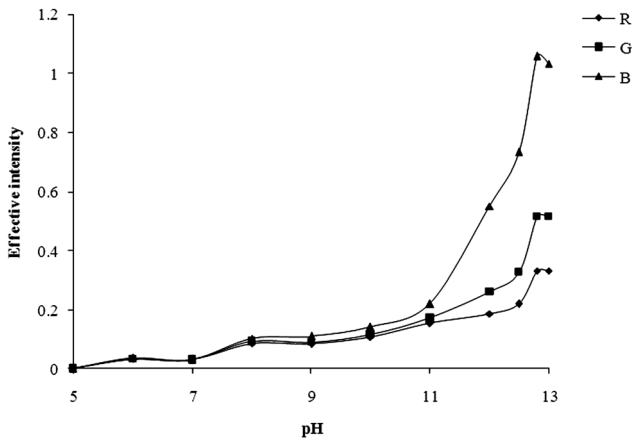


Fig. 4 Effect of pH on the oxidation of 5 mM dopamine with injection volume of 600 μL.

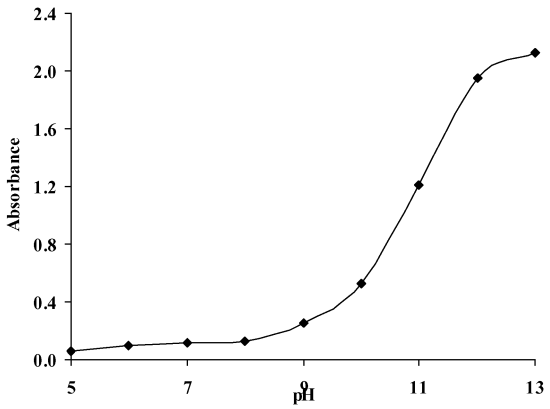


Fig. 5 Effect of pH on the oxidation of 5 mM dopamine at 390 nm.

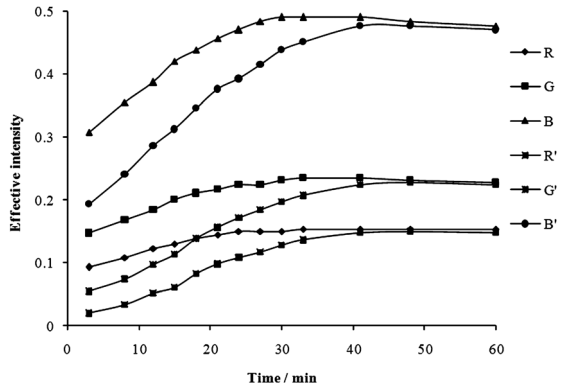


Fig. 6 Effect of time on the development of color in 1 mM dopamine solution at pH 13, R, G and B are the color values in the procedure with microwave treatment (10 s at 150 W), R′, G′ and B′ are the color values in the procedure without microwave treatment.

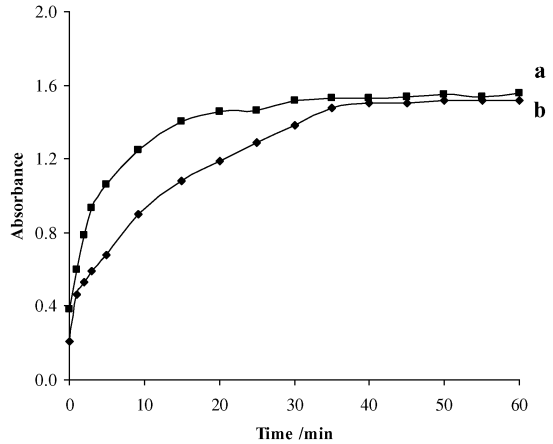


Fig. 7 Effect of time on the developing of color (spectrophotometry) in 1 mM dopamine solution at pH 13 and λ 390 nm, (a) the procedure with microwave treatment (10 s at 150 W) and (b) procedure without microwave treatment.

For decreasing this time, the solutions were warmed in a microwave at power of 150 W for 10 s. As Fig. 6 shows, following 10 s irradiation, the levelling off occurred after 20 min and the color intensity by microwave irradiation was higher for all colors. In order to compare kinetics as observed with the scanner to that observed with a spectrophotometer the absorbance as a function of time was obtained at a 390 nm wavelength with a UV-Vis spectrophotometer which is shown in Fig. 7. Comparing both Fig. 6 and 7 illustrate that both show the same trend. This means that the developed method and spectrophotometer show similar behaviour.

3.5 Spectrophotometric study of dopamine decomposition product

The absorbance of the dopamine decomposition product (0.1 mM) (benzoquinone) at pH 13 after a period of 60 min in the region of 350 to 800 nm was measured (against deionized water as a blank). As Fig. 8 shows, there is no sharp absorption peak or λ_{max} in this region, therefore the precise and accurate determination of dopamine with visible spectrophotometry is difficult at this condition and needs precisely controlled conditions. The λ_{max} of benzoquinone is near the 300 nm in the UV region.[13] Albeit some methods by using spectrophotometry based on decomposition of dopamine in alkaline media was successfully developed[5,6] by using flow injection analysis (FIA) with a good precision. In the current method we are not concerned about the λ_{max} since in this method we are dealing only with color intensity.

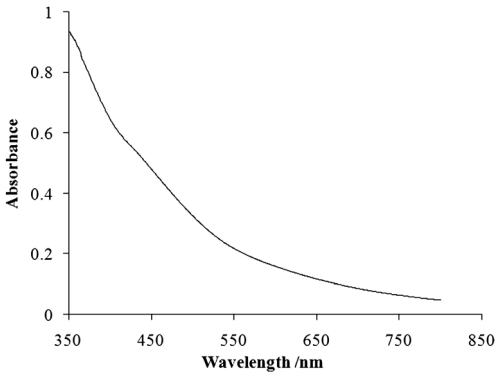


Fig. 8 Absorbance of the 0.1 mM dopamine decomposition product (benzoquinone) at pH 13.

3.6 Study of interference

To study the selectivity of the developed method for the determination of dopamine, the effect of the presence of several species that may coexist with dopamine in real samples (serum and urine) were investigated. Solutions containing 5.0×10^{-4} M dopamine and 100 folds higher concentration (5.0×10^{-2} M) of interfering species were prepared and injected into cells under the optimum conditions. Table 3 shows the errors in the R, G and B values for each interfering species. As illustrated in Table 3, no significant interference exists in all values for urea, glucose and lactose, whereas ascorbic acid interferes almost greater than five folds. The relative error for the B value was also greater than the other two color values. Therefore, it can be concluded, that the R and G value are more suitable for determination of dopamine relative to B value. Also the t test (n = 5, data are not shown)

Table 3 Interference study

Interference species	Concentration relative to dopamine (Folds)	Relative error %					
		Without microwave treatment			With microwave treatment		
		R	G	B	R	G	B
Urea	100	−2.8	−2.0	−7.4	−2.5	−2.4	−7.3
Glucose	100	+2.7	+2.0	−7.4	+2.9	+2.3	−7.5
Lactose	100	+2.8	+2.0	−5.9	+3.0	+1.9	−5.5
Ascorbic acid	5	+4.5	+4.3	−7.0	+5.0	+4.7	−7.5

showed that all results have no significant difference (probability of 99.9%), except in red and blue values for ascorbic acid, between with and without microwave treatment.

3.7 Calibration curve

Under optimum conditions for all color values the calibration curves were plotted. Fig. 9A and 9B show the calibration curves of the proposed method with and without microwave treatment, respectively. In the case of microwave treatment (10 sec in 150 W), the sensitivity of the method was improved by almost two fold relative to without microwave treatment. The linear range for both cases was 0.03–1 mM. In order to compare the behaviour of the development method with the spectrophotometer, the same procedures (with and without microwave treatment) were performed. Results are shown in Fig. 9C and are given in Table 4. As these results illustrate both methods reveal similar behaviour except that in the case of using the spectrophotometer the detection limit and RSD% are better.

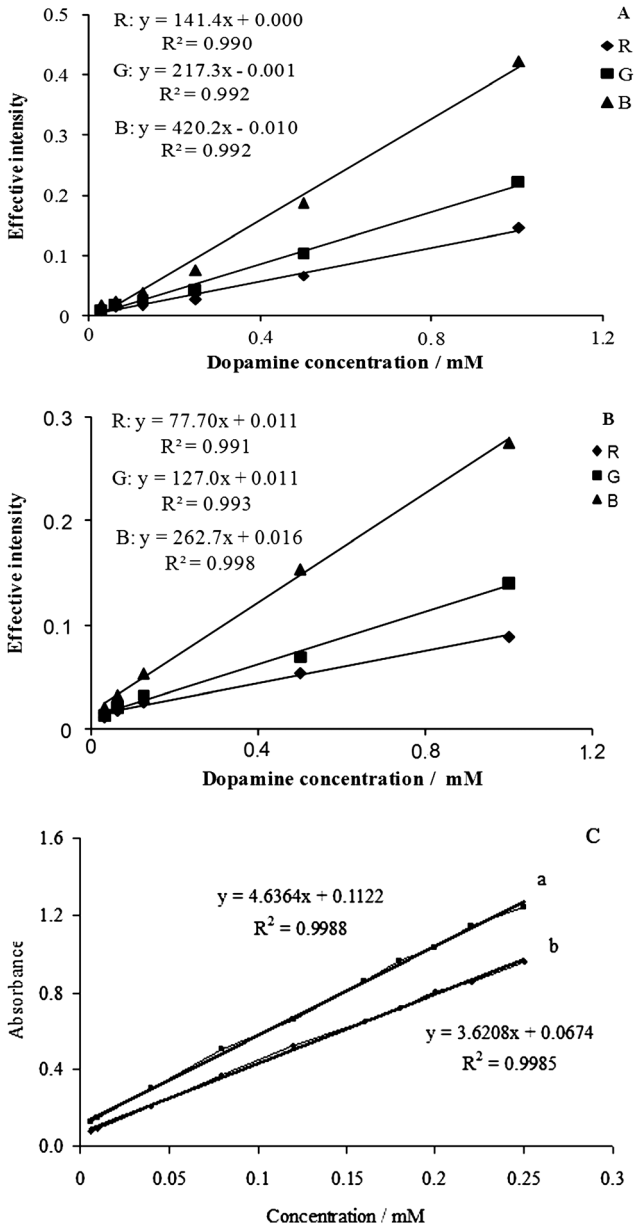


Fig. 9 Calibration curve for dopamine under optimum conditions by using scanner (A) with microwave treatment (10 s at 150 W), (B) without microwave treatment and (C) represents calibration curve of dopamine by using spectrophotometer, (a) with microwave treatment (10 s at 150 W) and (b) without microwave treatment.

Table 4 Detection limit and reproducibility

		Without microwave treatment			With microwave treatment		
Method	Parameter	R	G	B	R	G	B
Developed method	Detection limit (μM)	29	18	12	16	10	7.5
	RSD%	3.4	2.3	2.5	3.2	3.0	3.5
Spectrophotometry	Detection limit (μM)	3.8			3.0		
	RSD%	1.8			2.1		

Table 5 Determination of dopamine in real samples

		Relative error%					
		Without microwave treatment			With microwave treatment		
Sample	Spiked dopamine ($\times 10^{-4}$ M)	R	G	B	R	G	B
Serum	5	+1.57	+3.20	+7.50	+3.70	+4.51	+9.20
Urine	5	+1.45	+2.50	+5.62	+2.80	+3.15	+6.22

3.8 Detection limit and reproducibility

According to the suggested procedure, 10 sample solutions of 0.5 mM dopamine have been analyzed. Results for R.S.D. of three color values are presented in Table 4. A reagent blank was measured 10 times ($n = 10$) and a detection limit was obtained from three times of its standard deviation divided by the slope of the linear regression equation, these results (for developed and spectrophotometry methods) are also given in Table 4. As the results in this case also demonstrate, the detection limits for the procedure involving microwave treatment are better.

3.9 Application

The applicability of the method to assay dopamine in serum and urine was examined. The amounts of dopamine, which were spiked into the bovine serum and human urine samples, were measured without any pretreatment. Relative errors are given for three color values in Table 5. From results in this table it was concluded that again the R and G values were more suitable for accurate determination of dopamine in serum and urine.

4. Conclusion

A new, simple, fast and inexpensive method was developed as an alternative to visible spectrophotometry. Opposite to the

Table 6 Comparison of the proposed method with some other reported methods

Reagent	Linear range (μM)	RSD%	Detection Limit (μM)	Ref.
Alkaline medium (FIA)	Up to 200	0.481	2.5	5
Alkaline medium (FIA)	Up to 200	—	3.5	6
Ipomoea batatas (L.) Lam. (crude extract of sweet potato root)	200 to 6000	—	30	7
N-bromosuccinimide	15 to 74	—	—	10
Diazotised sulfanilamide and molybdate ions in acidic medium	0.1 to 14.8	0.98	—	12
Fluorimetry in acidic medium in methanol	0.53 to 18	2.37	0.43	13
Alkaline medium (Batch method)	6 to 250	1.8	3.8	—
Alkaline medium (Microwave treatment)	30 to 1000	R 3.2 G 3.0 B 3.5	16 10 7.5	This method

spectrophotometry, in this method the transparency of the solution is not important. Developed method only needs the scanner, a domestic microwave (optional) and a PC for analyzing the color. This method is suitable for inexpensive commercial optical sensors. In Table 6, comparison of this developed method with some other reported methods for determination of dopamine is presented. As this table shows not only the proposed method has a wider linear range in most cases, but also have a reasonable limit of detection, in addition no reagent is required except an alkaline media.

Acknowledgements

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References

1 A. Abbaspour, M. A. Mehrgardi, A. Noori, M. A. Kamyabi, A. Khalafi-Nezhad and M. N. Soltani Rad, *Sensors and Actuators B, Chemical*, 2006, **113**, 857–865.
2 A. Abbaspour, A. Khajezadeh and A. Noori, *Anal. Sci.*, 2008, **28**, 721–725.
3 M. S. Gomes, L. C. Trevizan, J. A. Nóbrega and M. Y. Kamogawa, *Quim. Nova*, 2008, **31**(6), 1577–1581.
4 A. Pezzela, M. d'Ischia, A. Napolitano, G. Misurata and G. Prota, *J. Med. Chem.*, 1997, **40**, 2211–2216.
5 J. J. B. Nevado, J. M. L. Gallego and P. B. Laguna, *J. Pharm. Biomed. Anal.*, 1996, **14**, 571–577.
6 J. J. Berzas, J. M. Lemus and P. Buitrago, *Anal. Lett.*, 1997, **30**, 1109–1120.
7 I. C. da Vieira and O. Fatibello-Filho, *Talanta*, 1998, **46**, 559–564.
8 D. W. Barnum, *Anal. Chim. Acta*, 1977, **89**, 157–166.
9 M. Murthy and M. Sundaram, *Indian J. Pharm. Sci.*, 1996, **58**, 124–126.
10 P. Nagaraja, K. C. Srinivasa Murthy, K. S. Rangappa and N. M. Made Gowda, *Talanta*, 1998, **46**, 39–44.
11 Q. Li, J. Li and Z. Yang, *Anal. Chim. Acta*, 2007, **583**, 147–152.
12 P. Nagaraja, R. A. Vasantha and K. R. Sunitha, *Talanta*, 2001, **55**, 1039–1046.
13 H. Y. Wang, Y. Sun and B. Tang, *Talanta*, 2002, **57**, 899–907.

