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Kinetic Spectrophotometric Determination of Morphine in Pharmaceutical Samples

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ABSTRACT

A new, sensitive, simple, inexpensive and fast kinetic spectrophotometric method was developed for the determination of trace amounts of morphine over the range of 12-60ng/mL. The method is based on the catalytic effect of morphine on the reaction of bromate and methylene bluein acidic media is reported. The reaction was monitored spectrophotometrically by 60 ng/ml measuring the decrease in absorbance of methylene blue at 665 nm with a fixed-time 0.5-2.5 min from initiation of the reaction. The detection limit is 0.8 ng/mL and relative standard deviation of 12and 52ng/mLmorphine for 6 replicate measurements was 1.50 and 0.87%respectively. The method 12 and 52 ng/ml was applied to the determination of morphine inpharmaceutical samples % respectively.

Key words: Morphine, Kinetic, Determination, Methylene blueCatalytic.

INTRODUCTION

Morphine extracted from the plant papaver somniferum[1]Morphine (MO) is a useful drug in relieving patients of severe pain, but it's excessive or habitual use frequently causes toxic symptoms[2]Morphine is the primary constituent of opium. It is the most important drug of the opiates group[3]. The use of morphine as an analgesic in pre-term newborns is very common, due to the many painful procedures and stressful circumstances they undergo[4, 24, 25]anditusedforthetherapy of reduce to severe pain, especially aftersurgical procedures. Toxic effects of morphine usage can be harmful for human.Morphine is a useful drug but it's excessive orhabitual use frequently is harmful.

Different methods have been reported for detecting morphine. These include: spectrophotometry[5, 6], immuno chromatography [7], potentiometry[8, 9], simultaneous voltammetric and amperometric[10], gc-mass[1, 11, 26], cyclicvoltametry and amperometry[12], cyclicvoltametry[13, 14], sequential injection analysis[4],chemiluminescence [15], kinetic potentiometric[9], high performance liquid chromatography[16-19], gas chromatography[20], capillary electrophoresis[21], ion mobility spectrometry[22, 23]. These methods are efficient, but

require expensive instrument and are expensive and many of them have needing time to complete the determination. Some of this methods have high limit of detection. Therefore, the need for a sensitive, simple, fast and selective method for the determination of morphine is obvious. In this paper, we developed and validated a rapid, sensitive kinetic spectrophotometric method for the determination of morphine based on its catalytic effect on the reaction of bromate and methylene blue in acidic media.Morphine sulfate $5H_2O$ has the following structure (Figure 1).

EXPERIMENTAL

Reagents and Apparatus

Doubly distilled water and analytical reagent grade chemicals were used during all of the experimental studies.Methylene bluesolution 3.1×10^{-4}M was prepared by dissolving 0.0100 g of the compound (Merck) in water and solution was diluted to the mark in a 100mL volumetric flask.Bromate stock solution 0.25 Mwas prepared by dissolving 4.1752 g of potassium bromate (M=167) in water and diluting to 100 mL in volumetric flask.Standard stock morphine solution10 µg/mL was prepared by dissolving 0.0013 g of morphine sulfate $5H_2O$(M=758.83) in water and diluting to 100 mL in volumetric flask.The working solutions were prepared by serial dilution of it in water. Sulfuric acid solution was prepared by appropriate dilution of concentrated sulfuric acid (Merck).All glassware were cleaned with detergent solution, rinsed with tap water, soaked in dilute HNO_3solution (2%V/V), rinsed with water and dried.

Apparatus

Absorption spectra were recorded with a CECIL model 7500 spectrophotometer with a 1.0cm quartz cell. A model pharmacia biotech (Novaspec II)spectrophotometer with 1.0 cm glass cuvettes was used to measure the absorbance at a fixed wavelength of at665 nm. A thermostat water bath (Gallen Kamp Griffin, BGL240 V) was used to keep the reaction temperature at 30°C±0.1.A stopwatch was used for recording the reaction times.

Recommended Procedure. All the solutions and distilled water were kept in a thermostated water batch at30 °C±0.1 for 20 min for equilibration before starting the experiment. An aliquot of the solution containing120-600ng/ mLmorphine was transferred into a 10mLvolumetric flask, and then 2.0mL0.5 M H_2SO_4, 1.0mL0.1 µg/mL morphine and 0.8mL3.1×10^{-4}Mmethyleneblue were added to the flask.The solution was diluted to 7.0mL with water. Then, 1.0mL 0.25 M bromate was added and the solution was diluted to the mark with water. The solution was mixed and a portion of the solution was transferred to the spectrophotometer cell. The reaction was followed by measuring the decrease in absorbance of the solution against water at 665 nm for 0.5–2.5 min from initiation of the reaction. This signal (sample signal) was labeled as ΔA_s.The same procedure was repeated without addition of morphine solution and the signal(blank signal) was labeled as ΔA_b. Time was measured just after the addition oflast drop of bromate solution. Analytical signal was deference between sample signal and blank signal ($\Delta A_{s-}\ \Delta A_b$).

RESULTS AND DISCUSSION

Methylene blue is a dye that can be oxidized with strong oxidizing agents.We found that trace amount of morphine have a catalytic effect on the this reaction. Therefore, by measuring thedecrease in absorbance of methylene bluefor a fixed time of 0.5-2.5min initiation of the reaction, the morphine contents in the sample can be measured. There are many methods, such as fixed-time, initial rate, rate constant and variable time methods for measuring the kinetic species. Among these, the fixed–time method is the most conventional and simplest, involving the measurement of ”A at 665

Table 1: Effect of foreign substances on the determination of 60 ng/mL morphine

Tolerance limit$w_{ion}/w_{morphine}$	Foreign ion
Na^+, K^+	1000
Glocose	700
Sucrose	500
Urea, NH_4^+	200
Citric acid, Zn^{2+}, Ag^+, Fe^{3+}	100
I^-, Io^{3-}	10
Pb^{2+}, $So_{3-}^{\ 2}$	5
Cl^-, Br^-, No_3^-, Pethidine, Tramadol, Methadone, Fentanyl	<1

nm(Figure 2).Methylene blue has the following structure (Figure3).

Influence of Variables

In order to take full advantage of the procedure, the reagent concentrations must be optimized. The effect of acid concentration, methylene blue concentration, bromate concentration and temperature on the analytical signal was studied.

The effect of sulfuric acid concentration on the analytical signal was studied in the range of 0.07 -0.13M (Figure4).The results show that the analytical signal increases with increasing sulfuric acid concentration up to 0.10M and decreases at higher con-centrations. Therefore, a sulfuric acid concentration of 0.10M was selected for further study.

The influence of methylene blue concentration on the analytical signal was studied in the concentration range of 1.2×10^{-5}- 4.3×10^{-5} M (Figure5).The results show that the analytical signal increases with increasing methylene blue concentration up to 2.5×10^{-5} M and decreases at higher concentrations. Therefore,a methylene blue concentration of 2.5×10^{-5} Mwas selected for further study.

Figure 6 shows the effect of the bromate concentration on the analytical signal for the range of 1.5×10^{-2}-3.5×10^{-2} M. This analytical signal increases with increasing bromate concentration

Table 2: Determination of free morphine in synthetic samples

Sample	Morphine added	Morphine found	RSD (n=4)	Recovery%
Ampoule	60.0	62.1±0.3	103.5	0.48
	12.0	11.2±0.1	93.3	0.89
	28.0	26.8±0.3	95.7	1.12
	44.0	46.5±0.3	105.7	0.64

Table 3: Comparison of some methods for determination of morphine with proposed method

Method	LDR/(ng/ml)	DL/(ng/ml)	Reference no
Kinetic spectrophotometry	48-76	1.8	propesedmethod
Kinetic spectrophotometry	1500-13500	-	6
Ion mobility spectrometry	-	60	16
High performance liquid chromatography	171.18-57060	28.5	21
Gc-mass	250-2000	250	4
Gc-mass	50-2000	20	15
Kinetic potentiometry	110-2900	41	12
Immunochromatography	-	10	11
High performance liquid chromatography	3.5-1000.0	3.5	14
Cyclic voltammetry	5.98-329.20	2.39	18
Gc-mass	5-500	1.0	17
Exploiting sequential injection analysis	100-2500	23	5
Simultaneous voltammetric and amperometric	570.6-285300	28.53	8
Cyclic voltammetry	57.06-11412	5.7	19
Kinetic spectrophotometry	570.6-285300	28.53	8

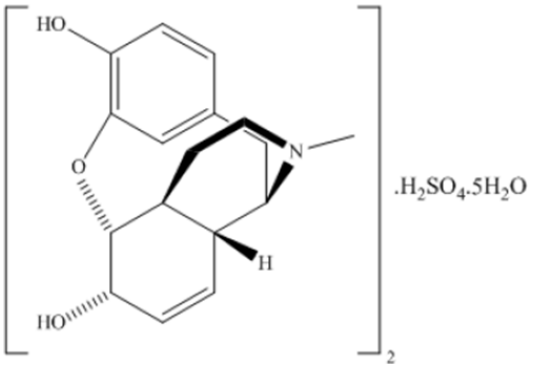

Fig. 1: Structure of morphine sulfate $5H_2O$

up to 2.5×10^{-2}M and decreases at higher concentrations. Therefore, a final concentration of 2.5×10^{-2} M of bromate was selected as the optimum concentration.

The effect of ionic strength on the analytical signal was studied. The results showed that, as the ionic strength increases, analytical signal slightly increases.

The effect of the temperature on the analytical signal was studied in the range 20–38°C with the optimum of the reagents concentrations. The results showed that, as the temperature

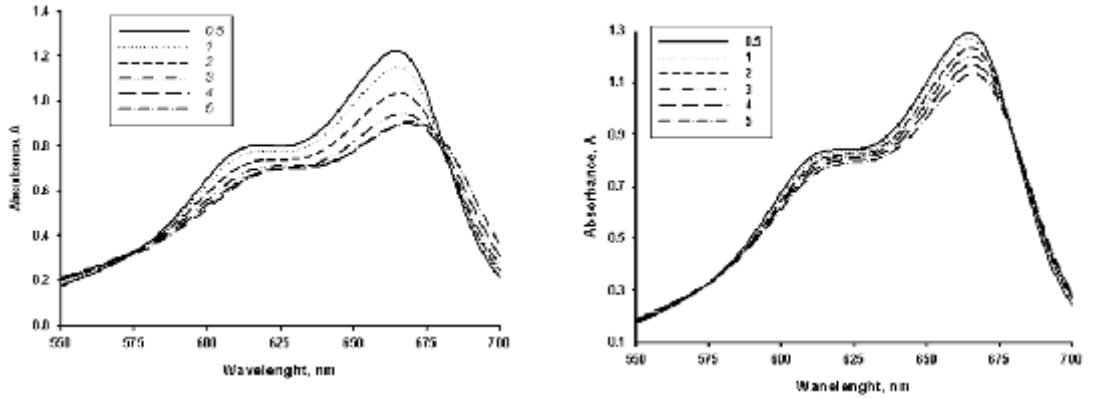

Fig. 2: Absorption spectrum for the morphinemethylene blue -BrO_3^- system with time. Conditions: H_2SO_4 , 0.10M; methylene blue, 2.5×10^{-5}M; BrO_3^-0.025 M; temperature, 30 °C; interval time for each scan, 0.5and2.5from initiation of the reaction. a- in presence of 20ng/mL of morphineb-in absence of morphine

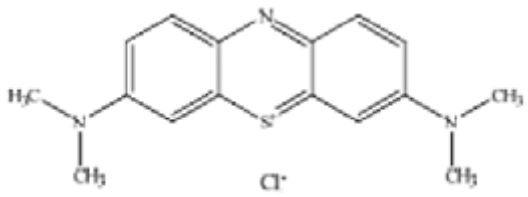

Fig. 3: Structure of methylene blue

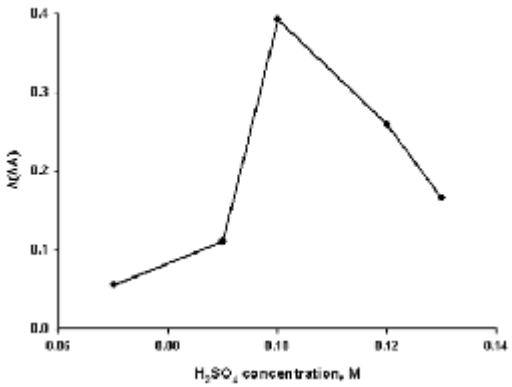

Fig. 4: Effect of H_2SO_4 concentration on the analytical signal. Conditions methylene blue3.1×10^{-5} M; BrO_3^-0.025 M;temperature, 30 °C and time of 3.5 min from initiation of the reaction

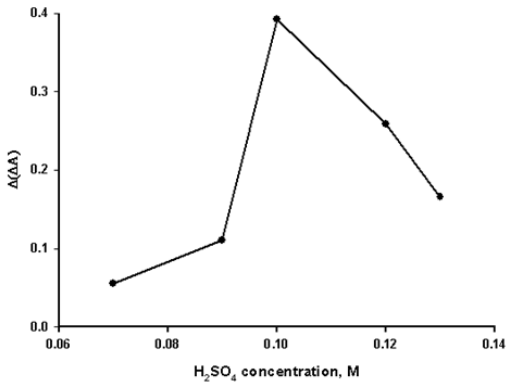

Fig. 5: Effect of methylene blue concentration(MB) on the analytical signal. Conditions: H_2SO_4, 0.10 M; BrO_3^-, 0.025M, temperature,30°C; and time of 3.5 min from initiation of the reaction

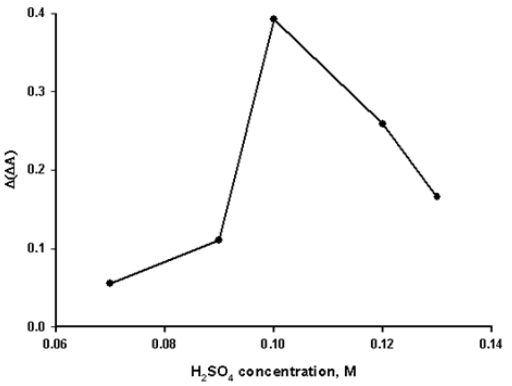

Fig. 6: Influence of BrO_3^- concentration on the analytical signal. Conditions: $H_2SO_4$0.10 M ; methylene blue2.5×10^{-5}M , temperature, 30 °C and time of 3.5 min from initiation of the reaction.

increases up to 30°C, the analytical signal increases, whereas higher temperature values decrease the analytical signal ($\Delta A=\Delta A_s - \Delta A_b$). Therefore,30°C was selected for further study.

Calibration Graph. Precision and Limit of Detection. Calibration graph were obtained using the fixed–time method. This method was applied to the change in absorbance over an interval of 0.5–2.5min from initiation of the reaction because it provided the best regression and sensitivity. The equation of the calibration graph is $\Delta A=0.0145C_{morphine} + 0.2827$(n=7, r =0.999) in the range of 12-60ng/mL. The calibration graph was constructed by plotted of ΔA_sat a fixed –time method versus morphine concentration.The limit of detection (Defined as $DL=3S_b/m$, where DL, S_b and m are limit of detection, standard deviation of the blank signal and slope of the calibration graph, respectively) is equal to 1.8ng/mL morphine.The relative standard deviation for five replicate determination of 12and 52 ng/mLmorphine was 1.50 and 0.87% respectively.

Interference Study. In order to assess the application of the proposed method to synthetic samples, the effect of various ions and substances on the determination of 20ng/mLmorphine was studied. The tolerance limit was defined as the concentration of a added ions causing a relative error less than 3% the results are summarized in Table 1.

Preparation of Real Samples

In order to evaluate the applicability of the proposed method to analysis of real sample the method was applied to pharmaceutical samples (ampoule) for determination of morphine.The results obtained by the proposed method are given in Table 2.

CONCLUSION

The kinetic-spectrophotometric method developed for the determination of morphine is inexpensive, uses readily available reagents, allows rapid determination at low operating costs and shows simplicity,good precisionandaccuracy compared to other kinetic procedures as shown in Table 3. With this method,it is possible to determine morphine at levels as low as 12ng/mL.

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REFERENCES

1. Van Thuyne, W., Van Eenoo, P., Delbeke, F.T., *Chromatography B*. **2003**, *785*, 245-251.
2. Sakaia, G. ,Ogataa, K., Udab, T., Miuraa, T. and Yamazoe, T., *Sensors and Actuators B: Chemical* 1998 , *49*, 5-12.
3. Idris, A.M., Alnajjar, A.O, *Talenta* **2008**,*77*, 522-526.
4. Cherry, D.A., Gourlay, G.K., *Agents Actions* **1994**, *42*, 173-174.
5. Malovanovic, G.A., Sekhtea, M.A., *Mikrochimica* 1984, *84*, 477-483.
6. Sheibani, A., Shishehbore, M.R., Mirparizi, E., *SpectrochimicaActa Part A* **2010**,77, 535-538**.**
7. Lyubavina, I.A., Zinchenko, A.A., Lapenkov, M.I., Nikolaeva, T.L. **2005**, *31*, 99-103.
8. Hassan, S.S.M., El-Naby, E.H., Elnemma, E.M., *Russian Journal of Bioorganic Chemistry* **1996,** *124*, 55-62.
9. Pejic, N., Anic, S., Mijatovic, M. Milenkovic, S., Ciric, J., Grozdic, T.,Nauka, tehnika,*bezbednost* **2003**,*13*, 67-74.
10. Xu, F., Gao, M., Wang, L., Zhou, T., Jin, L., Jin, J., *Talanta* **2002**, *58*, 427-432.
11. Melent'ev, A. B., *Journal of Analytical Chemistry* **2003**, *59*, 566-570.
12. Pournaghi-Azar, M.H., Saadatirad, A., *Electroanalytical chemistry*2008, 624, 293-298.
13. Ganjali, M.R., Norouzi, P., Dinarvand, R., Farrokhi, R., Moosavi-movahedi, A.A., *Materials Science and Engineering C.***2008,** *28*, 1311-1318.
14. Li, F., Song, J., Shan, C., Gao, D., Xu, X., Niu, L., *Biosensors and Bioelectronics***2010,** *25*, 1408-1413.
15. Francisa, P.S., Adcock, J.L., Costin, J., Purcell, S.D., Pfeffer, F.M., Barnett, N.,*J. Pharm. Biomed. Anal.***2008,** *48*, 508-518.
16. Rop, P. P., Grimaldi, F., Burle, J., De Saint Leger, M. N., Viala, A., *Journal of Chromatography. B.***1994,** 661, 245-253**.**
17. Domnguez-Ramrez, A. M., Cortes-Arroyo, A., y de la PeLa, M. H., Aoki-Maki, K., Medina Lopez, J. R., Ros-CastaLeda, C., Lopez-Munoz, F.J., *Journal of Pharmaceutical and Biomedical Analysis* 2006, *40*, 1172-1178.
18. Sato, K., Chiba, T., Chiba, R., Satou, S., Tanaka, S., *ANALYTICAL SCIENCES*.**2001,***17*, 1041-1043.
19. Berga, T., Lundanes, E., Chritophersen, A.S., Strand, D.H., *J.Chromatogr. B.***2009,** *877*, 421-432.
20. Hofmann, U., Seefried, S., Schweizer, E., Ebner, T., Mikus, G., Eichelbaum, M., *J.Chromatogr. B.***1999**, *727*, 81-88.
21. Mi, J.Q., Zhang, X.X., Chang, W.B., . *J. Immunoassay Immunochem***2004,***25*, 57-70.
22. McCooeye, M.A., Ells, B., Barnett, D.A., Purves, R.W., uevremont, R., . *Journal of Analytical Toxicology***2001,** *25*, 81-87.
23. Khyamian, T., Tabrizchi, M., Jafari, M.T., .*Talanta*Quantitative analysis of morphine and noscapine using corona discharge ion mobility spectrometry with ammonia reagent gas**2006,** *69*, 795**-**799**.**
24 .Breivik, H., *ActaAnaesthesiolScand* **2001**,*45*, 1059-1066.
25 .Lyun, A.M., Nespeca, M.K., Opheim, K.E., Slattery, J.T., *Anesth. Analg***1993**, *77*, 695-701.
26. Javidnia, K., Miri, R., Miri, D., *IJMS***2006**, *31*, 213-215.