Research Article

Flow Injection Photometric Assay for Determination of Methyl Dopa Using Hydrogel Approch as a Host for the Sodium Periodate Solution

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ABSTRACT

A new mode for the flow injection analysis method was developed for the determination of methyl dopa in pure and pharmaceutical formulations by using hydrogel bead. The method was based on the releasing of the oxidizing agent sodium periodate ($NaIO_4$) from the hydrogel beads body leads to oxidation of drug in alkaline medium to orange-reddish color product. A homemade flow injection photometer, single hydrogel bead and green light emitting diod were used for this application. The optimum conditions were 0.97ml.min^{-1} flow rate, 150µL injected sample volume, 12seconds as purge time of injected sample volume, 1mM of sodium hydroxide, 0.7mol.L^{-1} that gel bead soaked in and 3hours of soaking time for hydrogel bead inside sodium periodate solution. The linear range for the variation of instrument response with methyl dopa concentrations was 0.07-1.2mM, While the limit of detection was 0.23x10^{-9}M calculated from the stepwise dilution of the minimum concentration in the linear range. The correlation coefficient for the calibration graph was also equal to 0.9946. The method was applied successfully for the determination of methyl dopa in pharmaceutical preparations, so on that base the new method can be used as an alternative analytical method.

Keywords: Flow-Injection, Methyl dopa, Oxidation, Hydrogel beads.

INTRODUCTION

Methyl dopa is sesquihydrate of (-)-β-3,4 dihydroxy phenyl-α-methyl alanine. It is a white to yellowish-white crystalline powder or almost colorless crystals. It is odorless and almost tasteless. It is free soluble in dilute mineral acids and warm distilled water[1-2]. Methyl dopa have different pKa values each one related to functional groups exist in its structure including –COOH, -OH, -NH2, and, OH groups[3]. It was the mainstay of antihypertensive treatment, but it is use has declined on account of relatively severe adverse side effects , with increasing use of other safer and more tolerable agent such as alpha blockers, beta blockers, and calcium channel blockers[4-5].

Spectrophotometric and FIA were used for the determination of too many drugs such as Chlorpromzaine HCl[6], Rosuvastatin[7] and Chloroquine[8]. Many methods have been reported for the determination of methyl dopa in pharmaceutical formulations and or biological fluids. These methods include, spectrophotometry[9-12], Voltametery[13], high-performance liquid chromatography[14], and Titrimetry[15-16]. In this paper polyacrylic acid hydrogel bead was used as a host for the oxidant (sodium periodate) for a FIA spectrophotometric method for the determination of methyl dopa in aqueous solutions and pharmaceutical preparations.

MATERIALS AND METHODS

Chemicals

All chemicals used in this project were of analytical grade reagents unless otherwise stated. Table 1 tabulate the main chemicals used throughout this research work.

Table 1: The main chemical used throughout this research work.

Name	Formula	Supplier	Concentration (g/ml)
Sodium hydroxide	NaOH	BDH	0.1000g/250ml (10mM)
Sodium periodate	$NaIO_4$	BDH	10.9645g/50ml (1mole.L^{-1})
Methyl dopa	$C_{10}H_{13}NO_4$ $1_{1/2}H_2O$	SDI	0.1191g/100ml (5mM)

Gel beads (hydrogels) and cell housing unit

Gel bead (from local market was purchasing) was washed with distilled water. This was repeated with constant change of distilled water between time to time to insure a complete removal of any salt (if present) inside gel bead. No problem was met in introducing the gel bead into gel bead cell housing unit. Figure 1(a,b,c,d) shows a growth time for gel bead when it's soaked in distilled water.

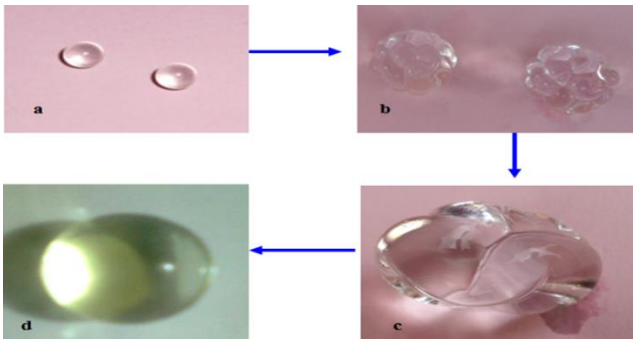

Figure 1: variable growth time for gel bead when it's soaked in distilled water.

a) Time of zero time (b) Time lapse of 4hours(
(c) Time lapse of 8hours (d) Time lapse of 16hours

While figure(2) shows the cell housing unit of hydrogel bead that used within the manifold of the flow injection analysis system.

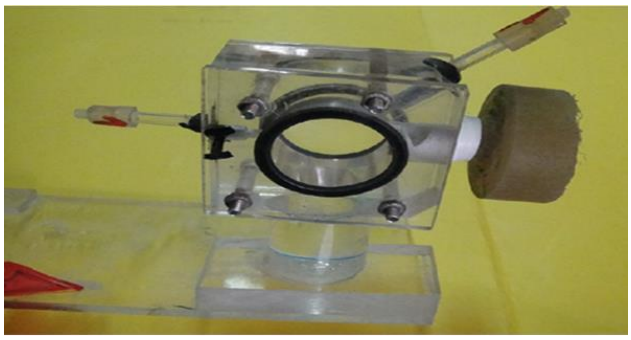

Figure 2: Gel bead cell housing unit.

APPARATUS

Peristaltic pump

An ismatec type (ISM796) peristaltic pump four channels was used throughout this project.

Connection Tubes

Various manifold parts made of either from rubber or polyproylene or even Teflon with inside diameter of 0.5-1mm were used.

Y- Junction Point

Homemade liquid junction point made of methylmethacrylate(Y-junction) for the mixing of chemical reactants in reaction pattern.

Rotary Six Ports Injection Valve

Six ports medium pressure(model,V-450) (upchurch scientific INC) valve was used with a variables sample volumes, fixed on the carrier stream line.

UV-vis Spectrophotometer

UV-Vis spectrophotometer digital double-beam type 3000-optima was used to scan the spectrum of colored complex and reactants using 1cm glass cell.

Measuring &Readout System

A homemade Ayah3Sx3-3D-solar FI photometer equipped with three different super bright light emitting Diod(LED) including; blue LED(470nm), green LED(525nm) and red(635nm) as sources with a detection using solar cell and flow cell of 2mm thickness was designed.. The readout of the system composed of X,Y-t potientiometric recorder(kompenso graph (C-1032) siemense(germany)) or digital AVO-meter(auto range)(0.00-2000mV)(China).

RESULTS AND DISSCUSION

Spectroscopic study

A study was carried out to scan the colored complex that was produced when methyl dopa is reacted with sodium periodate in sodium hydroxide medium to form an intense orange-reddish product. Figure (3) shows the height response of orange-reddish species using Ayah3Sx3-3D solar FI photometer at three different light Emitted Diod (LED) [blue(470nm), green(525nm), and red(635nm)]. A maximum response measured in mV obtained when using the high intensity green light Emitted diod (525nm) as a radiation source. Therefore, the super bright green light emitting diod was used throughout this work.

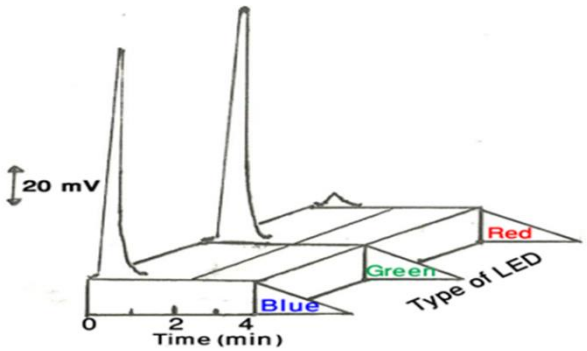

Figure 3: Profile of response(mV) versus light Emitting Diod[blue(470nm), green(525nm) and red(635nm)].

Preliminary investigations

A study was carried out in order to establish the ability of the hydrogel bead to entrapment followed by the release of sodium periodate solution whenever it is required as an oxidant in the reaction pattern for methyl dopa oxidation. A single hydrogel bead was placed into a plastic container containing sodium periodate solution for a period of time, after that the hydrogel bead was transferred to a beaker containing almost 10ml of distilled water which is equivalent to the volume occupied the accommodation cell that designed to accommodate the hydrogel beads. A few drops of methyl dopa was added to the beaker then few drops of sodium hydroxide was added, immediately an orange-reddish colored complex was formed indicating that the hydrogel bead has the tendency to entrap and the release of the sodium periodate solution via the diffusion phenomena. Also, the colored complex as it is observed was adsorbed on the surface of the hydrogel bead that accommodate sodium periodate solution .Due to this unique property noticed on the gel bead surface, it was decided to exploit this property via the use of this gel bead property in accommodation the oxidant sodium periodate inside the gel bead(water crystal). This observation needs for a detailed study to exploit the benefit property of the gel bead as an immobilizing host for sodium periodate solution. Therefore, in this research work the hydrogel bead was used as immobilizing agent for sodium periodate in the determination of methyl dopa.

Design of the manifold reaction

The block diagram of the whole reaction manifold system used for the determination of methyl dopa via it's oxidation in alkaline medium is shown in figure(4). The manifold system is composed of two lines, the first line supply distilled water leading to the gel bead cell unit that accommodate and encloses sodium periodate solution

inside the hydrogel bead. The oxidant is released from the hydrogel bead via diffusion phenomena and passes to the 6-port injection valve where the sample is injected. The second line is for sodium hydroxide solution. Both lines meet at Y-junction point leading to the formation of the colored complex. Ayah3Sx3-3D solar FI microphotometer was used to monitor the variation of the instrument response using green light emitting Diod (LED) as a source for irradiation throughout this reaction. The gel bead cell was placed before the injection valve to ensure that sodium periodate is released from the hydrogel bead when it's in contact with distilled water from distilled water line, and also to ensure that the injected sample volume is not lost via the adsorption and/or absorption on the surface of the hydrogel bead.

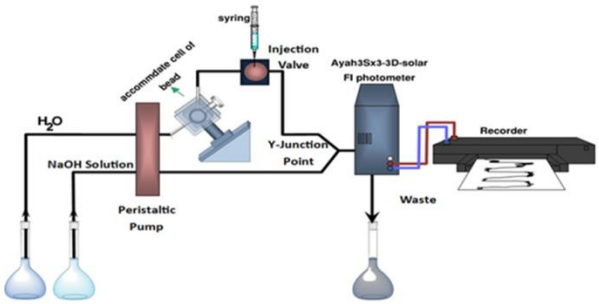

Figure 4: A schematic diagram of FIA for determination of methyl dopa was drawing by Photoshop program showing the gel bead cell housing unit (accommodate cell for gel bead).

Number & efficiency of hydrogel beads

A study were carried out to establish the number of hydrogel bead that is necessary for the entrapment of sodium periodate solution (0.5mole.L^{-1}) for this research work, using the manifold reaction system as shown in figure (4). A maximum measured response in millivolte was obtained when two hydrogel beads were used as immobilizing agent for sodium periodate; but the signal obtained was characterized by wide peak response compared with single hydrogel bead as shown in figure(5) also, the measurement time was longer. Therefore, single hydrogel bead was used throughout this research work. Also the repeatability of single hydrogel bead experiment was also investigated, using 150µl (0.8mM) as an injected sample volume, 1mM of sodium hydroxide solution and a flow rate of 1.7ml.min^{-1}.

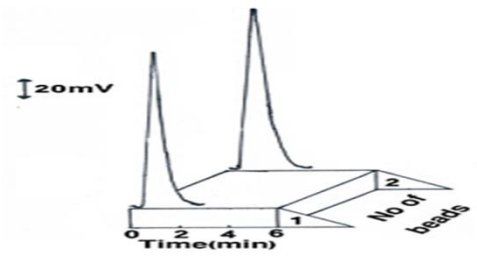

Figure 5: Profile of the variation of response(mV) of Ayah3Sx3-3D-solar FI microphotometer versus the number of hydrogel bead entrapped inside hydrogel bead cell(oxidant supplier).

The study shows that an increase in response was noticed and after three successive injections a steady state was achieved (45 injections). The response starts to decrease indicating that the amount of sodium periodate entrapped in the single hydrogel bead was depleted.

STUDY OF THE VARIABLES

The Physical Parameters

A study was carried out for the determination of preferred flow rate (0.5-2ml.min^{-1}) using 150µl as the injected sample volume (0.8mM of methyl dopa), and 1mM of sodium hydroxide. Eighteen seconds was used as the allowed permissible time for the departure of sample segment from the injection valve. A single hydrogel bead was imbedded into sodium periodate solution (0.5mole.L^{-1}) for two hours, and followed by the transfer to the gel bead cell leading to the manifold reaction system as mentioned earlier. From the obtained results 0.97ml.min^{-1} gave a higher, more sensitive and lesser base width this might be due at low flow rates there were an increase in peak base width (ΔtB) due to the dispersion and dilution which causes an irregular responses. Therefore, a flow rate of 0.97ml.min^{-1} was chosen as an optimum flow rate.

Variable sample volumes ranging from 40 to 300µl were also study adopting the same procedure which comprises the use of 0.97ml.mi^{-1} as flow rate. The obtained results shows that there is an increase in the responses up to 150µl sample volume, while no gain in the obtained responses was observed above 150µl in spite of the increase in base width of responses(i.e. increase analysis time). Therefore, a compromise was made between the sensitivity and analysis time leading to the use of 150µl as the optimum of volume sample. The purge time of injected sample segment from injection valve was also studied at variable time lapse (3-21seconds) using the achieved parameters. Twelve seconds was chosen as the optimum purge time. These studies conclude that 0.97ml.min^{-1} flow rate, 150µl sample volume and 12 seconds as purge time of injected sample volume will be used throughout this work.

The Chemical Parameters

A study was conducted to establish the optimum concentration of sodium hydroxide solution ranging from 0.1-5mM using the optimum physical parameters achieved in previous section. The obtained results were appeared that the values of responses increased regularly with increasing in the concentration of sodium hydroxide and then remained invariable between 1 and 5mM. Thus, a concentration of 1mM of sodium hydroxide was chosen as an optimum for complete oxidation of methyl dopa. A series of sodium periodate solutions (0.05, 0.1, 0.3, 0.5, 0.7 and 1mole.L^{-1}) were prepared. A single swollen hydrogel bead having a diameter of 18±0.02mm was placed into each solution in a plastic container for two hours, i.e. to give enough suitable time for hydrogel bead to enclose sodium periodate solution. The hydrogel bead is then transferred to the gel bead cell housing unit using

the manifold reaction system as mentioned previously. 0.7mole.L^{-1} was chosen as the optimum concentration that can be enclosed by hydrogel bead.

A single hydrogel bead was put into each of five separate individual plastic container (include a cover with screw thread) that containing sodium periodate solution (0.7mole.L^{-1}) for variable soaking time (1-5hours) then each of the single hydrogel bead was transferred to the gel bead cell fixed in the manifold reaction system. From the results obtained it was noticed that a maximum response was obtained when using a period of three hours as soaking time for the sodium periodate solution.

CONSTRUCTION OF THE CALIBRATION GRAPH

A series of methyl dopa solutions (0.0-2mM) were injected using all parameters whether chemicals or physicals were fixed in previous sections. A scatter plot diagram shows that a linear calibration graph range for the variation of the instrument response of Ayah3Sx3-3D-solar FI microphotometer with methyl dopa concentration was ranging from 0.07-1.2mM (fig(6)).

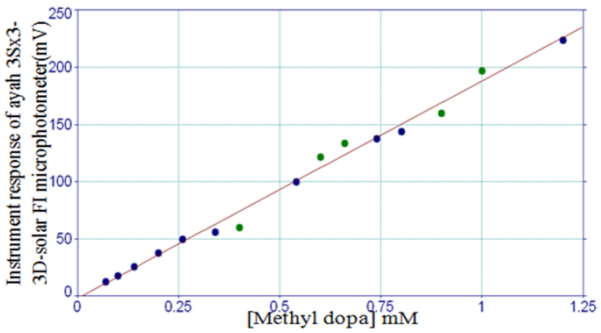

Figure 6: Linear calibration graph for the variation of the response versus methyl dopa concentration.

LIMIT OF DETECTION (L.O.D)

The limit of detection of methyl dopa was determined using the gradual dilution of the minimum concentration of the analyte in the calibration graph which was 0.07mM. The practical limit of detection of methyl dopa was 0.23nM/150µl injected sample volume.

THE APPLICATIONS

Thirteen tablets were weighted from each manufacture company (first column in table(2); these tablets were crushed, grinded using pistol and mortar until a fine powder was obtained followed by dissolving an equivalent amount to 0.74mM (0.0176g active ingredient) in enough amount of warm distilled water then complete the volume to 100ml in a volumetric flask with distilled water. The solution was filtrated on a washed filter paper (with distilled water) in order to get rid of any insoluble materials. Successive washing was carried out in order to ensure complete removal of any remaining drug. Methyl dopa in each filtrate for the three different manufacture company was determined using same method adopted in the construction of calibration graph. The results obtained are summarized in table(2).

The new method was compared with the quoted values using paired t-test. The obtained results indicate that there was no significant differences between the proposed method and the quoted method at 95% confidence interval as the calculated t-value is less than tabulated critical t-value. On this basis the new method using the hydrogel bead as an immobilizing agent for sodium periodate solution can be used as an alternative method for the determination of methyl dopa in pharmaceutical formulations.

Table 2: The recovery of the adopted method of different manufactures.

Pharmaceutical tablets, manufacture	Quoted content of active ingredient	Found content of active ingredient	Recovery%
Aldosame, SDI-Iraq	250mg	253.16mg	101.26
Aldomate, Algortham-Labanon	250mg	250.44mg	100.17
Methyl dopa, MBC-Syria	250mg	247.42mg	98.96

CONCLUSION

Flow injection analysis technique continue to be the most suitable method for routine analytical work because of it is simplicity and sensitivity beside of the number of samples that can be analyzed by time in compared with other classical analytical methods. The proposed method make use of the simple oxidizing agent sodium periodate were it retained inside hydrogel beads, which an ordinary analytical laboratory can afford and the procedures do not involve any critical reaction conditions or tedious sample preparation. It could be concluded that the developed method for methyl dopa determination is simple, sensitive, relatively precise, and accurate and can

be satisfactorily applied to the analysis of methyl dopa in bulk and pharmaceutical formulations. The proposed method was used for the routine analysis of the drug in the quality control.

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