GRAPHENE QUANTUM DOTS FOR SPECTROPHOTOMETRIC SENSING OF METOCLOPRAMIDE IN PHARMACEUTICAL AND WATER SAMPLES

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Abstract

An efficient sensing method based on a flow injection manifold has been established for spectrophotometric determination of metoclopramide. The method is based on simple diazotization reaction of metoclopramide with nitrite in acidic medium prior to azo coupling reaction with graphene quantum dots (GQDs). Absorbance was monitored at 423 nm. Effects of various chemical and instrumental parameters were investigated. The analysis of the results of central composite design showed that for obtaining the maximum sensitivity, concentrations of nitrite and hydrochloric acid, and dilution factor for GQDs should be as 1.31 mmol L^{-1}, 0.39 mol L^{-1} and 2-fold dilution, respectively. Two calibration linear ranges were observed for the determination method. The ranges were: 0.1-10 and 10-40 μg mL^{-1} of metoclopramide. The method was successfully applied to the determination of metoclopramide in pharmaceutical tablets, without any interference from common excipients. Also, the method was successful for quantitation of metoclopramide in different water matrices.

Key words: *central composite design, flow injection analysis, graphene quantum dots, metoclopramide*

1. INTRODUCTION

Metoclopramide (MCP), 4-amino-5-chloro-2-methoxy-N-(2-diethylamino-ethyl) benzamide is a dopamine-receptor antagonist active on gastrointestinal motility. It is used as an anti-emetic in the treatment of some forms of nausea and vomiting to increase gastrointestinal motility. It is also used at much higher doses for the prevention of cancer chemotherapy induced emesis (Tas et al. 2006).

Most of the analytical methods employed for the determination of MCP in dosage forms or in biological fluids, use different types of high performance liquid chromatography (HPLC) (Radwan 1998; Fatmi & Williams 1989; Kelly & Ni Kelly 1988; Vejendla et al. 2013), gas chromatography (GC)–mass spectrometry (MS) (Ghonim 2014), GC-electron capture detection (ECD) (Heftmann 1983), voltammetry (Farghaly et al. 2005) and chemiluminescence (Arfaj 2004; Fan, Wu & Zhang 2002), Spectrophotometry has also been widely used for MCP determination, especially for the analysis of pharmaceutical preparations. Some of the spectrophotometric methods are based on formation of charge-transfer complexes of MCP with chloranil and bromanil (Elgendy 1992), reaction with p-dimethyl aminocinnamaldehyde (Moussa 2000) and diazo-coupling with benzoylacetone in alkaline medium (Omran 2005). Automation of the analytical methods may make them faster, more efficient, and more environmentally friendly. Automation is a priority in routine and research pharmaceutical laboratories. The usefulness and compatibility exhibited by flow techniques make them ideal for chemical analysis.

More recently, a novel type of luminescent carbon nano-material, zero-dimensional graphene quantum dots (GQDs) has attracted much attention (Pan et al. 2010). GQDs are single atom-thick graphene sheets with sizes smaller around 10 nm. Similar to graphene nano-sheets, GQDs exhibit some excellent characteristics such as high surface area and good surface grafting by using the π–π conjugated network or abundant surface functional groups (Li et al. 2013; Williams et al. 2013; Zhang et al. 2012). Because of the good biocompatibility and low toxicity, graphene quantum dots have been used in analytical chemistry. Some of the methods use GQDs as sensing element for humidity (Alizadeh & Shokri 2016), glucose (Razmi & Mohammad-Rezaei 2013), free chlorine (Dong et al. 2012), Cd(II) (Li et al. 2012), tri-nitrotoluene (TNT) (Fan et al. 2012), Fe(III) (Wang et al. 2012),

pyrocatechol (Yang et al. 2012), glutathione, uric acid and tryptophan (Mazloum-Ardakani et al. 2015), immunoglobulin G (Zhao et al. 2013), and DNA sequences (Zhao et al. 2011). The analytical methods often have been developed based on fluorescence quenching of GQDs.

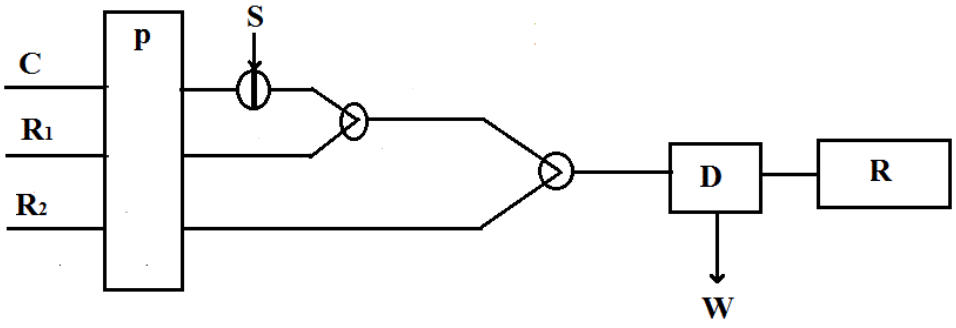
Diazotization-coupling reactions have been widely applied for determination of aromatic amines. In the methods, excess sodium nitrite must be removed prior to coupling reaction, because the excess nitrite reacts with the coupling reagents, and makes some serious drawbacks in the determination process. Sulfamic acid (Revanasiddappa & Manju 2001; Hosakere & Malligere 2006) or ammonium sulfamate (Kamalapurkar & Chudasama 1982; Kamalapurkar & Priolkar 1983), are the two common reagents which are used to remove the excess nitrite. Addition of the reagents not only dilutes the analytical solution but also adds an additional analytical step and makes the analytical time to be increased. In addition, by adding the reagents, the produced waste is increased. Introduction of a new coupling reagent enabling analyte determination in the presence of excess nitrite, makes the analytical method more efficient.

For the first time, GQDs are suggested as a coupling reagent in azo-coupling reaction for MCP determination by using a flow injection manifold. The developed method is based on the diazotization reaction of MCP with sodium nitrite in hydrochloric acid medium. The diazotized MCP is then coupled with GQDs to form an azo dye. During the determination process, sulfamic acid or ammonium sulfamate are not added, because the excess nitrite has no interferences on the monitoring process. The presented method is much more environmentally friendly, low-cost and sensitive.

2. EXPERIMENTAL

2.1. Apparatus

The flow system used in this work is shown in Scheme. 1.



Scheme. 1. The flow-injection (FI) system used for the determination of MCP.

Condition: C, carrier (water); R_1, nitrite 1.31 mmol L^{-1} in hydrochloric acid as 0.39 mol L^{-1}; R_2, GQDs (2-fold diluted); P, peristaltic pump; i. d. of tubes=0.8 mm; S, sample injection unit; length of $L_{1=}$ 76 cm; length of $L_{2=}$ 26 cm; D, detector; Wavelength=423 nm; W, waste; R, recorder.

The absorbance was measured with a Cecil model CE 1021 spectrophotometer, equipped with a flow-through cell of 20 µL inner volume and 1.0 cm optical path. An 8-channel peristaltic pump (Gilson, Miniplus 2) with three silicone rubber tubes (0.8 mm i.d.) was used. PTFE mixing joints were used. Nitrite containing sample solutions were injected with a 6-port Rheodyne sample injector. A Shimadzu 1650 PC spectrophotometer with 1.0 cm matching quartz cells was used for obtaining the sample spectra.Surface morphology of the prepared GQDs with scanning electron microscopy (SEM) was studied by using LEO 1430VP. The transmission electron microscopy (TEM) was performed by a Zeiss-EM10C instrument. The FT-IR spectra were obtained by a Perkin-Elmer Spectrum RX I instrument. Design and analysis of the central composite experiments were carried out by MINITAB (Minitab Inc. Release 17.0) statistical package.

2.2. *Reagents*

All chemicals used were of analytical grade and all solutions were prepared in distilled water. MCP hydrochloride was supplied from (Fluka). Citric acid anhydrous and sodium nitrite were supplied from (Loba-chemie, India). Hydrochloric acid and sodium hydroxide were purchased from (Merck, Germany).

2.3. Synthesis of GQDs

GQDs were selectively and successfully synthesized by controlling the degree of carbonization of citric acid and dispersing the carbonized product into alkaline solution according to the procedure reported by (Dong et al. 2012). In a typical procedure, 2.0 g of citric acid was put into a 250 mL beaker and warmed to 200°C. After 30 min, the color of the melted product became orange, hinting the formation of GQDs.

2.4. Sample handling

Three tablets containing MCP were weighed and grounded to fine powder. Then, an accurately weighed amount of the powdered tablets, were transferred to a 100 mL volumetric flask. The mixture was shaken with 60 mL water for about 20 min, then made up to the mark with water, mixed and filtered using a filter paper. The resulting solution was diluted appropriately and subjected to analysis by following the above discussed FI system.

3. RESULTS AND DISCUSSION

3.1. Characterization of GQDs

The scanning electron microscopic image of the synthesized GQDs is shown in Fig 1(I). The SEM image shows that GQDs are relatively spherical with diameters less than 30 nm. The obtained TEM micrograph was also used for more accurate size determination of GQDs. The TEM image is given in Fig 1(II). The size of GQDs is mostly distributed in the range of 10–20 nm, and an average size about 15 nm was calculated.

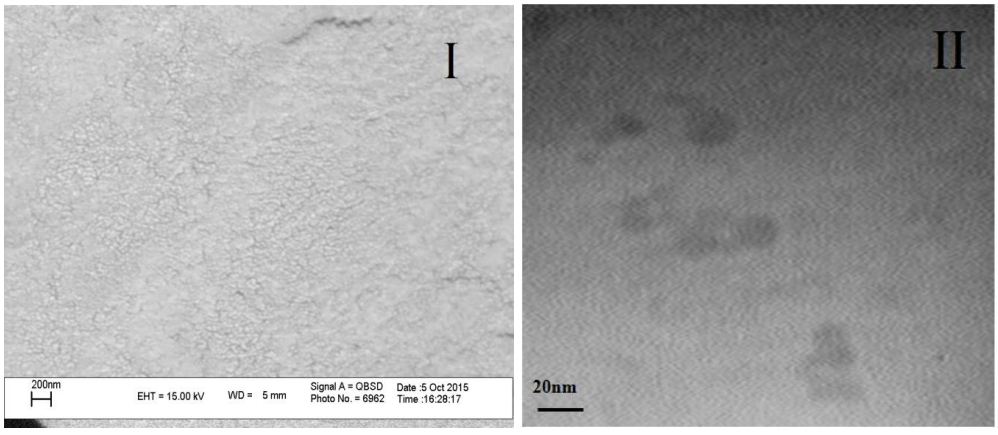


Fig. 1. SEM (I) and TEM (II) images of the synthesized GQDs.

Fig 2 shows the FT-IR spectrum of GQDs. As expected, it can be seen that GQDs contain the functional groups of OH and COOH. The strong and broadened peaks at wave number range of 3000–3500 cm^{-1} (assigned to OH stretching) and the strong paired peaks at 1634 and 1554 cm^{-1} (respectively, assigned to asymmetric and symmetric stretching of carboxylate functional groups) can

be clearly distinguished in the spectrum. These evidences show that the prepared GQDs possess a large density of hydrophilic functional groups such as hydroxyl and carboxylate groups (Alizadeh & Shokri 2016).

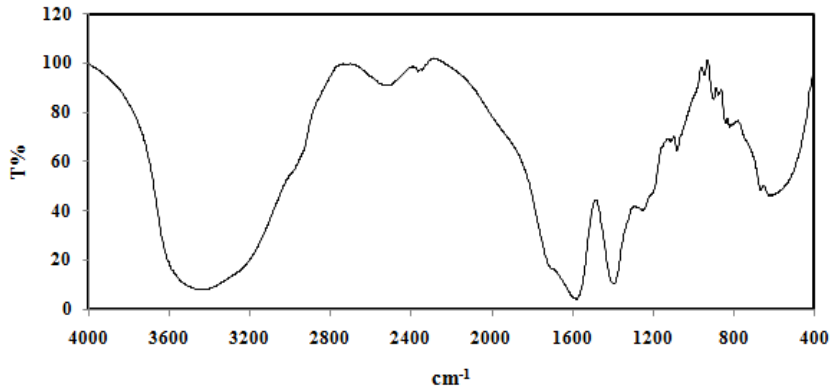


Fig. 2. FT-IR spectrum of the synthesized GQDs.

3.2. Absorption Spectra

The colorimetric nitrite determination method is based on the diazotization of metoclopramide hydrochloride in an acidic medium prior to coupling with GQDs. The product of the azo coupling reaction gives an absorption maximum at 423 nm, as can be seen in Fig 3. At this wavelength the blank is sufficiently small, and makes reproducible absorbance measurements. Hence, monitoring of absorbance was performed at 423 nm for all of the metoclopramide hydrochloride monitoring experiments.

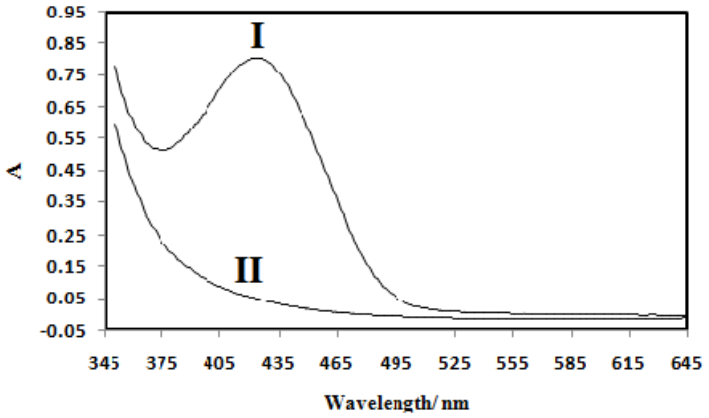
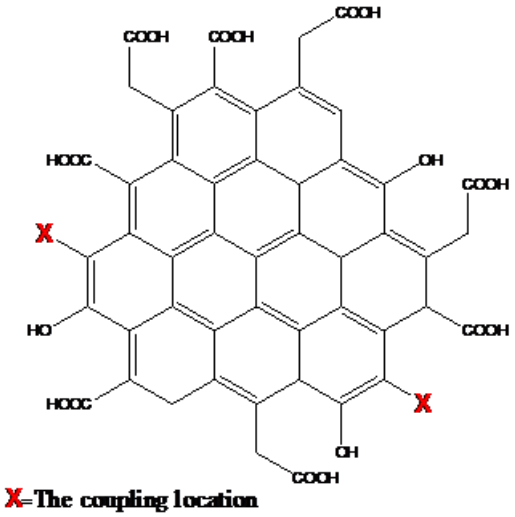


Fig. 3. (I) Absorption spectra of azo dye produced (6.7 μg mL^{-1} MCP) against water, and (II) blank against water.

The most probable reactive sites of GQDs (for azo coupling reaction) have been shown in Scheme 2.



Scheme 2. GQDs structure and its azo coupling reactive sites (have been assigned as X).

3.3. Optimization of the FI-system

The reaction of nitrite and metoclopramide in the presence of hydrochloric acid is occurred in L_1 reaction coil. When length of L_1 increases the diazotization reaction time is increased. But, by increasing the length of L_1, the more dispersion is occurred; therefore, sensitivity is decreased. As can be seen in Fig 4, length of L_1 as 76 cm has produced the best sensitivity. Therefore, L_1 with a length equal to 76 cm was selected for the next studies.

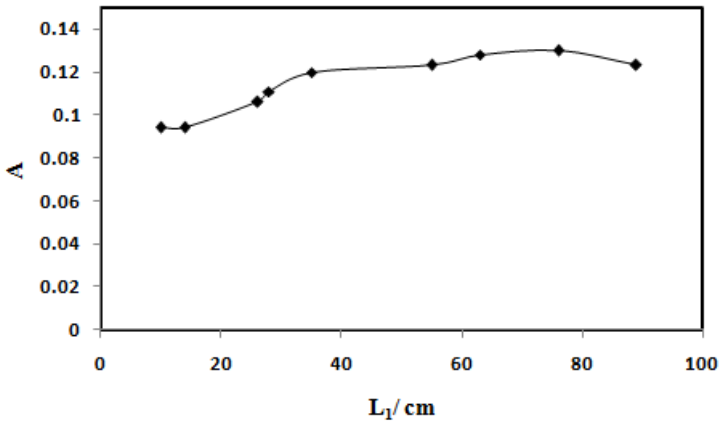


Fig. 4. Effect of length of L_1 on the sensitivity of the method.

Condition: nitrite, 1.0 mmol L^{-1}; hydrochloric acid, 0.2 mol L^{-1}; GQDs, 3-fold diluted; MCP, 10 µg mL^{-1}; flow rate, 2.1 mL min^{-1}; GQDs, 3-fold diluted; length of L_2, 50 cm; sample injected volume, 250 µL.

Investigation on the effect of length of L_2 (in the range of 26-60 cm) indicated a decrease in sensitivity, when the length of coil L_2 was increased. More dispersion is occurred when longer L_2 is used. As can be seen in Fig 5, length of L_2 as 26 cm has produced the best sensitivity. Therefore, the selected length for coil L_2 was 26 cm.

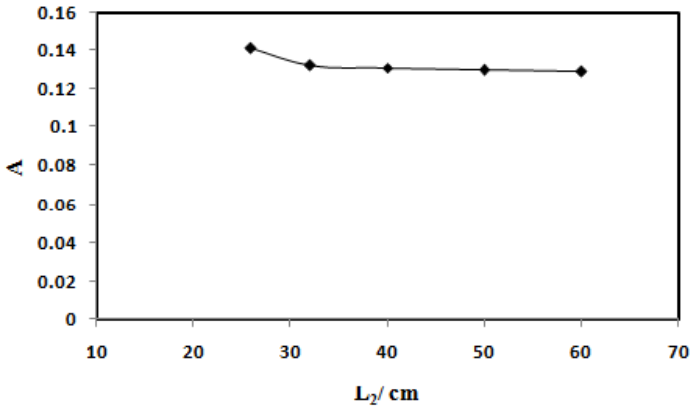


Fig. 5. Effect of length of L_2 on the sensitivity of the method.

Condition: nitrite, 1.0 mmol L^{-1}; hydrochloric acid, 0.2 mol L^{-1}; GQDs, 3-fold diluted; metoclopramide 10 µg mL^{-1}; flow rate, 2.1 mL min^{-1}; length of L_1, 76 cm; sample injected volume, 250 µL.

Flow rate also must be optimized. Various pump speed were applied to reach different flow rates. The results of the study are given in Fig 6. As can be seen, flow rate as 1.4 mL min^{-1} has produced the most sensitivity. When flow rate increased, dispersion is lowered but the reaction times are decreased. For the next studies, flow rate as 1.4 mL min^{-1} was selected.

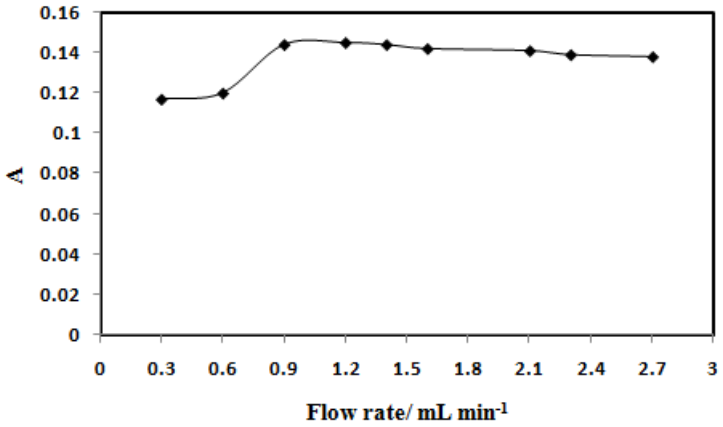


Fig. 6. Effect of flow rate on the sensitivity of the method.

Condition: nitrite, 1.0 m mol L^{-1}; hydrochloric acid, 0.2 mol L^{-1}, GQDs, 3-fold diluted; metoclopramide 10 µg mL^{-1}; length of L_1, 76 cm; length of L_2, 26 cm; sample injected volume, 250 µL.

The injected volume of the samples was varied, and sensitivity was monitored. Peak height increased by increasing the sample volume. Therefore, sensitivity has been increased by increasing the injected sample volume (Fig 7). Therefore, injected sample volume as 500 µL was selected to obtain appropriate sensitivity and rate of analysis.

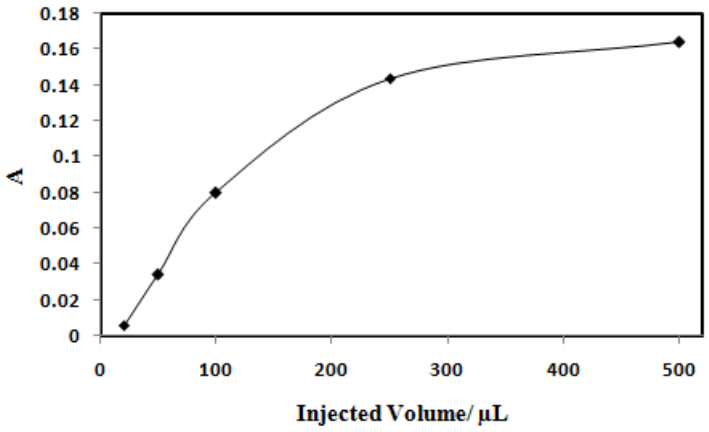


Fig. 7. Effect of sample injected volume on the sensitivity of the method.

Condition: nitrite, 1.0 mmol L^{-1}; hydrochloric acid, 0.2 mol L^{-1}, GQDs, 3-fold diluted; metoclopramide hydrochloride, 10 µg mL^{-1}; flow rate, 1.4 mL min^{-1}; length of L_1, 76 cm; length of L_2, 26 cm.

3.4. Modeling with Central composite experimental design and optimization of parameters

3.4.1. Effect of reagent concentration

In the reaction of metoclopramide, three factors must be optimized. These are concentration of nitrite, GQDs and hydrochloric acid. The purpose of the experimental design is extraction of the maximum amount of information of the system in an economical way (Kincl, Turk & Vrečer 2005). In experimental design, all variables are changed from one experiment to the next, simultaneously. The reason for this is that variables can influence each other and the optimum value for one of them might be related to the values of the others (Gunaraj & Murugan 1999). Full factorial design requires many experiments at multilevel mode. Central composite design (CCD) provides almost the same information that multilevel full factorial design gives, but with fewer experiments (Box, Hunter & Hunter 1978). CCD was originally developed by Box and Wilson (Box & Wilson 1952) and improved by Box and Hunter (Box & Hunter 1957). In CCD, it is assumed that the central point for each factor is 0 and the design is symmetrical around it. Factors and their considered levels of design are shown in Table 1.

Table 1. Experimental factors and their levels investigated.

Factor			Level		
	-1.68 (−α)	-1	0	+1	+1.68 (+α)
Conc. Of HCl (mol L^1)	2.00×10^{-2}	1.170×10^{-1}	26.00×10^{-2}	4.027×10^{-1}	5.00×10^{-1}
Conc. of nitrite (mol L^{-1})	5.00×10^{-5}	4.453×10^{-4}	1.025×10^{-3}	1.605×10^{-3}	2.00×10^{-3}
Dilution of GQDs	15.00	6.50	8.50	2.50	2.00

Results of the optimization of experimental factors have been collected in Table 2.

Table 2. Optimized values of the factors for response during the determination of MCP.

Factors	Optimized values
Concentration of hydrochloric acid (mol L^{1})	0.39
Concentration of nitrite (mol L^{-1})	1.31×10^{-3}
Dilution of GQDs	2-fold

3.5. Analytical data (linearity and sensitivity)

Under the optimized experimental conditions, two calibration curves were constructed covering a concentration range of 0.1-10 μg mL^{-1} and 10-40 μg mL^{-1} at 423 nm (Fig. 8).

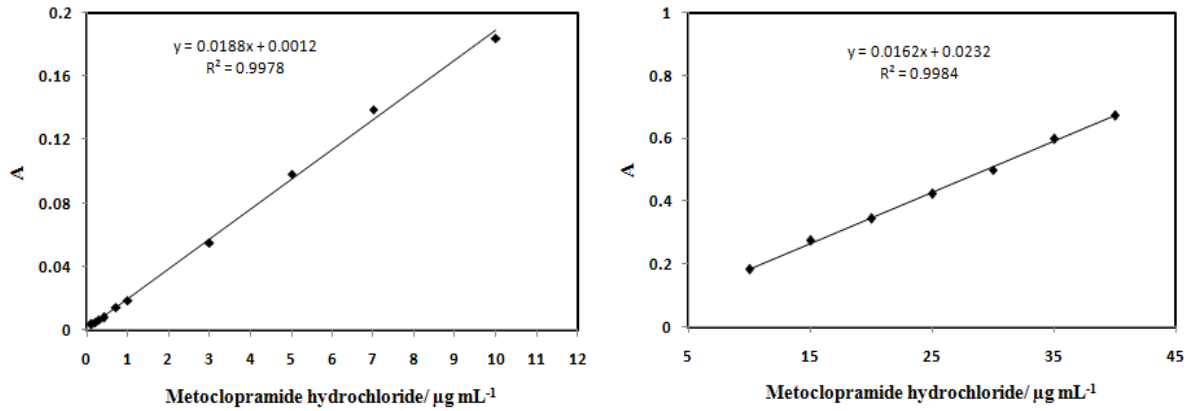


Fig. 8. The Plots of Absorbance vs. Concentration of MCP hydrochloride in μmol mL^{-1}.

The sampling rate comprises 30 h^{-1} for MCP hydrochloride. The repeatability (n=7), expressed by within-run precision, were 6.7%, 2.5%, 1.1%, 1.3% and 1.9% for 0.3, 5.0, 10.0, 10.0 and 35.0 μg mL^{-1} MCP hydrochloride, respectively for the two different linear ranges.

3.6. Interference of the foreign species

The effect of the common species and the additives in pharmaceuticals were investigated in the determination of 1.0 μg mL^{-1} of metoclopramide hydrochloride by the flow method. When the permitted relative deviation from A is less than ±7%, the permissible ratio of co-existing substance are showed in table 3. It is clear that the interference of the foreign species is very small except for urea and thiourea. This is advantageous to the determination of metoclopramide hydrochloride in pharmaceuticals.

Table 3. Effect of foreign species on the determination of nitrite 1.0 µg mL^{-1}.

Species	Tolerance limit (W_{ion}/W_{MCP})
Mg(II), Zn(II), Hg(II), Mn(II), Na(I), K(I), Cd(II), pb(II), CH_3COO^-, SO_4^{-2}, HCO_3^-, NO_3^-, $C_2O_4^{-2}$, Salicylic acid, Serine, tartrate, Glycine, Sucrose , glucose	>250*
NH_4^+, Co(II), Cu(II), Ca(II), Ni(II)	250
Ascorbic acid, L-Cysteine	125
Fe(II), Fe(III)	8
Urea, Thiourea	1

*: Maximum concentration which has been tested.

4. APPLICATIONS

The proposed method was used to the analysis of water samples and tablets. The water samples were examined directly using the procedure proposed without any pretreatment. The results are shown in Table 4. Further proposed method applied to the analysis of certain dosage forms containing metoclopramide hydrochloride in order to evaluate the analytical usefulness of the FIA method. Very good results with excellent recoveries were obtained based on 7 repetitive determinations for the same concentrations of each pharmaceutical preparation. The results are given in Table 5. The amount of active ingredient found in the samples agrees closely with the amount given on the label. The close agreement of the results indicates the applicability of the method to actual samples.

Table 4. Determination of MCP hydrochloride in water samples

Sample	**MCP (µg mL^{-1}) (n=7)**		**Recovery%**
	Taken	Found	
River	-	0.10	-
	1.00	1.16±0.03	106.0
	2.00	2.12±0.11	101.0
Well	-	ND*	-
	1.00	1.05±0.04	105.0
	2.00	2.00±0.09	100.0
Lake	-	ND*	-
	1.00	1.00±0.03	100.0
	2.00	2.12±0.03	106.0

*: Non-determinable

± means standard deviation

Table 5. Analysis of MCP hydrochloride in pharmaceutical preparations.

Sample	MCP (μg mL^{-1}) (n=7)		Recovery%
	Taken	Found	
Tablet 1	2.00	2.04±0.08	102.0
Tablet 2	2.00	2.01±0.07	100.5
Tablet 3	2.00	1.98±0.03	99.0
Tablet 4	2.00	2.13±0.06	106.5

± means standard deviation

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