<u>Regular Article</u>



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Calcium Hexacyanoferrate(II) as a Precipitating Reagent for the Determination of Mebeverine Using Multiple Flow Cells with Summed (S/N) Responses in NAG-4SX3-3D Instrument

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A novel and highly sensitive flow injection method was developed for the determination of Mebeverine. The method is based on the formation of an ion pair compound between Mebeverine hydrochloride and calcium hexacyanoferrate in an ammonium chloride medium, resulting in the formation of a whitish-yellow precipitate complex. The analysis was performed using a homemade NAG-4SX3-3D triple flow cell CFIA Analyzer. The experimental parameters were studied to optimize both the chemical and physical aspects of the method. The linear working range was obtained to be 0.01-40 mM, with an impressive correlation coefficient (r) of 0.9907. The limit of detection (LOD) was determined to be 0.349 μ g/150 μ l, and the repeatability of the method (RSD%) was demonstrated to be less than 1.5%. To further evaluate the performance of the newly proposed method, a comparison was conducted using the standard addition method in conjunction with a classical method. Overall, the developed flow injection method offers simplicity, sensitivity, and reliable analytical performance for the determination of Mebeverine, making it a promising alternative to the classical method.

Keywords: Flow injection analysis, Turbidity, Mebeverine hydrochloride

INTRODUCTION

Mebeverine hydrochloride (4-[ethyl-[1-(4-methoxyphenyl) propan-2-yl] amino]butyl3,4-dimethoxybenzoate) has an antispasmodic action on smooth muscle. Abnormal pain and spam associated with gastrointestinal disorders are mainly treated by Mebeverine hydrochloride [1] (Fig. 1). Many analytical methods were reported for the determination of Mebeverine such as HPLC [2-5], Elroctrochemistry [6], spectrophotometry [7-9], Liquid chromatography [10], potentiometric-flow injection analysis [11], turbidimetricflow injection analysis [12,13]. Flow injection analysis techniques are tools for the reduction of sample and reagent consumption, high sampling frequency, and



Fig. 1. Chemical structure of Mebeverine hydrochloride.

preconcentration, allowing coupling with several detection analytical systems, and improving sensitivity and selectivity in addition to the simplicity of the technique [14]. Timing in flow injection analysis plays an important role due to the selected variable scale of manifold units working procedure as can be adjusted since the main components are peristaltic

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pump, injection valve, coils (and all other separation or oxidation or reduction column) followed by final determination step *i.e.*; the instrument that is used for measurement. Turbidity is the optical measurement of the scattering light that results when light passes through a transparent medium and interacts with undissolved particles suspended in a liquid solution (most commonly water). It can be used in many fields of analytical chemistry to determine the concentration of solid particles in a sample and/or particle size distribution. It is a very qualitative measurement but, turbidity has the potential to become more quantitative. The intensity and direction of light scatter from solid material depends on the refractive index, wavelength of transmitted light, size, color, shape, concentration of the number of particles in solution, and the color of the suspending media. When measuring the scattered light by particles at right angles to the beam of incident light the measurements are called nephelometry (the angle between the detector and incident beam is 90°) [15,16]. A few published research has been reported on the use of turbidimetric-flow injection analysis in the estimation of pharmaceutical drugs [17-21]. The present study deals with the design of a homemade instrument (NAG-4SX3-3D-contineuos flow injection Analyzer) [22] for the measurement of the attenuation of incident light signals, resulting from the reaction of Mebeverine hydrochloride with calcium hexacyanoferrate. The instrument is characterized by simplicity, high sensitivity, low detection limit, and high repeatability of the results obtained.

EXPERIMENTAL

Chemicals

All the solutions of calcium hexacyanoferrate, ammonium chloride, sodium carbonate, sodium chloride, ammonium acetate, sodium nitrate, potassium iodide, acetic acid, ascorbic acid, tartaric acid, hydrochloric acid, and nitric acid were analytical grade unless otherwise stated. Mebeverine hydrochloride (MB. HCl) standards solutions were provided by Samara, Ltd. Iraq.

Commercial drugs (samples of MB.HCl) of Duspatalin[®] (Abbott, France), Meva[®] (Jamjoom Pharma, Saudi Arabia), and Colospasmin[®] (Eipico, Egypt) tablets were obtained from local drugstores. 20 tablets weighed out and ground.

Samples contain 135 mg of MB which weighs (0.6602, 0.6959, and 0.6182) g corresponding to 0.233 g of active ingredient (0.005 M) for MB in 100 mL volumetric flask.

Apparatus and General Procedure

NAG-4SX3-3D CFI Analyzer is regarded as a unique multiple continuous flow cell that can be used for flow injection analysis which is made to measure the turbidity at 0-180° angle [22].

The main measuring unit of the instrument is composed of four distinct parts can be followed:

- The whole system is placed in a brass structure surrounded, covered, and protected with non-transparent opaque lather or silicon rubber to eliminate the stray and scattered light that might reach the detector from an external source.

-Four sources of white snow LEDs were used.

-A triplet flow cell composed of three flow cells made of clear borax glass in the form of a tube, each of 60 mm length inlet diameter of 2 mm connected to this tube with a 600 mm -Teflon tube (hydrophobic properties) in two stages. Each stage is 300 mm in length of 1 mm inside diameter. Different diameters for different distances and different materials caused different fluid speeds and delayed movement causing much homogenization and good mixing of the fluid stream containing precipitated particles with one single response.

-Detector: Three detectors represented by three solar cells were used.

The Manifold system includes:

-Two-channel peristaltic pump type ISM796 (Switzerland).

-Connection tubes (silicon rubber or polypropylene)

-Rotary six ports injection valve (IDEX Corporation, USA)

-Y-junction point: homemade poly methylmethacrylate liquid junction point

-Readout system: x, y-t plotter (KOMPENSO GRAPH C-1032) Siemens (Germany)

Mebeverine hydrochloride assessment was based on the use of calcium hexacyanoferrate (CHCF) as a precipitating reagent. A whitish yellow precipitate is formed probably as an ion pair using two lines manifold design (Fig. 2). The first line is for ammonium chloride (20 mM, 1.7 ml min⁻¹) as a carrier stream passing through a sample loop (150 μ l) to carry Mebeverine hydrochloride segment (7 mM) while the second line stream is a precipitating reagent (10 mM, 1.7 ml min⁻¹). The two lines meet at the Y-junction point and then at the



Fig. 2. Two-line flow feed unit for the determination of Mebeverine hydrochloride.



Scheme 1. Simplified scheme of the proposed reaction mechanism for the ion pair formation between Mebeverine and calcium hexacyanoferrate

reaction coil (100 μ l) leading to measuring a turbidimetric S/N *via* the use of a NAG-4SX3-3D Analyzer. The proposed reaction mechanism is as follows in Scheme 1.

RESULTS

Chemical Variables

The initial concentrations for the MB-CHCF system are as follows: the concentration of MB was 7 mM and the sample loop volume was 85 μ l; with open valve mode. 1.2 ml min⁻¹ flow rate for each line. Each measurement was repeated three times.

Effect of calcium hexacyanoferrate concentration. A study was conducted to optimize the concentration of calcium hexacyanoferrate (precipitating reagent) using the



Fig. 3. Effect of CHCF concentration.

1-13 mM concentration range. Figure 3 indicates the variation of S/N with a variation of CHCF concentration using NAG-4SX3-3D Analyzer reaching 10 mM > 10 mM leads to a decrease of S/N which can attributed to an increase of density of the MB-CHCF product leading to restriction of the flow. 10 mM of CHCF concentration was regarded as the optimum concentration for the determination of MB that was used for subsequent experiments.

Effect of medium reaction. The effect of various acids, salts, and deionized water (D.W) as a carrier stream on S/N was examined. The obtained results indicated (Fig. 4) that NH₄Cl was found to be the most appropriate carrier stream than other media which gave lower responses this might be attributed to the increase in the amount of particles in the presence of NH₄Cl as a catalyst for MB-CHCF ion pair formation. So NH₄Cl was chosen as the best medium for completing the reaction of CHCF with MB.

Effect of ammonium chloride concentration. The effect of NH₄Cl (carrier stream) concentration on the turbidimetric measurements was checked by recording the S/N of the NAG-4SX3-3D for solutions containing 5-50 mM of NH₄Cl and 7 mM MB.HCl-10 mM CHCF system. The plot of S/N versus NH₄Cl concentrations (Fig. 5) explains an increase in the S/N with an increase of NH₄Cl concentration up to 20 mM then decrease in S/N at > 20 mM might be the dissociation of some of the precipitate particles. Therefore 20 mM was the best concentration of ammonium chloride stream.



Fig. 4. Effect of different media (salts (A) and acids (B)) on S/N.



Fig. 5. Effect of [NH₄Cl] on S/N.

Physical Parameters

The physical parameters that affect the pattern of reaction in achieving high sensitivity and reliable measurements were examined. As the sample moves the effect of loading mode causes the movement of the MB segment through the stream of carrier solution, and as the sample segment moves it will suffer a dilution as soon as it leaves the sample loop until the measuring instrument unit. Therefore, the calculation of the dilution factor was taken into consideration.

Flow rate. Under optimal chemical conditions, the flow rate in the range of 0.7-2.5 ml min⁻¹ of both lines was checked. The experimental results showed (Table 1) that the peak height increased with an increase in flow rate up to 1.7 ml min⁻¹ for both lines. Maximum S/N was observed at 1.7 ml min⁻¹ for the carrier stream and CHCF stream. Therefore 1.7 ml min⁻¹ was found to be the most suitable flow rate to compromise between peak shape, and consumption of chemicals with the best analysis time (complete the precipitate reaction).

Sample volume. The effect of sample volume was studied by varying the sample loops from 50-200 μ l. Considerable responses were observed when the sample loop increased with increased analysis time (Table 2) and long duration of precipitate particles in front of three solar cells (detector) with increased particle size causing a slow movement of precipitate species. Hence 150 μ L was selected as a suitable sample loop and gave regular responses for quantitative purposes.

Reaction coil. To investigate the reaction coil effect on the completion of the reaction between MB and CHCF in the ammonium chloride medium, different coil lengths ranging from 13-28 cm (after the junction point) were used. The results of this study (Table 3) show that the S/N increased with increasing reaction coil length and increasing base width of response Δt_B up to 100 µl (13cm). It can be seen clearly that 25-38 cm will increase peak base width due to the increase of dispersion and accumulation of precipitate particles, so 13 cm (100 µl) length as reaction coil was chosen for further analysis.

Linear Range and Detection Limit

Under the optimum conditions, there is a linear relationship between the analytical result and MB concentration in the range of 0.01-40 mM (r = 0.9907, n = 17).

Flow rate for each line	$S/N \pm t_{0.025,2} \; \sigma/\sqrt{n}$	t ^a	$\Delta t_{\rm B}{}^{\rm b}$	${ m V}_{ m add}{ m c}$	\mathbf{C}^{d}	D.F ^e
(ml min ⁻¹)	$(t_{0.025} = 4.303)$	(s)	(s)	(ml)	(mM)	
	(mV)				$(C_{initial} = 7 \text{ mM})$	
0.7	600 ± 3.21	30	45	1.135	0.524	13.36
1.2	617 ± 2.98	28	39	1.645	0.362	19.34
1.5	637 ± 3.17	25	35	1.835	0.324	21.60
1.7	665 ± 2.97	23	33	1.955	0.304	23.03
1.9	600 ± 3.05	22	30	1.985	0.299	23.41
2.1	587 ± 2.75	21	28	2.045	0.291	24.05
2.3	530 ± 2.55	20	27	2.155	0.276	25.36
2.5	498 ± 2.72	18	25	1.585	0.375	18.67

Table 1. Effect of Flow Rate

^aDeparture time for MB segment from sample loop to measuring cell. ^bBase width of response. ^cVolume of the sample at the flow cell. ^dConcentration of sample at the flow cell. ^cDilution factor.

Sample volume	$S/N \pm t_{0.025,2} \; \sigma/\sqrt{n}$	$\Delta { extsf{t}_{ extsf{B}}}^{ extsf{a}}$
(µl)	$(t_{0.025} = 4.303)$	(s)
	(mV)	
50	388 ± 2.97	27
85	666 ± 3.10	33
100	680 ± 3.23	35
125	690 ± 3.67	36
150	710 ± 3.23	38
175	720 ± 3.58	39
200	725 ± 3.86	40

Table 2. Effect of Sample Volume

^aDeparture time for MB segment from sample loop to measuring cell.

Reaction coil	$S/N \pm t_{0.025,2} \; \sigma/\sqrt{n}$	$\Delta t_{ m B}{}^{ m a}$	$V_{add}{}^{b}$	C^{c}	$D.F^d$
μl (cm)	$(t_{0.025} = 4.303)$	(s)	(ml)	(mM)	
r = 0.5 mm	(mV)			$(C_{initial} = 7 \text{ mM})$	
Without coil	710 ± 3.14	38	2.303	0.456	15.35
100(13)	780 ± 3.10	39	2.460	0.427	16.39
200(25)	777 ± 3.57	45	2.900	0.362	19.34
250(32)	776 ± 4.23	49	3.177	0.331	21.48
350(38)	773 ± 4.97	53	3.503	0.299	23.41

Table 3. Effect of Reaction Coil

^aBase width of the response. ^bVolume of the sample at the flow cell. ^cConcentration of sample at the flow cell. ^dDilution factor.

The repeatability of the NAG-4SX3-3D CFI Analyzer was checked with two concentrations (7 and 15 mM) n = 7 and the coefficient of variation was less than 1.5%. The LOD of standard MB by the proposed method was determined using a gradual dilution of minimum concentration in the linear range and slope method (based on the value of slope). Table 4 summarizes all results of linearity and LOD.

Analysis of Commercial Samples and Statistics

Three samples of MB (label claim 135 mg) were analyzed by the NAG-4Sx3-3D CFI Analyzer and by the UV-Vis spectrophotometric classical method ($\lambda_{max} = 219$ nm). The accuracy of the methods was analyzed by applying the standard addition procedure (transferring 2 ml of 5 mM sample solution to 5 volumetric flasks followed by gradual volumes of 0, 0.4, 0.6, 0.8, and 1 ml of standard solution (50 mM)). The results were obtained and tabulated in Table 5.

A comparison was made between the two methods via the use of paired t-test (P = 0.05) and the ANOVA one-way test was carried out at a 95% confidence level for comparison between different the mean of samples. The results (Table 6) show undistinguishable differences between the two methods (t_{cal} (-3.946 << t_{tab} 4.303) and no difference between three different samples (0.520 > 0.05).

Tabl	e 4.	Summary	of	Linear	Equation	and	LOI	D
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Results parameter	Obtained value from the proposed method	
Linear range	0.01-40 mM	
Slope	85.272	
Intercept	144.092	
r ^a	0.9907	
r ^{2 b}	0.9815	
r ² % °	98.15%	
$\mathrm{LOD}^{\mathrm{d}}$	0.349 µg/150 µl (0.005 mM)	
LOD ^e	3.69 µg/150 µl	

^aCorrelation coefficient. ^bCoefficient of determination. ^cPercentage linearity. ^dLimit of detection depending on gradual dilution. ^eLimit of detection depending on the slope method.

Table 5. The Analytical Results of Two Methods by Standard Addition Technique

No.	Commercial samples	Theoretical content ^a	Practical content in tablets	Practical content in tablets
		(mg)	(mg)	(mg)
			(Recovery%) ^b	(Recovery%) ^c
1	Duspatalin®	135 ± 1.165	134.344	140.869
	Abbott		(99.51)	(104.35)
	France			
	Meva®	135 ± 0.938	130.445	140.048
2	Jamjoom pharma		(96.62)	(103.74)
	Saudi Arabia			
	Colospasmin®	135 ± 1.959	139.545	143.329
3	Eipico		(103.37)	(106.17)
	Egypt			

Table 6. Paired	l t-test and	l ANOVA	Results
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Paired t-test	ANOVA one-way					
$t_{cal}\!=\!\overline{X}_{\!d}\;{}^a\!\sqrt{n}\!/\!\sigma_{n\!-\!1}\;{}^b$	Source	SSq ^c	df^d	MSq ^e	F	Sig.
	Between groups	26.029	2	13.014	0.731	0.520
-3.946 < 4.303	Within groups	106.749	6	17.791		

 ${}^{a}X_{d}$ Difference means. ${}^{b}\sigma_{n-1}$ Difference standard deviation. ${}^{c}Sum$ of squares. ${}^{d}degree$ of freedom and ${}^{e}Mean$ of square.

CONCLUSION

A flow injection-turbidimetric method was developed for the assessment of MB using calcium hexacyanoferrate in an ammonium chloride medium, resulting in the formation of a whitish-yellow color precipitate. The analysis was performed using a homemade instrument. The utilization of the NAG-4SX3-3D CFI Analyzer proved to be highly successful, demonstrating excellent repeatability of response, a wide linear range, a low detection limit, and cost-effectiveness compared to other methods.

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