



## Continuous Flow Injection Analysis for The Photometric Determination of Metformin Drug Via The Release of Copper(II) ion from Charged Gel Bead Crystal

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### Abstract

A newly developed analytical method characterized by its speed and sensitivity for the determination of metformin-HCl via the formation of complex for metformin-HCl-OH<sup>-</sup>-copper(II) ion from the gel bead system by continuous flow injection analysis. The method is based on the imbedded copper(II) ion in the gel bead structure can be used in the reaction for the formation of red –magneta colour complex ( $\lambda_{max} = 530\text{nm}$ ) formed by direct reaction of the drug with the released copper (II) ion from the gel bead in alkaline medium. Linear dynamic range for the absorbance versus metformin concentration was  $0.001-1 \text{ mmol.L}^{-1}$  while C.O.D was ( $r^2\% = 95.33\%$ ). The L.O.Q was  $0.868 \text{ mmol.L}^{-1}$ . L.O.D ( $S/N=3$ ) =  $0.5 \mu\text{mol.L}^{-1}$  from the step wise dilution for the minimum concentration of lowest concentration in the linear dynamic ranged of the calibration graph with R.S.D% lower than 0.5% for  $0.01 \text{ mmol.L}^{-1}$  ( $n=8$ ) concentration of metformin- HCl, throughput  $30 \text{ sample.hr}^{-1}$ . The method was applied successfully for the determination of metformin in three pharmaceutical drugs. A comparison was made between the newly developed method of analysis with the classical method (Uv-spectrophotometry at wave length =  $241\text{nm}$ ) of analysis using the standard addition method via the use of paired t-test. It shows that there was no significant difference at  $\alpha = 0.05$  (95% confidence) between the two methods. Therefore the newly developed method (using the Metformin- OH<sup>-</sup> - Cu(II) entrapped inside gel bead) can be adopted as an alternative method for the analysis of metformin.

**Keywords:** Flow injection analysis, Metformin-HCl, Spectrophotometric method.

## التحليل الفوتومتري بالحقن الجرياني المستمر لعقار الميتافورمين من خلال تحرير ايون النحاس ( II ) من بلورة حبة الجيل المشحونة

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### الخلاصة

تم تطوير طريقة تحليلية سريعة وحساسة لتقدير الميتافورمين بواسطة تكوين معقد لنظام - ميتافورمين - هيدروكسيد الصوديوم -ايون النحاس(II) المحتوى داخل حبة الجيل وباستخدام منظومة الحقن الجرياني. استندت الطريقة على تكوين معقد بين ايون النحاس المزاح من حبة الجيل والعقار الميتافورمين في وسط

قاعدي وبلون عنابي محمر والذي يمتص عند 530 نانومتر. تم الحصول على علاقة لتغير الامتصاصية مع التركيز للميتافورمين باستخدام معادلة الخط المستقيم وكان مدى منحنى المعايرة (1-0.001) مللي مول. لتر<sup>-1</sup> ومعامل التقدير (C.O.D) (%<sup>2</sup> = 95.33%)، اما حد التقدير الكمي (L.O.Q) 0.868 مللي مول . لتر<sup>-1</sup> ويحد كشف (S/N=3) 0.5 مايكرو مول. لتر<sup>-1</sup> من التخفيف التدريجي لاقبل تركيز في منحنى المعايرة والانحراف القياسي النسبي المئوي اقل من 0.5% لتركيز 0.01 مللي مول. لتر<sup>-1</sup> ول n=8 لمحلول الميتافورمين ومعدل النمذجة 30 انموذج/ ساعة . طبقت الطريقة لتقدير الميتافورمين في ثلاث نماذج من المستحضرات الصيدلانية . اجريت مقارنة بين الطريقة المستحدثة والطريقة التقليدية للقياس الطيفي (مطيافية الاشعة فوق البنفسجية وعند طول موجي 241 نانومتر) باستخدام نتائج منحنى الاضافات القياسية وذلك باختصاصها لاختبار t- المزدوج وبين انه لا يوجد فرق جوهري بين الطريقتين وبالامكان استخدام نظام: ميتافورمين- OH<sup>-</sup> - ايون النحاس(II) المحتوى داخل حبة الجيل كبديل اقل كلفة في استهلاك المواد الكيميائية مقارنة بالطريقة التقليدية.

## Introduction

Metformin hydrochloride (MTF-HCl) chemically 1,1-dimethylbiguanide hydrochloride is white crystalline powder, hygroscopic and freely soluble in water, used as a hypoglycemic drug[1,2]. MTF-HCl is an oral biguanidine, which reduces the elevated blood glucose concentration in patients with diabetes but dose not increase insulin secretion. It dose not lower the blood glucose in non-diabetic subjects[3]. Augmentation of muscular glucose uptake and utilization, and reduction of increased hepatic glucose production through an anti gluconergic action explain the blood glucose lowering effect[4]. MTF-HCl is safe and not teratogenic in many of the species studied[5]. Several methods are available for determination of MTF-HCl either alone or in combination with various drugs in bulk, pharmaceutical preparations and biological fluids, Spectrophotometric methods were developed for the determination of MTF-HCl via charge-transfer complex with iodine[6]. The primary amino group of MTF-HCl was oxidized using hydrogen peroxide to form a yellow chromogen, which is determined spectrophotometrically at 400 nm[7], conductometric titrations are based on the copper-biguanide reaction in the basic medium which gives a pink soluble complex[8]. Poly(vinyl chloride) (PVC) matrix membrane sensors based on the use of ion association complexes of metformin with tetra phenyl borate, tungstophosphate and molybdophosphate have been used for direct potentiometric determination of MTF-HCl[9]. Many HPLC method with UV detection is described for determination of MTF-HCl in plasma samples, chromatographic separation

was performed at 40°C by pumping a mobile phase of a mixture of phosphate buffer and acetonitrile through a silica column[10] or on a reversed-phase phenyl column[11]. Chemiluminometric determination of MTF-HCl was implemented on the basis of induced inhibition (metformin acts as a Cu(II) scavenger) of the catalytic effect of Cu(II) ions on the reaction [12]. Metformin have two imine groups in cis position thus acting as a chelating agent, it posses an excellent capacity for coordination with many elements of the transition series thus giving highly colored chelate complexes, especially Cu(II), Ni(II), Co(II) and Pt(II). As these metal ion complexes can be measured spectrophotometrically[13-15].

In this study, the use of new mode of continuous flow injection analysis comprising the entrapment of copper(II) ion inside the water crystal gel bead for the determination of metformin concentration in pharmaceutical preparation. A single gel bead (or many) located in a specially designed cell which aims to the liberation of copper(II) ion from this water crystal to the carrier stream for the completion of the reaction of the metformin complex with copper(II) ion in alkaline medium[16].

## Experimental

### • Chemicals

All chemicals were of analytical reagent grade and distilled water used to prepare solutions.

MTF-HCl stock standard solution (C<sub>4</sub>H<sub>12</sub>N<sub>5</sub>.Cl, 165.63 g.mol<sup>-1</sup>, SDI, 100 mmol.L<sup>-1</sup>) was prepared by dissolving 4.1408 g /250mL distilled water. A 100 mmol.L<sup>-1</sup> NaOH solution (BDH) was prepared by dissolving

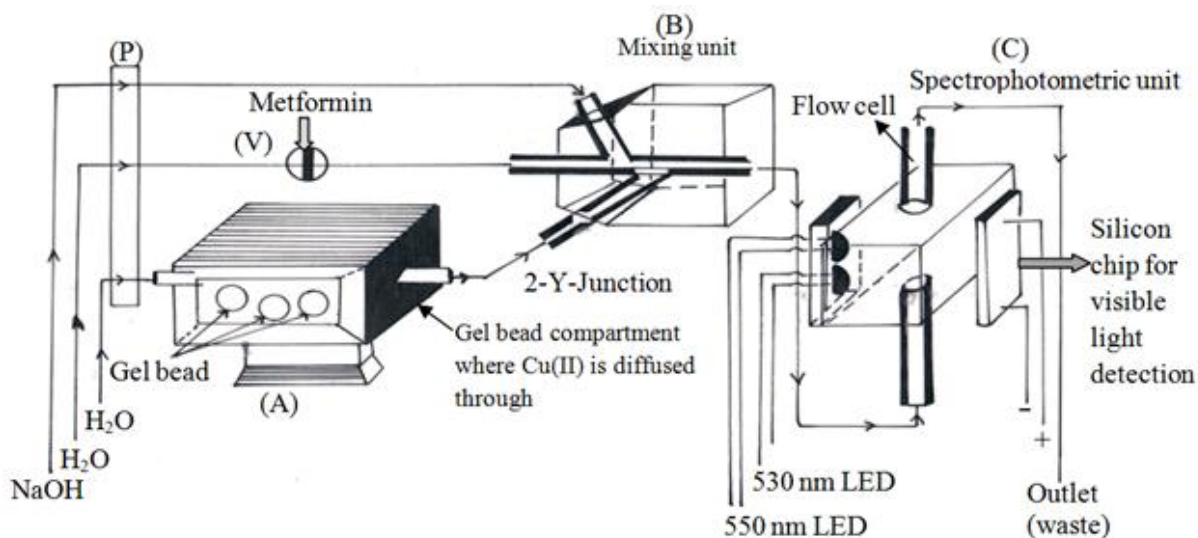
approximately more than 4.0g of NaOH was washed using pre-boiled distilled water, then dissolved in pre-boiled distilled water to 1 L. This solution was standardized with standard hydrochloric acid which was prepared previously to obtain  $100 \text{ mmol.L}^{-1}$  NaOH. A stock solution of Cu(II)(BDH),  $1000 \mu\text{g.mL}^{-1}$ : 1.90116g from  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  in 500mL distilled water for the supply of copper(II) ion that is encapsured into the water crystal gel as shown in Figure.1.

### • Sample Preparation

Thirteen tablets chosen randomly for each drug from different strips and packets (to ensure complete randomness). The tablets were crushed, and grinded to fine mist; followed by weighing an amount equivalent to 0.1656g active ingredient (0.01M) for each pharmaceutical preparation. The powder was dissolved in deionized water followed by filtration to remove any undissolved residue affecting the response. The filtrate was completed to 100 mL volumetric flask. 1.5 mL of this solution was transferred to each of the five 100 mL volumetric flask with addition of gradual increase of the standard metformin solution to obtain 0.0, 0.05, 0.07, 0.1, 0.3 and 0.5  $\text{mmol.L}^{-1}$ . Both spectrophotometric methods and developed methods were used

### Apparatus and Manifold

The flow system used for the determination of MTF-HCl, shown schematically in Figure.1 [17]. A peristaltic pump: three channels (Ismatec, Switzerland), 6-port medium pressure injection valve (upchurch scientific, U.S.A) with a sample loop (0.5 mm i.d, Teflon, variable length). The gel bead unit cell that contains the water crystal containing copper(II) ion as shown in Figure.1A; which is made of poly methyl methacrylate  $20 \times 30 \text{ mm}$ , 22mm thickness with all necessary 3-way valve system to insure ideal use of charging the water crystal bead with copper(II) ion and using the charged bead for completion of reaction for metformin- $\text{OH}^-$  gel bead charged with copper(II) ion system. Mixing unit is formed of three inlets with one out put as shown in Figure. 1B. This unit insure mixing in the required order. The microphotometer is supplied with two light emitting diod having 530, 550nm with a selective use of either source with a variable intensity monitored with  $1\text{k}\Omega$  and a photosilicon detector as shown in Figure 1C. The signal was recorded by recorder (Siemens, Germany, range (1-500)mV or (1-500)volt). UV spectra were measured with an UV-VIS (CARY 100 conc.) spectrophotometer (Japan).



**Figure 1-** Schematic flow gram for the determination of metformin P: Peristaltic pump V: Injection valve

### Methodology

Metformin flow injection system Figure.1 for the reaction of  $\text{MTF-HCl-OH}^-$ -Cu(II) is composed of three lines: the first line supply sodium hydroxide solution ( $5 \text{ mmol.L}^{-1}$ ) at 1.5

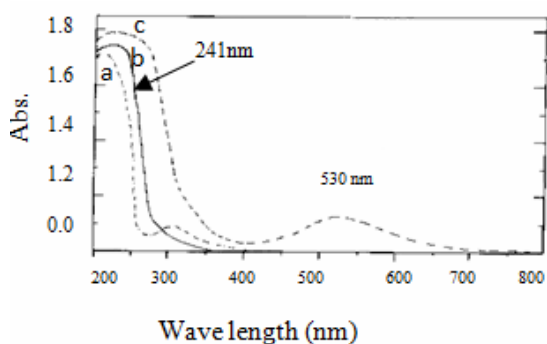
$\text{mL.min}^{-1}$  flow rate; while the second line is the carrier stream (distilled water) which leads to the injection valve to carry metformin sample of  $50 \mu\text{L}$  at  $1.25 \text{ mL.min}^{-1}$  flow rate; while the third line is also a carrier stream (distilled water)

which leads to the gel bead cell unit that is the supply of copper(II) ion Figure.1A at 1.5 mL.min<sup>-1</sup> flow rate. The drug solution mixes with hydroxides ion then followed by copper(II) ion via a specially home designed mixing unit (made from poly methyl methacrylate polymer) which has three inlet that mixes the solution in a required order leading to a single outlet. (i.e formation of red-magenta solution. The outlet is fed directly to the measuring flow injection cell at  $\lambda$ 530 nm [16]. Sampling rate 30 sample.hr<sup>-1</sup>.

## Results and Discussion

### Spectroscopic Study of Metformin Complex System

Figure 2 shows the various spectrum obtained for (a) Cu (II), (b) metformin and (c) MTF-OH-Cu (II) system. It shows the disappearance of both absorption maxima of metformin and Cu (II). It might be attributed to the total consumption of both reactant and involves reaction of metformin with Cu(II) in basic medium to form Cu(II)-metformin complex having red-magenta coloured which gave a maximum absorption at 530nm [17].



**Figure 2-** Absorption spectra for all chemical used for the determination of metformin using optimum experimental parameters: Metformin (1.2 mmol.L<sup>-1</sup>)-OH<sup>-</sup> (8mmol.L<sup>-1</sup>)-Cu(II) (50 $\mu$ g.mL<sup>-1</sup>).

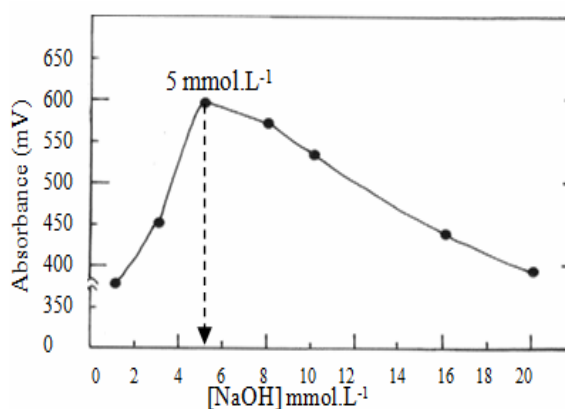
- a:** Absorption spectra (---) for Cu(II) in aqueous medium.  
**b:** Absorption spectra (—) for Metformin in aqueous medium.  
**c:** Absorption spectra (-----) for Metformin-OH<sup>-</sup>-Cu(II) system.

### Optimization of Experimental Conditions

#### Chemical Variable

##### Effect of NaOH Concentration

A series (1-20)mmol.L<sup>-1</sup> of sodium hydroxide solution were prepared. A selected concentration of metformin having the concentration of 0.7 mmol.L<sup>-1</sup> was also prepared. The absorbance was plotted vs. concentration of sodium hydroxide solution as shown in Figure.3, which shows that optimum concentration of sodium hydroxide solution was 5 mmol.L<sup>-1</sup>. This might be attributed that the increase of the base increase the duration of the



**Figure 3-** Effect of variation of sodium hydroxide conc. On absorbance, injection volume 50 $\mu$ L

coloured species for metformin complex in the measuring cell and on this basis it was noticed during the measurement that there is an broadening of the maxima and the base of the response. In addition to that copper(II) might be precipitated and it is one of the reactant for the formation of metformin complex.

#### Physical variable

##### Variation of Flow Rate

Using the system for the determination of metformin via the :MTF (0.7mmol.L<sup>-1</sup>)-OH<sup>-</sup> (5mmol.L<sup>-1</sup>)-gel bead charged with copper(II) ion; with variable flow rates controlled by the peristaltic pump as shown in Figure.1 The results obtained were summarized in Table.1. It was noticed that at slow flow rates, the dilution factor will affect peak profile in every aspect characterized by broadening of the base as well as the peak maxima. While when using high flow rates, not enough time is given for the diffusion of copper (II) ion from the gel bead

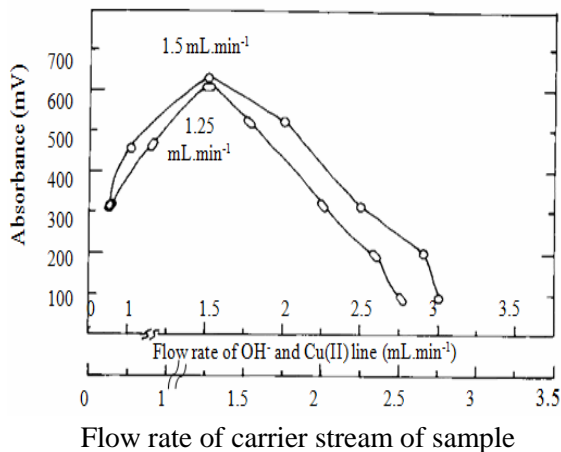
due to successive equilibrium that is necessary to supply enough copper to the reaction medium. This cause a decrease in the absorbance as shown in Figure.4A and broadening in the response of the base as shown

in Figure.4B. A flow rate of 1.5 mL.min<sup>-1</sup> was chosen for the hydroxide and the carrier stream(i.e transporting copper(II) ion from gelbead cell), while 1.25 mL.min<sup>-1</sup> was chosen for the metformin line

**Table 1-** Effect of the variation of flow rate on MTF-OH<sup>-</sup> - gel bead charged with copper(II) ion system.

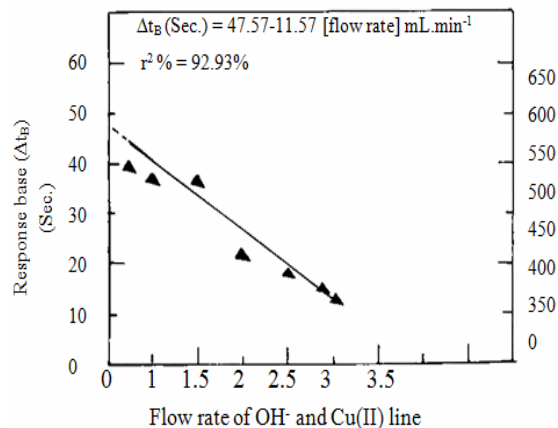
Peristaltic pump speed (indication approximate)	Flow rate (mL.min <sup>-1</sup> )			Response n=3 $\bar{y}$ (mV)	Peak base width $\Delta t_B$ (sec.)	t (sec.)
	OH <sup>-</sup>	Carrier stream of metformin	Carrier stream of copper(II)			
10	0.5	0.25	0.5	324	0.65	18
15	1.0	0.9	1.0	468	0.62	15
20	1.5	1.25	1.5	630	0.60	14
25	2.0	1.5	2.0	526	0.35	12
30	2.5	2.0	2.5	320	0.30	11
35	2.9	2.3	2.9	200	0.25	9
40	3.0	2.5	3.0	96	0.20	6

t = time for the departure of sample segment from injection valve reaching to the measuring cell



**Figure 4A-** Effect of variation of flow rate on absorbance using: MTF-OH<sup>-</sup> -gel bead charged with copper(II) ion.

- Flow rate for both OH<sup>-</sup> and the carrier stream of Cu(II) line.
- Flow rate for the carrier stream of sample, injection volume 50μL

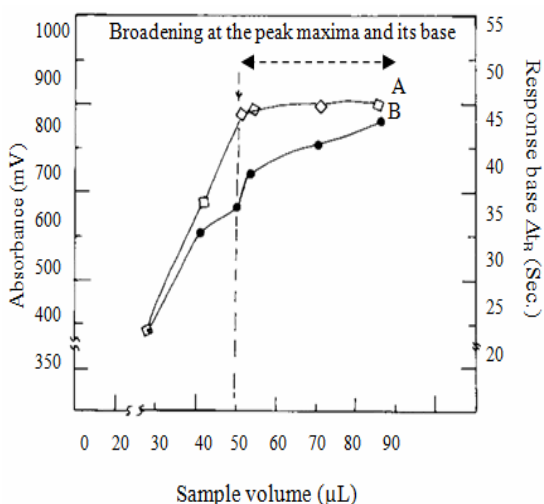


**Figure 4B-** Effect of variation of flow rate on base width ( $\Delta t_B$ ).

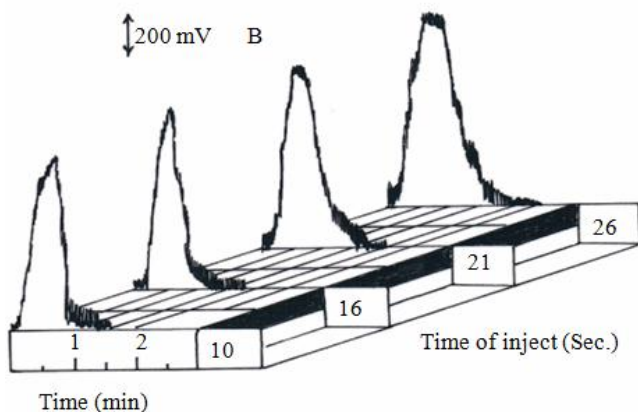
**Effect of Sample Volume**

Using MTF (0.7mmol.L<sup>-1</sup>)-OH<sup>-</sup>(5mmol.L<sup>-1</sup>) -gel bead charged with copper ion system, with the optimum flow rate. A variable volume (39-100) μL was used for this study, the plot of absorbance vs. change in sample volume is shown in Figure 5A. while Figure 5B shows the variation of the absorbance vs.  $\Delta t_B$  of the response. It shows that the optimum volume is 50μL for better response profile. While increasing sample volume more than 50μL

gave irregular peak response increase in  $\Delta t_B$  as shown in Figure 5B.



**Figure 5-** Effect of variation of sample volume segment of metformin on:



**Figure 6 -** Effect of variation of injection time on A: Absorbance of metformin complex B: Response profile Using optimum parameter: MTF (0.7mmol.L<sup>-1</sup>)-OH(5mmol.L<sup>-1</sup>)-gel bead charged with copper(II) ion (100μg.mL<sup>-1</sup>) system , sample volume 50 μL

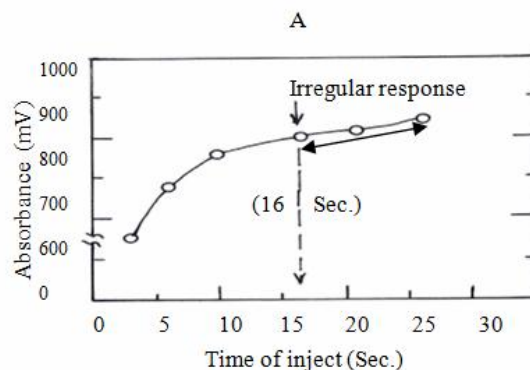
**Calibration Graph**

Series of metformin solution ranging from (0.001-5)mmol.L<sup>-1</sup> were prepared for the purpose of using them for the preparation of scatter plot followed by the choice of calibration graph. All physical and chemical variables were fixed at their optimum values. The results obtained were tabulated in Table 2A and was represented in Figure 7. which shows

**A:◇◇:Absorbance of Metformin complex.**  
**B:●●:Broadening of its base width, flow rate 1.5 mL.min<sup>-1</sup> for OH & carrier stream of Cu(II & 2mL.min<sup>-1</sup> for carrier stream of sample.**

**Effect of Purge Time**

A study was carried out to determine the optimum duration of the injection time i.e. allowed permissible time for purging of the sample from the injection valve unit. 3-26 seconds were used to support this study . Figure 6A shows that optimum purge time is 16 seconds . using purge time of more than 16 seconds, gave a distorted peak profile as shown in Figure 6B. As the resistance in the injection valve might be effect the physical variable to more or to a lesser extent.

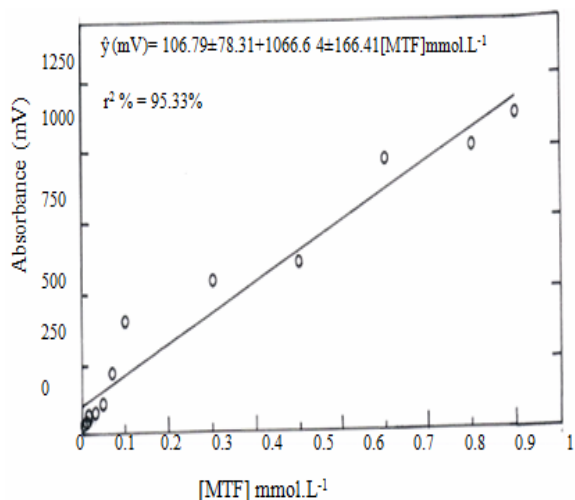


the variation of response with concentration of metformin. Analysis of variance was carried out as shown in Table 2B which indicate that  $F_{tab.} = F_{v2} = F_{10} = 6.94 \ll F_{stat.}(203.95)$ .

Therefore it can be concluded that there is a significant relation between the concentration variation of metformin and the response obtained.

**Table 2A-** Summary of calibration graph results for the determination of metformin using MTF-OH<sup>-</sup>-gel bead charged with copper(II) ion.

Measured [MTF] mmol.L <sup>-1</sup>	[MTF] range for (n=12) mmol.L <sup>-1</sup>	$\hat{y}(mV) = a \pm tS_a + b \pm tS_b [MTF] \text{ mmol.L}^{-1}$ at confidence interval 95%, n-2	r, %r <sup>2</sup>	t <sub>tab.</sub>	$t_{cal} = \frac{ r \sqrt{n-2}}{\sqrt{1-r^2}}$
				at 95%, n-2	
0.001-5	0.001-1	$106.79 \pm 78.31 + 1066.64 \pm 166.41 [MTF] \text{ mmol.L}^{-1}$	0.9764 95.33	2.228 << 14.29	



**Figure 7-** Effect of variation of [MTF] on absorbance at optimum conditions MTF (variation of conc.)-OH<sup>-</sup>(5mmol.L<sup>-1</sup>)-Gel bead charged with copper ion (1000µg.mL<sup>-1</sup>), flow rate: 1.5 mL.min<sup>-1</sup> for NaOH line and 1.25mL.min<sup>-1</sup> for both carrier stream(copper (II) ion and Metformin segment), sample volume 50µL, time for injection 16 Sec.

**Table 2B-** ANOVA[18-20] for linear equation results

Source	Sum of squares	Df	Mean square	F <sub>stat.</sub> = S <sub>1</sub> <sup>2</sup> /S <sub>2</sub> <sup>2</sup>
Regr( $\hat{y}_i - \bar{y}$ )	1748968.2	$\nu_{1=}$ 1	1748968.2	203.948
Error( $y_i - \hat{y}_i$ )	85755.46	$\nu_{2=}$ 10	8575.546	
Total ( $y_i - \bar{y}$ )	1834723.7	11		

Study of the detection limit for the metformin drug using four different ways of its stimation was carried out all. Values were tabulated in Table 3, in addition of calculation of limit of quantitation.

**Table 3-** Limit of detection of metformin at optimum parameter for MTF-OH<sup>-</sup>-gel bead charged with copper(II) ion, sample volume 50µL.

Gradual dilution for the minimum conc.	Based on dilution factor(d <sub>f</sub> )	Based on the value of slope $X = \frac{3S_B}{Slope}$	Linear equation $\hat{y}(mV) = y_B + 3S_B$	L.O.Q= $\hat{y}(mV) = y_B + 10S_B$
4.141ng	0.156ng	0.233ng	2.16 µg	7.19µg

X= value of L.O.D based on slope

S<sub>B</sub>= Standard deviation of blank solution

y<sub>B</sub>= Average response for the blank solution

(equivalent to intercept in straight line equation) .

L.O.D= limit of detection

L.O.Q= limit of quantitation

Repeatability study was conducted. Table 4 tabulates the data obtained for eight successive

injections of selected metformin solution. It gave %RSD of better than %0.5.

**Table 4-** Repeatability of metformin results.

[MTF] mmol.L <sup>-1</sup>	$\bar{y}(n=8)$ (mV)	$\sigma_{n-1}$	Repeatability %RSD	$\bar{y}_i \pm t_{0.05/2, n-1} \frac{\sigma_{n-1}}{\sqrt{n}}$ $t_{0.05/2, 7}=2.365$
0.01	69	0.15	0.22	96 ± 0.130
0.1	435	0.05	0.011	435 ± 0.045
0.7	953	0.83	0.087	953 ± 0.740

**Application of The Method**

The developed method was applied to the available drugs in the market (drug names :Glyciphage, Metforal, Glucophage) and was compared with classical spectrophotometric method. The standard addition method was

applied and the data were treated statistically; tabulated in Table 5. the data were presented at 95% and 99% confidence. Also paired t-test was carried out for both method as shown in Table 6. It shows no significant difference between the two methods.

**Table 5 -** Results for the determination of Metformin in pharmaceutical preparation

Sample no.	Commerical name Content country	*Confidence interval for average weight $\bar{w} \pm 1.96 \frac{\sigma_{n-1}}{\sqrt{n}}$ at 95% $\bar{w} \pm 2.58 \frac{\sigma_{n-1}}{\sqrt{n}}$ at 99% (g)	Sample weight equivalent to 2.484 mg (0.15mmol.L <sup>-1</sup> ) of the active ingredient (g)	Theoretical content for the active ingredient at 95% and 99% for n=∞ (mg)	Equation of standard addition curve at 95% for n-2 $\hat{y}_i = a \pm S_a t + b \pm S_b \pm x$	Practical conc. (mmol.L <sup>-1</sup> ) and what is equivalent of active ingredient (mg)	Practical content of active ingredient at 95% and 99% for n=∞ (mg)	Efficiency of determination (%Rec.)
					Spectro.-FIA			
1	Glyciphage 500 mg medical Bahri company Syria	0.75108 ± 0.0052 0.75108 ± 0.0068	0.00373	500±3.33 500±4.53	178.43±56.57+1177.18±231.79x	0.145 2.402	483.59 ± 1.109 483.59 ± 1.459	96.72%
					0.183±0.033+1.542±0.136x	0.142 2.352	473.60 ± 2.63 473.60 ± 3.46	94.72%
2	Metforal 500 mg Laboratori Guidotti s.p .A-pise Italy	0.5508± 0.0013 0.5508± 0.0016	0.00274	500±1.18 500±1.45	186.71±62.32+1181.72±255.34 x	0.149 2.468	496.09 ± 2.693 496.09 ± 3.545	99.22%
					0.217± 0.149+1.493±0.608x	0.148 2.451	492.77 ± 3.66 492.77 ± 4.81	98.55%
3	Glucophage 500 mg Merck France	0.5289 ± 0.0039 0.5289 ± 0.0051	0.00263	500±3.69 500±4.82	193.62±67.90+1204.94±278.18x	0.152 2.518	506.29 ± 2.85 506.29± 3.75	101.26%
					0.256±0.164+1.525±0.675x	0.153 2.534	509.59 ± 3.67 509.59 ± 4.83	101.92%

x : [MTF] mmol.L<sup>-1</sup>

$\hat{y}_i$ : absorbance or absorbance in mV.

\* $t_{0.025, \infty} = 1.96$  at 95%,  $\alpha = 0.05$ .

$t_{0.005, \infty} = 2.58$  at 99%,  $\alpha = 0.01$ .



**Table 6** - Paired t-test results for spectrophotometric-FIA with classical UV-method using standard addition method for the determination of metformin in pharmaceutical preparation.

Sample no.	A moment found X(mg)±RSD at 95% (n=3)		Xd	X d	Sd	t <sub>tab.</sub> at 95%, n-1	T <sub>cal</sub> = $\frac{\bar{x}_d \cdot \sqrt{n}}{S_d}$ n=3
	Proposed method(SP.-FIA)	UV-method					
1	483.59±1.109	473.60±2.63	9.99	3.34	6.65	4.303>>0.87	
2	496.09±2.693	492.77±3.66	3.32				
3	506.29±2.85	509.59±3.67	-3.3				

SP: Spectrophotometric

Sd: Standard deviation of the different

### Conclusions

The method adopted in the procedure used through this research work is completely new in its approach via the use of immobilised copper(II)ion that was introduced through the gel bead structure vacancies. The imbedded copper(II)ion in the gel bead structure can be used in the reaction for the formation of red-magneta colour complex formed by direct action of the drug with the released copper(II) ion from the gel bead in alkaline medium. The release of copper(II) ion from the gel bead is no problem in having the necessary amount for the reaction and this was achieved via the optimization of the time for this release. Excellent reproducibilities for various ranges of concentrations were achieved in all the studied carried out through out this work. It compared quite well with traditional spectrophotometric method. The method reliability based on the repeatability of successive measurements gives a great confidence for the use of this approach as an analytical tool for determination of metformin-HCl.

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